Growth of biopellets on glucose in upflow anaerobic sludge bed (UASB) systems

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Abstract

This paper reports on an investigation into the response of a UASB system using a defined substrate, glucose. Glucose was selected because its biochemical fermentation pathways are well known and this allows process identification from product formation. It also eliminates the presence of a polymer in the influent as a possible cause for pellet formation. Excellent pellet formation was observed in the single UASB reactor system. The single reactor system was separated into two in-series UASB reactor system. The single reactor system was separated into two in-series reactors with the first operating in the high hydrogen partial pressure (high $\overline{p}H_2$) phase. In the high $\overline{p}H_2$ reactor it was found that:

- Butyrate was generated at a sludge age less than about 2 d; when the sludge age exceeded about 2,3 d no butyrate was observed, apparently because butyrate oxidisers became established.
- Soluble COD removal was about 21 per cent on average.
- The overall volatile solids (pellet solids plus suspended solids) yield was 0,47 mgVSS/mgCOD removed.
- From a glucose balance the hydrogen flux was estimated and the gross yield of the hydrogenotrophs (organism plus polymer) calculated, 0,23 mgVSS/mgCOD (H_2)— this is about 6 times greater than the reported values for hydrogenotrophs [0,043 mgVSS/mgCOD(H_2)] and is due to polymer formation.

Introduction

In an investigation into the behaviour of a UASB system treating a carbohydrate-type (apple juice) waste water consisting principally of sugars, Sam-Soon *et al.* (1987) concluded that:

(1) There was very little intermixing of the bed material and that the fluid mixing regime was essentially of a plug flow type.

(2) The bed consisted basically of three zones:

- A lower active zone in which
  - acidogens generate short-chain fatty acids (SCFA), principally acetic (HAc) and propionic (HPr); carbon dioxide (CO_2) and hydrogen (H_2). The hydrogen is generated at such a rate that a high hydrogen partial pressure (high $\overline{p}H_2$) is created;
  - hydrogenotrophic methanogens generate methane (CH_4) from H_2 and CO_2; and
  - acetoclastic methanogens convert some HAc to CH_4 and CO_2.
- An upper active zone which commences when the $\overline{p}H_2$ has been reduced to, and maintained at, such low values due to the action of hydrogenotrophs that
  - acetogens can convert HPr to HAc, H_2 and CO_2; and
  - acetoclastic methanogens convert all the HAc to CH_4 and CO_2
- An upper inactive zone in which no further significant biochemical reactions take place.

From the above, it is apparent that the differentiation of the sludge bed into lower (high $\overline{p}H_2$) and upper (low $\overline{p}H_2$) active zones by Sam-Soon *et al.* (1987) is based on whether HPr oxidation to HAc occurs or not. For the purpose of this paper, these definitions will be accepted, i.e. lower active (high $\overline{p}H_2$) zone is where no apparent HPr oxidation to HAc takes place; upper active (low $\overline{p}H_2$) zone is where HPr oxidation takes place. The parameter that can be used to separate these zones is $\overline{p}H_2$; for $\overline{p}H_2 > \pm 10^{-4,1}$ no HPr oxidation takes place (lower active, high $\overline{p}H_2$ zone), for $\overline{p}H_2 < \pm 10^{-4,1}$ HPr oxidation takes place (upper active, low $\overline{p}H_2$ zone) (see later).

This approach is a macro one in which the overall average behaviour is described. A micro point of view would be to consider the pellet itself. Exactly what happens in the pellet can only be surmised: Microphotographs of the pellet indicate passageways which would imply that there are channels for entry and exit of the compounds through which movement of compounds is assisted by a pumping action due to escaping gases. Thus, diffusion processes alone do not impose limitations on mass transfer of substrate and products. However, it is conceivable that internal to the pellet the average conditions change to some degree with depth below the pellet surface. Accordingly, it is possible that $\overline{p}H_2$ might be lower or higher at some point in the pellet interior leading to different product formation.

(3) Pellet generation was confined to the lower active (high $\overline{p}H_2$) zone; pellet break-up took place in the upper active (low $\overline{p}H_2$) and inactive zones.

(4) In the high $\overline{p}H_2$ zone no pellet debri discharged from the bed: the pellet mass generated was approximately 0,42 mgVSS/mgCOD removed which is an abnormally high specific volatile suspended solids (VSS) yield value, on par with that for **aerobic** growth ($\approx$0,45 mgVSS/mgCOD removed. **See Appendix 3.**

(5) Free and saline ammonia uptake in the high $\overline{p}H_2$ zone was 12 times that normally observed for anaerobic bio-growth.

(6) Augmentation of the feed by the amino acid cysteine, immediately reduced the generation of pelletised mass.

From (4) and (5) it was postulated that the high VSS yield was due to the generation of extracellular polymer which enmeshed the organism mass into pellets. From (5) and (6) it was postulated, *inter alia,* that the polymer was composed predominantly of peptides. Sam-Soon *et al.* (1987) concluded that this polymer, most likely,

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Received 12 June 1989; accepted in revised form 7 February 1990

was secreted by an H_2-utilising methanogen, *Methanobacterium* Strain *AZ (Methanobrevibacter arboriphilus),* under high $\overline{p}H_2$ conditions. This organism utilises H_2 as its sole energy source and can produce all its amino acid requirements for growth except cysteine - it is dependent on an external supply of cysteine for cell synthesis. Apparently under the high H_2 substrate there is a high production of all the amino acids except cysteine; due to insufficient cysteine, an internal metabolic imbalance in the amino acids is created. Adjustment is by release of some of the excess amino acids to the surrounding medium and linking of the rest in polypeptide chains which are stored extracellularly by extrusion from active sites. They concluded that the following conditions must be satisfied for pellet formation:

- An environment with a high $\overline{p}H_2$.
- A nitrogen source, in the free and saline ammonia form, well in excess of the metabolic requirement of the organisms.
- A limited source of cysteine either from the feed or becoming available from the action (e.g. death) of other organisms.
- A near neutral pH.

The following situations were identified under which one could expect pelletisation or not:

(1) Pelletisation in systems where the substrate yields hydrogen and the operation allows a zone of high H_2 partial pressure to build-up, e.g. carbohydrates and proteins in plug flow reactors.

(2) No pelletisation in systems where the influent substrate does not yield hydrogen in the fermentation process, e.g. acetate as sole substrate.

(3) No pelletisition in systems where the substrate yields hydrogen but, in order to obtain complete conversion to methane, operation requires a low hydrogen partial pressure, e.g. carbohydrates and proteins in completely mixed reactors.

(4) Limited pelletisation where the substrate can generate a high $\overline{p}H_2$ but some of the H_2 generated is utilised by other organisms, such as sulphate reducers.

(5) No pelletisation in systems where the influent substrate can be broken down only under low H_2 partial pressure conditions, e.g. propionate and lipids.

(6) Limited pellet production where there is a limitation on free and saline ammonia in the influent.

The situation under (6) above was not stated explicitly by Sam-Soon *et al.* (1987) but follows logically from the hypothesised behaviour of *M.* Strain *AZ.*

The mechanism for pellet formation proposed by Sam-Soon *et al.* (1987) is probably one of a number of mechanisms which may lead to bio-conglomerate accumulation in a UASB system. Dolfing (1987), for example, identifies three types of conglomerates:

- Flocs: Conglomerates with a loose structure.
- Pellets: Conglomerates with a well-defined structure (similar in appearance to lead shot) that settle rapidly.
- Granules: Pellets having a granular appearance.

It is possible that these different conglomerates could arise from different mechanisms, due to different flow regimes, operational modes and influent substrates.

As regards the type of polymer formed, Ross (1984) examined, by electron microscope, pellets formed in a clarigester treating a glucose/starch waste. He identified a polymer matrix binding the pellets together composed of carbohydrates and proteins, but found that the polymer constitution was approximately the same as a polymer present in the influent. He concluded that the polymers in the pellets may have been be derived in part from polymers in the influent. Dolfing *et al.* (1985), also using electron microscopy on pelletised sludge from a UASB system treating sugar processing waste waters, found large amounts of extracellular material of which only 1 to 2 per cent was polysaccharide in nature; other types of polymers were present but these were not identified. Dolfing (1987) implicated both a polysaccharide type of extracellular polymer and a filamentous methanogen, *Methanothrix,* as being responsible for conglomerate formation (as flocs) where the influent substrate consisted of a mixture of short-chain fatty acids. The conglomerate (flocs) generated appeared fluffy compared with the granular conglomerate (pellets) generated from carbohydrate substrate, as reported by Hulshoff Pol *et al.* (1984) and Sam-Soon *et al.* (1987). Other workers have observed polymer matrices but have not identified the polymer types. From TKN/COD and COD/VSS ratio measurements Sam-Soon *et al.* (1987) concluded the pellets formed with a carbohydrate substrate were made up 90 per cent by polypeptide mass.

In the investigation of Sam-Soon *et al.* (1987), a behaviour deviant from that expected in anaerobic fermentation was observed -no butyrate production in the profiles in the high $\overline{p}H_2$ zones of both the single and two in-series UASB reactor systems even at the maximum loadings. From reported work on fermentation of carbohydrates, in addition to acetate and propionate, usually a substantial butyrate production is reported in the acidogenic stage (Thauer *et al.,* 1977; Zoetemeyer *et al.,* 1982). Sam-Soon *et al.* (1987) however, did not attempt to find an explanation for this behaviour.

In the literature, all the work reported had made use of influents that were in effect mixtures of substrates, such as SCFA, carbohydrates and proteins. For example, the substrate utilised by Sam-Soon *et al.* (1987) was an apple juice waste water supplemented with excess free and saline ammonia and a trace metal solution; this waste water was not a clearly defined substrate, almost certainly containing various organic carbon species in addition to sugars and SCFA (acetic and propionic). With such an undefined substrate the biochemical reactions occurring are uncertain - in particular it is not possible to assess the hydrogen flux from end product formation (because the biochemical pathways cannot be identified fully); and the influent feed may contain polymers that can promote pellet formation (Ross, 1984). These difficulties would be resolved if a defined substrate is used. The carbohydrate, glucose, for example, should be a particularly appropriate substrate because extensive knowledge of its biochemical pathways with associated product formation is available, and certainly the presence of polymers in the influent would be eliminated. Accordingly it was decided to study the response of a UASB system using glucose instead of apple juice as substrate.

The investigation devolved into the following main tasks:

- Response of a UASB system to glucose as substrate feed.
- Enquiry into the appearance or non-appearance of butyrate in UASB systems.
- Behaviour of the hydrogen-utilising organisms in the high $\overline{p}H_2$

zone of a UASB system.

Fermentation of glucose

Fermentation of glucose under the conditions normally encountered in methane fermentation processes occurs in three distinct phases: Acidogenesis; acetogenesis; and methanogenesis. Product formation arising in these three phases depends to a large degree on the $\overline{p}H_2$. The three phases in the fermentation of glucose are set out briefly below.

Acidogenesis

This is the initial phase in fermentation of soluble carbohydrates carried out by a group of organisms, the acidogens. When the $\overline{p}H_2$ is $< 10^{-3,7}$ atmospheres (atm), acetic and butyric acids, hydrogen and carbon dioxide are generated, and when the $\overline{p}H_2$ is $> 10^{-3,7}$ atm acetic, propionic and butyric acids, hydrogen and carbon dioxide are generated (Fig. 1a, b and c). That is, butyrate is a normal fermentation product from a carbohydrate substrate irrespective of the $\overline{p}H_2$ (Thauer *et al.*, 1977). The stoichiometric reactions describing these pathways are as follows:

For $\overline{p}H_2 < 10^{-3,7}$ atm
production of acetic acid:

$$C_6H_{12}O_6 \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

and production of butyric acid:

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

For $\overline{p}H_2 > 10^{-3,7}$ atm
production of acetic and propionic acids:

$$C_6H_{12}O_6 \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2 \quad (3)$$

and production of butyric acid, as in Eq. 2.

In terms of the definition of Sam-Soon *et al.* (1987) for the high $\overline{p}H_2$ zone ($\overline{p}H_2 > \pm 10^{-4,1}$), all the SCFA generation pathways (Eqs. 1 to 3) can be expected to be operative in this zone; in the low $\overline{p}H_2$ zone propionic acid generation (Eq. 3) is not expected. However, as described earlier, the micro $\overline{p}H_2$ conditions within the pellet itself may differ (probably less) from that at the surface, giving rise to production of SCFAs not expected from the macro average $\overline{p}H_2$.

The acidogens have a generation time of about 2 h with a specific yield on glucose of approximately 0,12 to 0,14 mgVSS/mg glucose processed (Zoetemeyer *et al.*, 1982). Acidogens are not pH sensitive down to pH 3,5, but in the lower pH region product formation changes markedly, to solvent production such as ethanol, butanol, etc.

Acetogenesis

Short-chain fatty acids, butyric and propionic, generated in the acidogenic phase, are oxidised by a specific group of organisms, the acetogens, to acetic acid and hydrogen. Conversion of butyric and propionic acids can take place only at low $\overline{p}H_2$, but at different partial pressures respectively:

For acetic acid formation from butyric acid, the reaction is:

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (4)$$

Thermodynamically this reaction is feasible for $\overline{p}H_2 < 10^{-2,7}$atm (McInerney *et al.*, 1979). The generation time for butyrate oxidisers is approximately 2,3 d (Gujer and Zehnder, 1983) with a specific yield of about 0,03 gVSS/gCOD butyrate processed.

For acetic acid formation from propionic acid, the reaction is:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (5)$$

Thermodynamically this reaction is feasible for $\overline{p}H_2 < 10^{-4,1}$ atm (McInerney *et al.*, 1979). The generation time for propionate oxidisers is between 4,6 to 5,8 d (Boone and Bryant, 1980; Koch *et al.*, 1983) with a specific yield of between 0,023 to 0,034 gVSS/gCOD propionate processed (Dolfing, 1987).

At present, pH sensitivity of acetogens is not established.

Methanogenesis

Three groups of methanogens have been identified:

- Obligate acetoclastic methanogens that utilise acetic acid only as energy source.
- Obligate hydrogenotropic methanogens (also called H_2-utilisers) that utilise H_2 only as energy source (with CO_2 as the carbon source). The hydrogenotrophs can operate over a wide range of hydrogen partial pressure.
- Hydrogenotrophic/acetoclastic methanogens that can utilise acetic acid or hydrogen as energy source, but preferentially hydrogen.

The reactions for hydrogen and acetate oxidations are:

For hydrogen:

$$4H_2 + H_2CO_3 \rightarrow CH_4 + 3H_2O \quad (6)$$

and for acetic acid:

$$CH_3COOH + H_2O \rightarrow CH_4 + H_2CO_3 \quad (7)$$

In the presence of SCFA these methanogens grow optimally in the neutral pH range. In the absence of SCFA growth is optimal down to pH 5,4 (Attal *et al.*, 1988).

Single high/low $\overline{p}H_2$ UASB reactor system

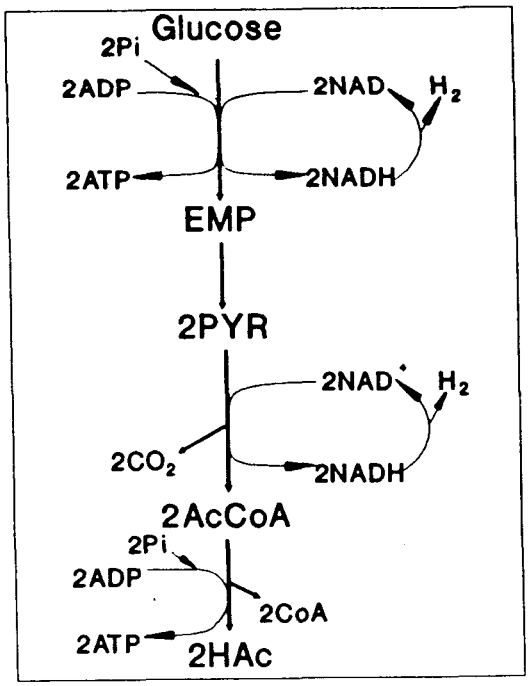
Experimental

A UASB reactor, effective volume 9 ℓ, was seeded with 3 ℓ of pelletised sludge developed on an apple juice waste water (Dold *et al.*, 1987). The reactor was fed with glucose as sole organic carbon source with COD concentration approximately 2 750 mgCOD/ℓ. The feed was supplemented with trace elements and nutrients for organism growth (details of composition are given by Sam-Soon *et al.*, 1987) with excess NH_3-N (65,8 mgN/ℓ), and was buffered by addition of 10 g $NaHCO_3$ per litre of feed. The effects of loading on the system were investigated for the range 8,3 to 26,7 kgCOD/m³ reactor volume.d; pertinent operating data for the system are given in Table 1.

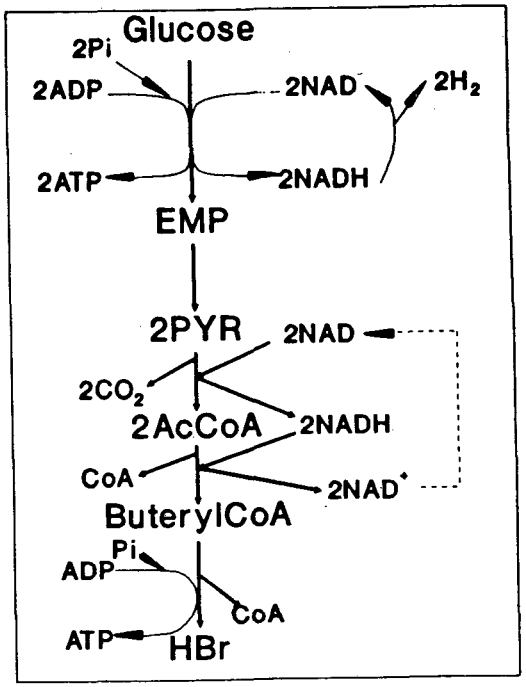
After each increment of loading, steady state was assumed to have been established when COD removal, NH_3-N removal and gas production remained constant for more than five consecutive

TABLE 1
OPERATING DATA FOR SINGLE HIGH/LOW H_2 PARTIAL PRESSURE UASB REACTOR WITH GLUCOSE AS INFLUENT (VOLUME 9 ℓ, DIAMETER 100 mm)

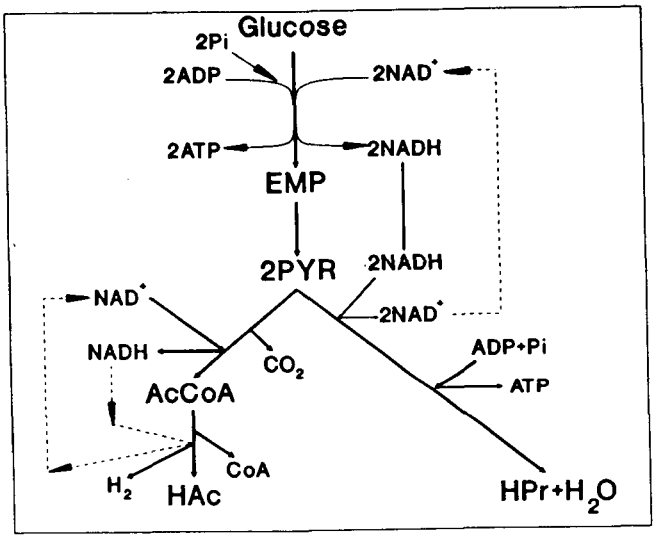
Influent COD concentration, mgCOD/ℓ	2 712 — 5 345
Volumetric flow rate, ℓ/d	15 — 45
Nominal velocity of flow in reactor, cm/min	0,13 — 0,40
Hydraulic retention time, h	14,4 — 4,8
Organic loading, kgCOD/m³ reactor. d	8,3 — 26,7
Operating temperature, °C	30
Influent total alkalinity, mg/ℓ as $CaCO_3$	5 000
Influent pH	8,12
Influent free and saline ammonia (NH_3-N),mgN/ℓ	65,8



(a) Acetic acid only generation ($\bar{p}H_2 < 10^{-3,7}$atm)



(b) Butyric acid generation ($\bar{p}H_2 < 10^{-3,7}$atm and $> 10^{-3,7}$atm)



(c) Acetic + propionic acid generation ($\bar{p}H_2 > 10^{-3,7}$atm).

Figure 1
Acidogenic phase pathways for glucose fermentation to form acetic acid, butyric acid and propionic acid. H_2 and CO_2 formed in all pathways. (Abbreviations: EMP - Embden-Meyerhof pathway; PYR - pyruvic acid; AcCOA - acetyl coenzyme A; NAD⁺ - nicotinamide adenine dinucleotide (oxidised form); NADH - nicotinamide adenine dinucleotide (reduced form); Butyryl CoA - butyryl coenzyme A; HAc -acetic acid; HBr - butyric acid; HPr - propionic acid)

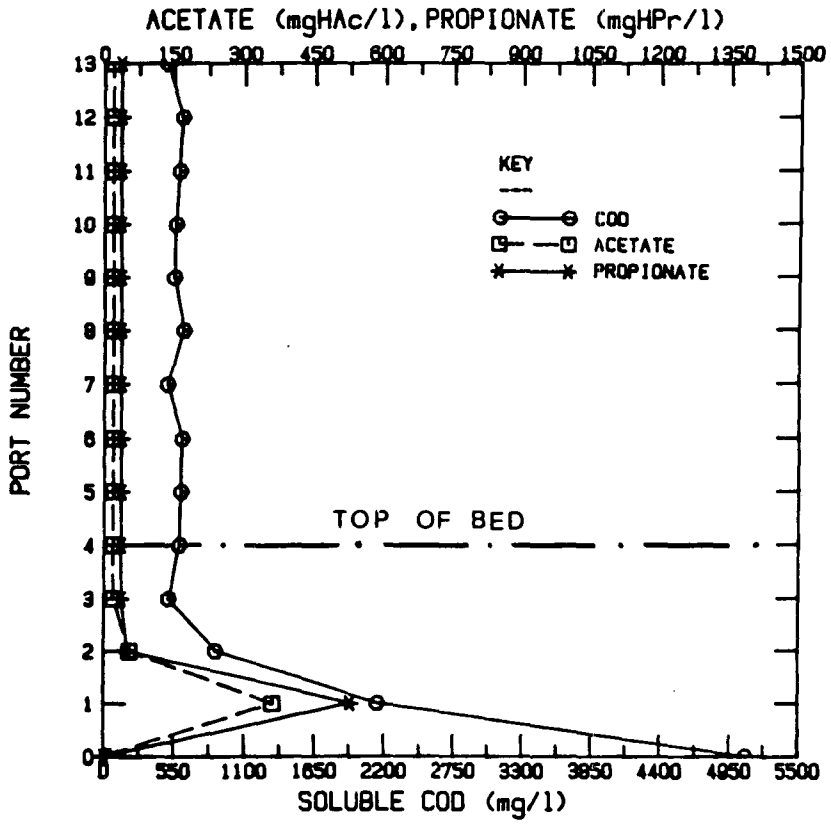
days. Once steady state had been attained, samples were taken along the line of flow and total soluble COD, short-chain fatty acids, free and saline ammonia, total Kjeldahl nitrogen (hence organic nitrogen by difference) and pH were determined.

Results

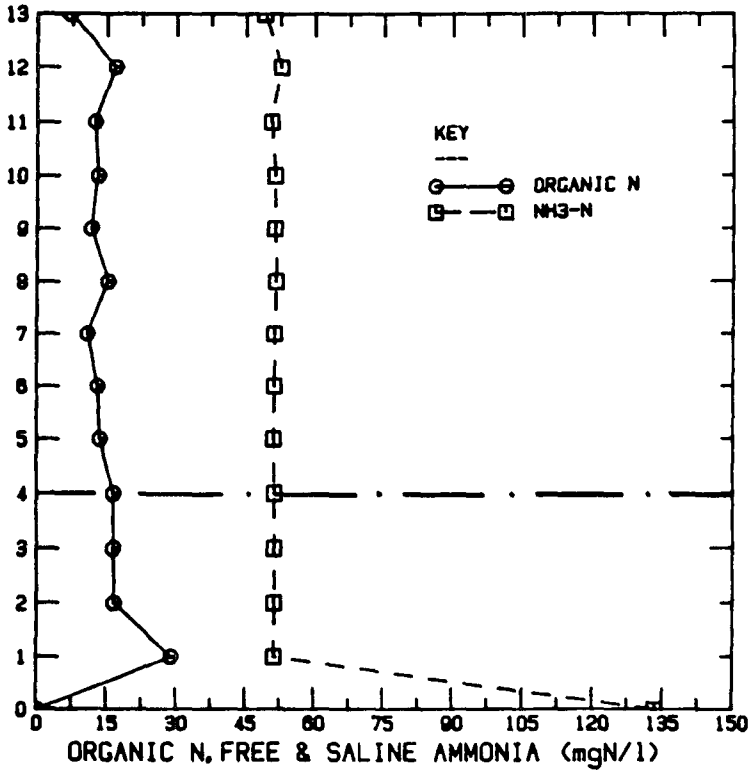
For the range of loadings investigated excellent pelletisation was obtained. In all cases the COD removal exceeded 90 per cent. Concentration profiles of the various parameters measured along the line of flow in the reactor are shown in Fig. 2a, b and c and Fig. 3a, b and c for the lowest and highest loading respectively. In general, the profiles were similar to those obtained with apple juice waste water as substrate. Acetic and propionic acids were the only SCFA identified in the profile, and two distinct active zones and one inactive zone were identified:

- ***A lower active zone:*** In this zone, the total soluble COD concentration decreased to about half the initial value; acetate and propionate increased monotonically to maximum values; no butyrate formation was observed; NH_3-N concentration dropped sharply to a minimum; organic nitrogen was released into the aqueous phase; and pH declined to a minimum. The monotonic increase in propionate defines a high $\bar{p}H_2$ zone (Sam-Soon *et al.*, 1987).

- ***An upper active zone:*** In this zone, the total soluble COD concentration reduced further to a minimum value; acetate and

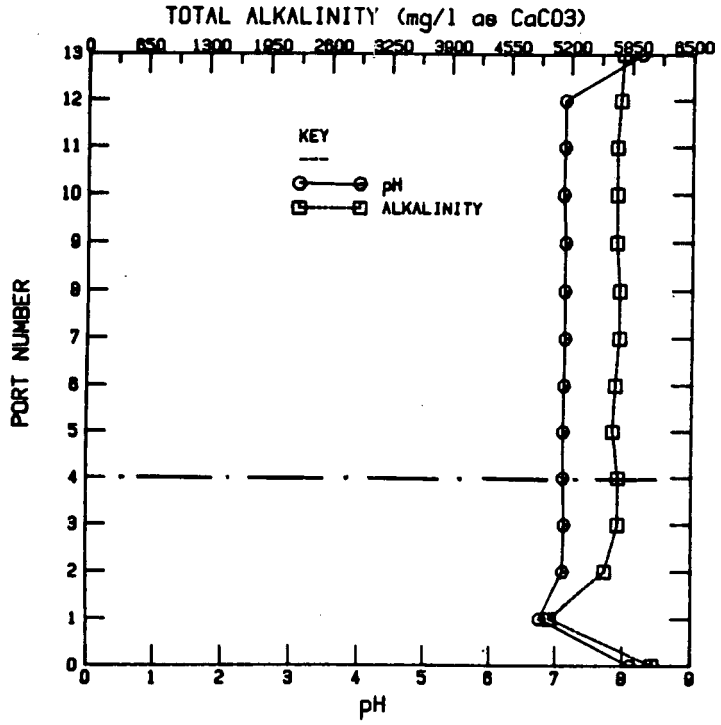


(a)



(b)

Figure 2
Product concentration and pH profiles observed in the single reactor UASB system on glucose substrate (loading: 8,3 kgCOD/m³ reactor volume.d)



(c)

propionate decreased monotonically to minimum values and remained constant thereafter; NH_3-N concentration remained virtually constant; organic nitrogen concentration showed a slight decrease; and pH increased slowly to a stable value. The monotonic decrease in propionate defines a low $\overline{p}H_2$ zone (Sam-Soon *et al.*, 1987).

- ***An inactive zone:*** Above the upper active zone, up to the top of the sludge bed, no observable product formation was detected, defining an inactive zone.

From the results above it would appear that the fermentation of glucose in the UASB system follows a pattern similar to that observed in the fermentation of apple juice waste water. Pellet formation with glucose as substrate clearly indicates that pelletisation is not due to extraneous organic polymers present in the influent feed (as hypothesised by Ross, 1984) but is due to some other biochemical mechanism, such as that proposed by Sam-Soon *et al.* (1987). As with apple juice waste, butyrate was not detected even though this is a normal fermentation product to be expected in acidogenesis from glucose.

Having established a very similar response between the glucose and apple juice fed UASB systems, it could be expected that, as in the apple juice fed system, pellet generation in the glucose fed system takes place in the high $\overline{p}H_2$ zone of the reactor. Accordingly, it was decided to investigate the high $\overline{p}H_2$ zone of a UASB system with glucose as substrate.

High $\overline{p}H_2$ UASB reactor system

The enquiry into the high $\overline{p}H_2$ phase of the UASB system with glucose as substrate set the following specific tasks: Pellet production; non-production of butyrate; hydrogenotrophic organism behaviour; and nature of polymer.

Experimental

A UASB reactor, to operate under high $\overline{p}H_2$ conditions, was set up as follows: A reactor with an effective volume of 3 ℓ was inoculated with 1 ℓ of pelletised sludge obtained from the high $\overline{p}H_2$ zone of a single high/low $\overline{p}H_2$ UASB reactor fed with the same glucose substrate as described in the section above. The reactor supplying the inoculum had been operated at a loading rate of 8,3 kgCOD/m³ reactor.d, with influent COD concentration ≈ 5 000 mg/ℓ, flow rate = 15 ℓ/d. Measurements of the pelletised sludge inoculum gave a mean pellet density = 35 349 mgVSS/ℓ.

In operating the high $\overline{p}H_2$ reactor, the COD concentration of the glucose substrate was changed to be approximately half that of the single high/low $\overline{p}H_2$ UASB reactor system, i.e. an influent COD concentration of around 2 800 mg/ℓ. Increased COD load on the unit was achieved by an appropriate increase in the influent flow rate. To ensure that the system comprised virtually only the high $\overline{p}H_2$ zone, the volume of pelletised sludge was selected by measuring propionate concentration along the line of flow and adjusting the bed volume to a value so that propionate concentration increased continuously up the bed. This gave a bed volume of 1 ℓ; each day the volume of pellets in excess of 1 ℓ bed volume was wasted. The following parameters were measured daily: Influent COD, filtered effluent COD, free and saline ammonia, TKN, pH of the reactor, gas production (corrected to standard temperature and pressure, STP), volume of sludge generated, VSS and total suspended solids (TSS). Once steady state appeared to have been attained, short-chain fatty acids (acetic, propionic and butyric acids) in the effluent, and the percentage of carbon dioxide in the gas were measured (hence by difference, the percentage methane could be estimated assuming the gas phase consisted only of CO_2 and CH_4).

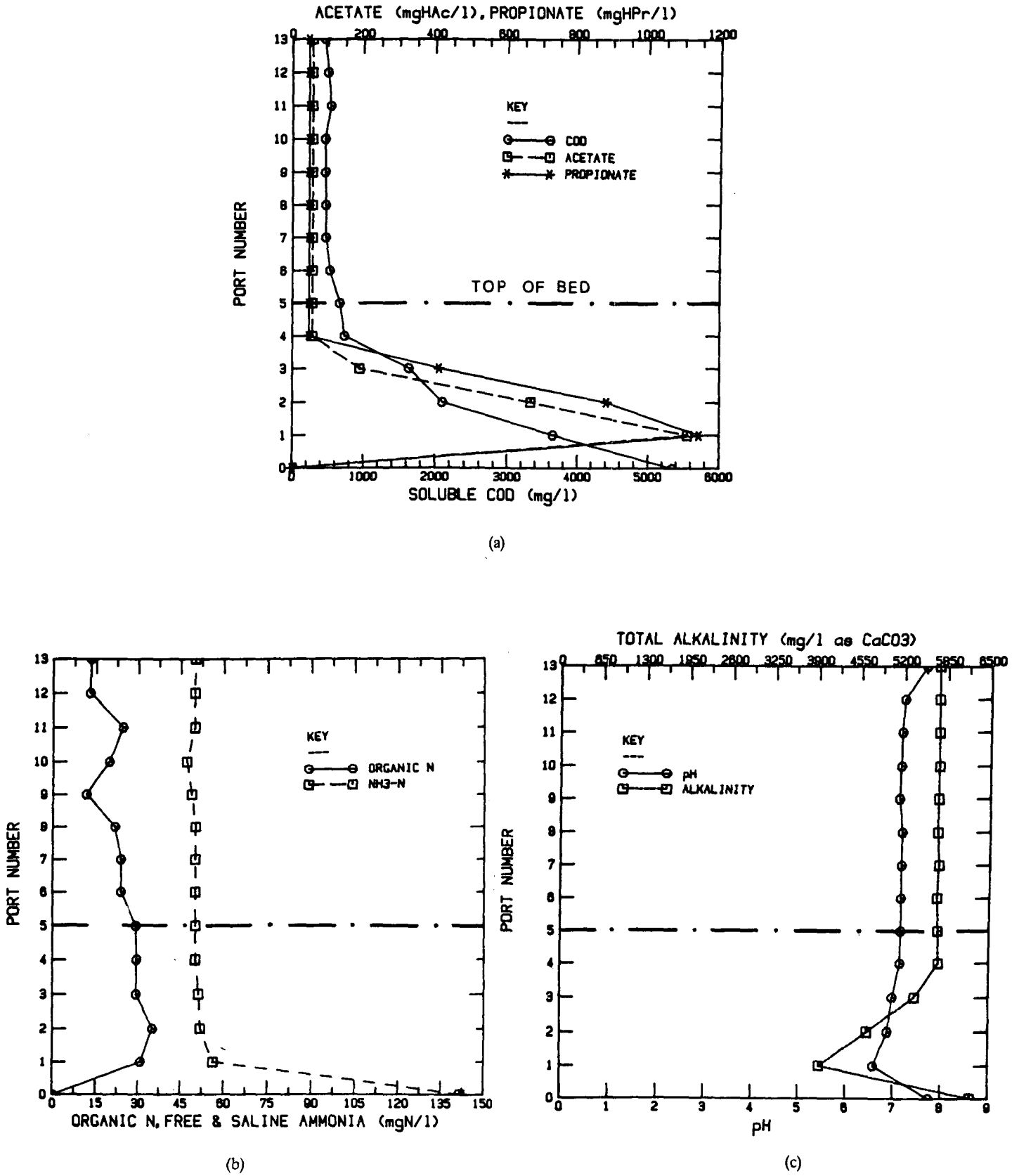


Figure 3
Product concentration and pH profiles observed in the single reactor UASB system on glucose substrate (loading: 26,7 kgCOD/m³ reactor volume.d)

Results

The steady state response of the high $\overline{p}H_2$ reactor, to loading rates of 0,166, 0,203 and 0,238 kgCOD/d respectively, are shown in Table 2. The sludge wasting procedure automatically established a sludge age for the system as follows:

$$\text{sludge age} = \frac{\text{(mass of pelletised bed)}}{\text{(mass of pellets plus pellet debris wasted per day)}} \quad (8)$$

From the sludge mass in the high $\overline{p}H_2$ reactor and the mass wasted daily, the sludge ages for the three loadings ranged from 2,0 to 2,1 d. These sludge ages are below the generation time of propionate oxidisers (4,6 to 5,8 d, Boone and Bryant, 1980; Koch *et al.*, 1983) and butyrate oxidisers (2,3 d, Gujer and Zehnder, 1983).

Soluble COD removal in the high $\overline{p}H_2$ reactor ranged from 17,6 to 22,7 per cent; the average was 20,6 per cent.

Pellet production in high $\overline{p}H_2$ reactor

In the high $\overline{p}H_2$ reactor the VSS production rate was estimated by daily removing excess pellets and measuring their VSS content. No debri discharged from the bed. The principal fermentation reactions taking place were:

- acidogenesis;
- methane formation from H_2 and CO_2; and
- a degree of methane formation from acetic acid cleavage.

Acetogenesis did not appear to take place (see later). Accordingly, in the high $\overline{p}H_2$ reactor the microbial content comprised principally acidogens, hydrogenotrophs and acetoclastic methanogens.

With glucose as substrate, in acidogenesis:

- Approximately 10 per cent (molar or COD basis) of the glucose processed reappears as acidogenic mass (Zoetemeyer *et al.*, 1982).
- Approximately 12 per cent (COD basis) of the glucose processed is released as molecular hydrogen (Cohen *et al.*, 1979).
- The balance of the glucose is transformed to short-chain fatty acids, such as acetic, propionic and butyric, and CO_2.

The hydrogen generated serves as substrate for the hydrogenotrophs. These organisms have a specific yield of 0,043 mgVSS/mgCOD(H_2) removed (Shea *et al.*, 1968), that is, approximately 0,043 x 0,12 = 0,0052 i.e. 0,5 per cent of the glucose (COD) processed reappears as hydrogenotrophic VSS mass.

Some of the acetic acid is converted to methane by the acetoclastic methanogens. Even if all the acetic acid generated should be converted, the mass of acetoclastic methanogens still will be very small relative to the mass of acidogens because the acetoclastic organism yield is only 0,03 mgVSS/mgCOD(acetic) removed. Later in the paper, by appropriate mass balance, a method to estimate the mass of acetic acid utilised by the acetoclastic methanogens will be presented.

Measurements of the VSS generated in the system, for the three loadings, were well in excess of the expected values using the reported yields above. For example for the 60 ℓ/d feed (Table 2), glucose processed was 0,867 mol/d. The expected VSS generation was:

For acidogenic organisms:

VSS generated = 0,867 x 1,067 x 180 x 0,1
= 16,65 gCOD/d

For hydrogenotrophic organisms:

VSS generated = 0,867 x 1,067 x 180 x 0,12 x 0,043
= 0,86 gVSS/d
= 1,22 gCOD/d

For acetoclastic organisms (from Table 2 and the section below):

VSS generated = 4,48 x 0,03
= 0,134 gVSS/d
= 0,19 gCOD/d

Total expected VSS production
= 16,65 + 1,22 + 0,19
= 18,06 gCOD/d

For this loading the observed VSS generated was 25,54 gCOD/d. For each of the loadings investigated similar discrepancies arose. One explanation for the unusually high observed VSS production is that polymer generation took place in the system. In view of the high nitrogen to COD removal (see later) it would appear that the polymer was a peptide type.

For the 60 ℓ/d feed the COD removal in the high $\overline{p}H_2$ reactor was 22,8 per cent (Table 2), i.e.

COD removal = 0,228 x 2774 x 60 = 37,95 gCOD/d.

With an observed VSS production of 25,54 gCOD/d (17,99 gVSS/d), the overall specific yield was

25,54/37,95 = 0,67 gCOD/gCOD removed = 0,47 gVSS/gCOD removed.

In a single high/low $\overline{p}H_2$ reactor (or in-series high and low $\overline{p}H_2$ reactor system) some of the VSS generated may be broken down in the low $\overline{p}H_2$ zone (reactor) so that the specific yield calculated here would form an upper limit. For the high/low $\overline{p}H_2$ glucose reactor, with a COD removal of 95 per cent, the overall specific yield would be approximately

25,54/(0,95x2774x60) = 0,16gCOD/gCOD removed (0,11 gVSS/gCOD removed)

With respect to the influent COD, a maximum of approximately 15 per cent of the influent COD would be converted to volatile solids (as COD), i.e. 10 per cent as VSS.

Butyrate production and oxidation

For each of the three loadings investigated, butyric acid was observed in the high $\overline{p}H_2$ glucose reactor, in addition to acetic and propionic acids (Table 2). The presence of butyric acid was a feature not observed previously in either the parent single high/low $\overline{p}H_2$ reactor maintained on a glucose substrate, or, in either the single high/low $\overline{p}H_2$ or in-series (high and low $\overline{p}H_2$) reactor systems operated on apple juice waste (Sam-Soon *et al.*, 1987). In search of a cause for this difference in behaviour, the operational sludge age of the high $\overline{p}H_2$ glucose reactor was compared with the sludge ages of the other systems: In the high $\overline{p}H_2$ glucose reactor the sludge age ranged between 2,0 and 2,1 d whereas for

TABLE 2
STEADY STATE RESPONSE OF THE HIGH H_2 PARTIAL PRESSURE UASB REACTOR TO DIFFERENT LOADING RATES WITH GLUCOSE AS SUBSTRATE

Flow rate	Infl.* COD	SCOD	Effl. COD gCOD/d		COD recovery	Glucose utilised	Effl. SCFA+ mg/ℓ			NH_3-N In	NH_3-N Out	OrgN Effl	Gas production ℓ/d STP	fraction CO_2	fraction CH_4	COD/VSS mgCOD/ mgVSS	TKN/COD mgN/ mgCOD	Sludge age days
ℓ/d	g/d		Sludge wasted	CH_4	%	mol/d	HAc	HPr	HBr	mgN/ℓ	mgN/ℓ	mgN/ℓ						
60	166,44	128,52	25,54	11,85	99,7	0,867	722	389	333	86,8	40,3	17,7	8,5	0,51	0,49	1,42	0,1260	2,1
75	203,85	167,85	26,90	8,70	99,8	0,965	750	367	250	86,8	40,3	19,5	5,8	0,59	0,41	1,30	0,1296	2,0
90	237,78	186,84	30,47	18,68	99,2	1,140	589	350	278	86,8	40,3	23,0	12,3	0,47	0,53	1,23	0,1126	2,0

*To convert COD (gram) to glucose (mol) divide by (180 x 1,067); 1 g glucose = 1,067 g COD.
+To convert HAc; HPr; HBr (mg) to COD (mg) multiply by 1,067; 1,512; 1,714 respectively.
To convert HAc; HPr; HBr (mg) to (mmol) divide by 60; 74; 88 respectively.

the other systems the sludge ages were greater than 3 d. The generation time for acetogenic butyrate oxidising bacteria is about 2,3 d (Gujer and Zehnder, 1983). Therefore, with a sludge age of about 2 d (as in the high $\overline{p}H_2$ glucose reactor) one may expect washout of these butyrate oxidisers, and hence appearance of butyric acid in the profiles. In UASB systems with both high and low $\overline{p}H_2$ zones present in the same reactor operating on glucose, the sludge age always would be well in excess of 2,3 d so that butyrate oxidisers could become established in the system. Accordingly, one would still expect butyric acid generation but also its oxidation, to give a pattern similar to that for propionic acid, i.e. generation in the lower zone and disappearance (oxidation) in the upper zone. However, in these systems butyrate had **not** been observed at the sample ports in the lower (high $\overline{p}H_2$) zones, possibly due to the following: Observing the movement of the pellets in the sludge bed under the disturbing influence of both gas bubble evolution and upward motion of the fluid flow, there is a slow downward exchange of pellets. In this fashion butyrate oxidisers are introduced to the region where butyrate is generated, and, provided the $\overline{p}H_2$ in this region is less than $10^{-2,7}$ atm, butyrate oxidation should take place. Propionate oxidation was not observed in this region; this occurs only at $\overline{p}H_2 < 10^{-4,1}$ atm. Hence, the disappearance of butyrate, but not propionate, would infer a $\overline{p}H_2$ of between $10^{-4,1}$ and $10^{-2,7}$ atm at the sample ports.

If a sludge age is established at a level lower than that required for butyrate oxidisers (as in the high $\overline{p}H_2$ reactor, sludge age = 2 d), these organisms are washed out of the system and even intermixing of the pellets would not affect the butyrate oxidation.

Unfortunately, at the time these experiments were undertaken the hypothesis on the presence or absence of butyrate had not yet been formulated and consequently no experimental evaluation was undertaken to check this explanation. Such an evaluation will require closer spacing of the sampling ports in the UASB reactor, or appropriate batch tests using the pellets from the UASB reactor.

Hydrogenotrophic organism behaviour in the high $\overline{p}H_2$ UASB reactor

The principal objective was to calculate the yield of the hydrogenotrophs. To do this the mass of the hydrogen generated needs to be estimated from the mass of glucose converted to the various short-chain fatty acids, and the mass of acidogens. We will now develop the procedures that allow these to be estimated.

Conversion of glucose to SCFA and acidogen mass

In the high $\overline{p}H_2$ UASB reactor, controlled to a sludge age of about 2 d, the only fermentation processes would be acidogenesis and methanogenesis. Acetogenesis would not be established because the generation time of the acetogens (propionate and butyrate oxidisers) is longer than the sludge age of 2 d.

Glucose will be processed via the various acidogenic pathways set out in Fig. 1a, b and c. Provided none of the propionic and butyric acid is oxidised, one could readily make a glucose balance (using the fermentation pathways, Eqs. 1 to 3). The glucose is processed to the short-chain fatty acids, acetic (HAc), propionic (HPr) and butyric (HBr), to hydrogen and to acidogenic mass.

- From Eq. 3, for every one mol HPr appearing one mol glucose is processed and one mol HAc also appears, i.e.

  if [G1] = molar mass of glucose processed to HPr and HAc, then:
  $[HPr]_{G1} \equiv [HAc]_{G1} \equiv [G1]$

- From Eq. 1, for every two mol HAc appearing one mol glucose is processed, i.e.

  if [G2] = molar mass of glucose processed to HAc (only), then
  $^1/_2\ [HAc]_{G2} = [G2]$

- From Eq. 2, for every one mol HBr appearing one mol glucose is processed, i.e.

  if [G3] = molar mass of glucose processed to HBr, then
  $[HBr]_{G3} = [G3]$

- The molar mass of glucose processed and reappearing as acidogenic mass [G4] is given approximately empirically by:

  $[G4] \approx Y_{acid}[GT]$
  where Y_{acid} = yield of acidogens
  $\approx 0,1$ mol glucose/mol glucose (Zoetemeyer *et al.*, 1982).

Hence $[GT] = [G1] + [G2] + [G3] + [G4]$ (9a)

Substituting for [G1], [G2], [G3] and [G4] from above:

$$[GT] = [HPr]_{G1} + {}^1/_2\,[HAc]_{G2} + [HBr]_{G3} + Y_{acid}\,[GT] \quad (9b)$$

Now, the total acetic acid, $[HAc]_T$, is made up of $[HAc]_{G2}$ and $[HAc]_{G1}$, i.e.:

$$[HAc]_T = [HAc]_{G2} + [HAc]_{G1}$$
$$= [HAc]_{G2} + [HPr]_{G1} \quad \text{since } [HAc]_{G1} = [HPr]_{G1}$$

i.e. $[HAc]_{G2} = [HAc]_T - [HPr]_{G1}$

Substituting for $[HAc]_{G2}$ in Eq. 9b:

$$[GT] = [HPr]_{G1} + {}^1/_2\{[HAc]_T - [HPr]_{G1}\} + [HBr]_{G3} + Y_{acid}[GT] \quad (10)$$

Usually some of the HAc generated will be oxidised to methane, $[HAc]_{ox}$, thereby reducing the total HAc, $[HAc]_T$, to give the observed HAc, $[HAc]_{obs}$. To incorporate $[HAc]_{obs}$ into Eq. 10 we note that:

$$[HAc]_T = [HAc]_{obs} + [HAc]_{ox}$$
i.e. $$[GT] = [HPr]_{G1} + {}^1/_2\,\{[HAc]_{obs} + [HAc]_{ox} - [HPr]_{G1}\}$$
$$+ [HBr]_{G3} + Y_{acid}[GT] \quad (11)$$

All the terms in Eq. 11 except $[HAc]_{ox}$ can be measured directly or estimated. [GT] can be measured directly, or alternatively, if no glucose measurements are available on the effluent, [GT] can be estimated as set out in **Appendix 1.**

For 60 ℓ/d system, from Eq. 11, acetic acid oxidised for methane production ($[HAc]_{ox}$) is:

$$[0,867] = [0,315] + {}^1/_2\{[0,722] - [0,315]\} + {}^1/_2[HAc]_{ox} + [0,227] + [0,0867]$$

(Data obtained from Table 2, suitably converted to molar form)

i.e.:
$[HAc]_{ox} = 0,07$ mol/d
$= 4,48$ gCOD/d

The $[HAc]_{ox}$ for the three loadings are listed in Table 3.

Hydrogen flux

Having determined the mass of acetic acid oxidised to methane ($[HAc]_{ox}$) at a point in the high $\overline{p}H_2$ zone, it is possible to determine the hydrogen flux, $[H_2]$, at that point. This is done by using the same approach as in setting up the glucose balance equation (Eq. 11), except that glucose processing implies hydrogen generation with the stoichiometry between SCFA generated and hydrogen generated appropriately adjusted, as set out in the fermentation pathways in Fig. 1a, b and c, i.e.:

$$[H_2] = [Hpr]_{G1} + 2[HBr] + 2\{[HAc]_{obs} - [HPr]_{G1} + [HAc]_{ox}\} \quad (12)$$

where [ ] = mol/day

For the 60 ℓ/d system, the values for $[HPr]_{G1}$, [HBr] and $[HAc]_{obs}$ can be obtained from Table 2, and $[HAc]_{ox}$ from the calculation above i.e.:

$$[H_2] = [0,315] + 2[0,227] + 2\{[0,722] - [0,315] + [0,070]\}$$
$$= 1,723 \text{ mol/d}$$
$$= 27,57 \text{ gCOD/d}$$

The $[H_2]$ fluxes associated with the three loaldings are listed in Table 3.

VSS of hydrogenotrophs

Knowing the acidogenic mass generated per day ($VSS_{acidogens}$) and the measured total VSS generated per day, one can estimate the VSS mass generated by the hydrogenotrophic organisms ($VSS_{H_2\text{-utilisers}}$) if the assumption is made that the acetoclastic organism mass is negligible. (This assumption is acceptable as shall be apparent from the calculation below). With this assumption the VSS generated would stem only from acidogens and hydrogenotrophic organisms, i.e.

$$VSS_{H_2\text{-utilisers}} = \text{Total VSS measured} - VSS_{acidogens} \quad (13)$$

For example, using the data for the feed of 60 ℓ/d

$VSS_{measured}$ = 25,54 gCOD/d
$VSS_{acidogens}$ = 16,65 gCOD/d (see earlier)
i.e. $VSS_{H_2\text{-utilisers}}$ = 25,54 - 16,65 gCOD/d
= 8,89 gCOD/d
= 8,89/1,42 = 6,26 gVSS/d.

Specific yield of hydrogenotrophs

It is now possible to determine the specific yield associated with the H_2-utilising methanogens ($Y_{H_2\text{-utilisers}}$), from the VSS ascribed to the H_2-utilisers (VSS_{H_2}-utilisers) and the hydrogen flux ($[H_2]$) determined above, i.e.

$$Y_{H_2\text{-utilisers}} = VSS_{H_2\text{-utilisers}}/[H_2] \quad (14)$$

For the 60 ℓ/d system,

$Y_{H_2\text{-utilisers}}$ = 6,26/27,57
= 0,23 gVSS/gCOD(H_2)

For the loading rate 0,203 kgCOD/d, repeat calculations using

TABLE 3
CALCULATED HYDROGEN FLUX, ACETATE CLEAVAGE, HYDROGEN OXIDATION AND VSS YIELD OF H_2-UTILISERS IN HIGH H_2 PARTIAL PRESSURE UASB REACTOR

Flow rate	Glucose processed	Glucose not processed	Methane generated	HAc oxidised to CH_4	Hydrogen oxidised to CH_4	Hydrogen flux	Total VSS generated	VSS generated by H_2-utilisers	Hydrogen used for* amino acids	Yield H_2-utilisers gVSS/gCOD
ℓ/d	gCOD/d	gCOD/d	gCOD/d	gCOD/d	gCOD/d	gCOD/d	gCOD/d	gCOD/d	gCOD/d	
60	166,46	0	11,85	4,48	7,37	27,57	25,54	8,89	9,87	0,24
75	185,28	18,53	8,70	0	8,70	30,88	26,90	8,28	13,61	0,21
90	218,88	17,91	18,68	11,14	7,54	36,13	30,47	8,58	19,27	0,19

*See **Appendix 2** for computation

Eq. 11 show that $[HAc]_{ox}$ was zero. For this loading rate the calculated hydrogenotroph specific yield value was $Y_{H_{2\text{-utilisers}}}$ = 0,21 mgVSS/mgCOD(H_2) utilised. This value is very close to that calculated above when acetate oxidation did take place ($[HAc]_{ox}$ = 4,48 gCOD/d). Hence, the calculated hydrogenotroph specific yield was not influenced significantly by the generation of acetoclastic organism mass and, accordingly, neglecting the acetoclastic mass in the calculations is acceptable. The acetoclastic mass can be neglected because their specific yield is only 0,03 mgVSS/mgCOD (acetate oxidised) (Ten Brummeler *et al.,* 1985; Dolfing, 1987).

The hydrogenotrophs yield values calculated above [0,23 gVSS/gCOD(H_2)] are **approximately 6 times** that normally observed for these organisms (0,043 gVSS/g COD(H_2) removed, Shea *et al.,* 1968). The reason is that the calculated yield in fact includes the polymer mass formed - for the purpose of distinction we could speak of a **gross** specific yield, to distinguish it from the specific yield of the hydrogenotrophs of 0,043 mgVSS/mgCOD(H_2). (The gross specific yield value for the hydrogenotrophic organisms, determined above, is in fact conservative because it is based only on the measured VSS of the pellets generated. Organic nitrogen is observed in the effluent (Table 2) and this is ascribed to amino acids released by the hydrogenotrophic organisms (Sam-Soon *et al.,* 1987). Converting this organic nitrogen to a COD value (**Appendix 2**) and adding this to the pelletised VSS gives a gross specific yield for the H_2 utilisers of approximately 0,56 gVSS/gCOD(H_2) generated, i.e. a value about 14 times higher than the specific yield of the hydrogenotrophs normally expected in anaerobic systems).

Hydrogen balance

To cross-check the calculations detailed above, a hydrogen balance. can be conducted on the system. The hydrogen that is generated (hydrogen flux, $[H_2]$) follows three paths of utilisation:

- Oxidised to methane, $[H_2]_{CH4}$
- Incorporated into hydrogenotroph cell mass and associated polypeptide mass, $VSS_{H_{2\text{-utiliers}}}$.
- Incorporated into free amino acids released by hydrogenotrophs, $COD_{amino\ acids}$.

Thus, a hydrogen balance equation can be set up, i.e.:

$$[H_2] = [H_2]_{CH_4} + VSS_{H_{2\text{-utilisers}}} + COD_{amino\ acids} \quad (15)$$

Now, for the calculation example:

$[H_2]$ = 27,57 gCOD/d, from Eq. 12

$[H_2]_{CH_4}$ = total methane generated — $[HAc]_{ox}$

where total methane generated = 11,85 gCOD/d (Table 3)

$[HAc]_{ox}$ = 4,48 gCOD/d, calculated from Eq. 11

= 11,85 — 4,48 = 7,37 gCOD/d

$VSS_{H_{2\text{-utilisers}}}$ = 8,89 gCOD/d, from Eq. 13

$COD_{amino\ acids}$ = 9,87 gCOD/d, from Appendix 2

Thus, percentage hydrogen recovery

$$= \frac{7,37+8,89+9,87}{27,57} \times 100$$

= 95 per cent

Nature of polymer

The results from the previous sections provide support for the pelletisation hypothesis of Sam-Soon *et al.* (1987). In this hypothesis they proposed that the polymer formed in the pelletisation process consisted of polypeptides. Examination of the nitrogen behaviour in the UASB system with glucose as influent may substantiate this proposal.

Nitrogen was added to the high $\bar{p}H_2$ reactor as NH_3-N in the influent. For the three loadings investigated, the NH_3-N removed in VSS synthesis, and the dissolved organic N generated are listed in Table 2. It is now of interest to partition the NH_3-N removal between the two principal groups of VSS generated, i.e. the acidogenic and hydrogenotrophic organisms.

The mass of NH_3-N removed by the glucose utilising acidogens can be determined from the mass of these organisms generated by accepting the TKN/COD ratio for this organism of 0,086 mgN/mgCOD (McCarty, 1972). The balance of the NH_3-N removed can be ascribed to the action of the hydrogenotrophic organisms, i.e. removal for cell synthesis and conversion into organic nitrogen in the form of amino acids which are either released to the surrounding medium or extruded as polypeptide chains, or both.

Taking, for example, the data for the 60 ℓ/d feed shown in Table 2:

Nitrogen removed from the system per day, $\Delta M(NH_3\text{-}N)$:

$$\Delta M(NH_3\text{-}N) = M(NH_3\text{-}N)_{in} - M(NH_3\text{-}N)_{out}$$
$$= (86{,}8 \times 60 - 40{,}3 \times 60)$$
$$= 2\ 790\ mgN/d$$

where $M(NH_3\text{-}N)_{in,\ out}$ = mass of free and saline ammonia per day in the influent, effluent respectively.

Nitrogen incorporated in acidogenic mass per day, $M(NH_3\text{-}N)_{acid}$.

$$M(NH_3\text{-}N)_{acid} = VSS_{acidogens} (TKN/COD)$$
$$= 16{,}65 \times 0{,}086$$
$$= 1\ 432\ mgN/d$$

where $VSS_{acidogens}$ = mass of acidogens (COD) generated/d

Nitrogen incorporated into hydrogenotrophic mass (cell mass + polymer mass), $M(NH_3\text{-}N)_{hyd}$:

$$M(NH_3\text{-}N)_{hyd} = \Delta M(NH_3\text{-}N) - M(NH_3\text{-}N)_{acid}$$
$$= 2\ 790 - 1\ 432$$
$$= 1\ 358\ mgN/d$$

Nitrogen removed by the hydrogenotrophs for **cell synthesis** can be determined from the hydrogen flux ($[H_2]$); the normally expected yield value should be about 0,043 mgVSS/mgCOD(H_2) removed (Shea *et al.*, 1968) i.e. **expected** mass of H_2-utilisers generated per day, ΔXv_{H_2}, is:

$$\Delta Xv_{H_2} = Y_{H2}[H_2]$$
$$= 0{,}043 \times 27{,}57$$
$$= 1{,}19\ gVSS/d$$

where Y_{H_2} = specific yield of hydrogenotrophs, mgVSS/mgCOD(H_2).

Assuming a TKN/COD = 0,086 and with a measured COD/VSS = 1,42 (Table 2), then the TKN/VSS = 0,122 mgN/mgVSS for organism cell mass, and the expected N removal by hydrogenotrophs for cell synthesis, $M(NH_3\text{-}N)_{hyd.cell}$ is:

$$M(NH_3\text{-}N)_{hyd.cell} = \Delta Xv_{H_2} (TKN/VSS)$$
$$= 1{,}19 \times 0{,}122$$
$$= 145\ mgN/d.$$

This value (145 mgN/d) is about one-tenth of the total nitrogen estimated to have been removed by the hydrogenotrophs (1 358 mgN/d). The difference is hypothesised to be due to the formation of polypeptides and the discharge of amino acids to the surrounding liquid. This would support the proposals of Sam-Soon *et al.* (1987), that the polymer formed in pelletisation is predominantly made up of polypeptides.

Discussion

This study is illustrative of the different kinds of difficulties associated with enquiry into the behaviour of **mixed cultures** in anaerobic systems from those encountered in the study of **specific organisms** in such systems. With regard to the latter, discovery, identification and characterisation of specific anaerobic organisms presents major problems in itself — one can only reflect with admiration on the discovery of the species *M.* Strain *AZ* with its unusual characteristics. With regard to the former, the art in process description in anaerobic systems lies in recognising the function of such organisms amid the complex interactions between organisms within the environment "imposed" on them (for example the imposition of a plug flow or a completely mixed regime). The information to establish a model of the system behaviour, is largely inferential, for example, although hydrogen partial pressure is crucial to the behaviour of the organism in the system, direct measurement of the hydrogen partial pressure in the liquid in a pellet forming system is likely to provide little quantitative information because the situation in the pellet will differ substantially from that in the surrounding liquid and the transfer of hydrogen between the H_2-producers and H_2-utilisers takes place at microscopic proximity (Gujer and Zehnder, 1983; McCarty and Smith, 1986). As a consequence the procedures used in the description of UASB behaviour in this paper, are indirect and inferential. The measure of success attained by the description will be the measure of its justification.

Conclusions

From the study of UASB systems with glucose as substrate feed:

- The response of a high/low $\overline{p}H_2$ reactor UASB system fed with glucose as sole substrate, was similar to that obtained with apple juice waste water.
- Excellent pellet formation was observed with glucose as substrate indicating that the presence of extraneous organics such as polymers is not a prerequisite for pellet formation.
- In the high/low $\overline{p}H_2$ UASB reactor fed with glucose no butyrate was observed along the line of flow, a response similar to that observed with apple juice waste water.
- Separating the high/low $\overline{p}H_2$ UASB system into a two in-series UASB reactor system, with the first reactor operating as a high $\overline{p}H_2$ reactor and the second as a low $\overline{p}H_2$ reactor, with glucose as substrate, butyrate was formed in the high $\overline{p}H_2$ reactor, whereas no butyrate was observed with apple juice waste water in a similar system. This difference in behaviour is attributed to the difference in sludge ages between the high $\overline{p}H_2$ reactors in the two systems: In the glucose fed system the sludge age ranged between 2,0 to 2,1 d whereas in the apple juice fed system the sludge age was 3,0 d. Recognising that the generation time of butyrate oxidisers is about 2,3 d, one would expect washout of these bacteria when the sludge age falls below about 2 d and hence appearance of butyrate.
- In the high $\overline{p}H_2$ reactor with sludge age of 3 d and in the single high/low $\overline{p}H_2$ UASB reactor system where the sludge age was well in excess of 2,3 d, one would have expected butyric acid generation and utilisation to take place and follow a similar pattern to propionic acid, i.e. generation in the lower and disappearance in the upper region. The fact that this was not observed indicates the presence of butyrate oxidisers in the lower region and this in turn indicates a degree of solids back mixing. The flow regime therefore appears to be closely plug flow with respect to the liquid phase but not necessarily so for the solid phase.
- Using organism acidogenic yield values reported in the literature, from a glucose balance on the high $\overline{p}H_2$ reactor the

fraction of acetic acid oxidised and hence the hydrogen flux could be estimated, and hence the gross specific yield (organism mass + polymer) of the hydrogenotrophs determined. The gross specific yield ranged from 0,19 to 0,24 mgVSS/mgCOD(H_2) removed. These values are approximately 6 times larger than reported hydrogenotroph yield values; this high yield is attributed to polymer generation. The mass concentration of NH_3-N removed indicates the polymer to be composed predominantly of peptides.

Acknowledgements

This research was supported jointly by the Foundation for Research Development and the Water Research Commission of South Africa and this paper is published with their permission.

References

ATTAL, A, EHLINGER, F, AUDIC, JM and FAUP, GM (1988) pH inhibition mechanisms of acetogenic, acetoclastic and hydrogenophilic populations. *Procs. 5th IAWPRC Symposium on Anaerobic Digestion,* Bologna (Eds.: ER Hall and DN Hobson). Advances in Water Pollution Control, 13-23.

BOONE, DR and BRYANT, MP (1980) Propionate-degrading bacterium, *Syntrophobacter Wolinii* sp. *nov. gen. nov.,* from methanogenic ecosystems. *Appl. Environ. Microbiol.* **40**(3) 626-632.

COHEN, A, ZOETEMEYER, RJ, VAN DEURSEN, A and VAN ANDEL, JG (1979) Anaerobic digestion of glucose with separated acid production and methane formation. *Water Res.* **13** 571-580.

DOLD, PL, SAM-SOON, PALNS, PALMER, IH and MARAIS, GvR (1987) Anaerobic treatment of an apple processing waste water. *Wat.Sci.Tech.* **19** 237-247.

DOLFING, J, GRIFFWEN, A, VAN NEERVEN, ARW and ZEVENHUIZEN LPTM (1985) Chemical and bacteriological composition of granular methanogenic sludge. *Can. J. Microbiol* **31** 744-750.

DOLFING, J (1987) Microbiological aspects of granular methanogenic sludge. Ph.D Thesis, Agricultural University of Wageningen, Holland.

GUJER, W and ZEHNDER, AJB (1983) Conversion processes in anaerobic digestion. *Wat. Sci. Tech.* **15** 127-167.

HULSHOFF POL, LW, DOLFING, J, VAN STRATEN, K, DE ZEEUW, WJ and LETTINGA, G (1984) Pelletization of anaerobic sludge in upflow anaerobic sludge bed reactor on sucrose-containing substrates. In: *Current Perspectives in Microbial Ecology.* MJ Klug and CA Reddy (Eds.) American Society for Microbiology, Washington DC.

KOCH, M, DOLFING, J, WURHMANN, K and ZEHNDER, AJB (1983) Pathways of propionate degradation by enriched methanogenic cultures. *Appl. Environ. Microbiol.* **45**(4) 1411-1414.

McCARTY, PL (1972) Energetics of organic matter degradation. In: *Water Pollution Microbiology.* Mitchell (Ed.). Wiley Interscience, New York.

McCARTY, PL and SMITH, DP (1986) Anaerobic wastewater treatment. *Environ. Sci. and Tech.* **20** 1200-1206.

McINERNEY, MJ, BRYANT, MP and STAFFORD, DA (1979) In: *Anaerobic Digestion.* DA Stafford, BI Wheatley and DE Hughes (Eds.) Applied Science Publishers Ltd., Ripple Road, Barking, Essex, England.

ROSS, WR (1984) The phenomenon of sludge pelletization in the anaerobic treatment of a maize processing waste. *Water SA* **10**(4) 197-204.

SAM-SOON, PALNS, LOEWENTHAL, RE, DOLD, PL and MARAIS, GvR (1987) Hypothesis for pelletization in the upflow anaerobic sludge bed reactor. *Water SA* **13**(2) 69-80.

SHEA, TG, PRETORIUS, WA, COLE, RD and PEARSON, EA (1968) Kinetics of hydrogen assimilation in the methane fermentation. *Water Res.* **2** 833-848.

TEN BRUMMELER, E, HULSHOFF POL, LW, DOLFING, J, LETTINGA, G and ZEHNDER, AJB (1985) Methanogenesis in an upflow anaerobic sludge blanket reactor at pH 6 on an acetate-propionate mixture. *Appl. Environ. Microbiol* **49** 1472-1477.

THAUER, RK, JUNGERMANN, K and DECKER, K (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bact. Rev.* **41** 100-180.

ZEHNDER, AJB and WUHRMANN, K (1977) Physiology of a *Methanobacterium Strain AZ. Arch. Microbiol.* **111** 199-205.

ZOETEMEYER, RJ, VAN DEN HEUVEL, JC and COHEN, A (1982) pH influence on acidogenic dissimilation of glucose in an anaerobic digestor. *Water Res.* **16** 303-311.

Appendix 1

Determination of unprocessed glucose in a high hydrogen partial pressure zone

If glucose measurements are not available an estimate can be made by the following procedure:

Measure the following on the filtered effluent:

- Total COD concentration.
- SCFA concentrations (i.e. acetate, propionate and butyrate), hence the COD_{SCFA}.
- Organic nitrogen concentration (from the difference between TKN and NH_3-N).

Thus the total effluent COD comprises:

$$\text{Total COD}_{eff} = COD_{SCFA} + COD_{orgN} + COD_{\text{glucose not fermented}} \quad (1.1)$$

From the hypothesis on pelletisation, Sam-Soon *et al.* (1987) proposes that the high organic nitrogen (orgN) in the effluent is derived from amino acids released by *M.* Strain *AZ.* The COD associated with this orgN, (COD_{orgN}), can be estimated as set out in **Appendix 2.** Knowing the effluent total COD_{eff}, COD_{SCFA} and COD_{orgN} the COD of the glucose not processed can be estimated from Eq. 1.1. The amount of glucose processed is then calculated from the difference between the **total** glucose influent (as COD) and the $COD_{\text{glucose not processed}}$.

The procedures above were applied to the observations obtained on the three loadings listed in Table 2 and the results are listed in Table 3. As an example to illustrate the procedures, consider the flow rate 60 ℓ/d with influent glucose COD of 2 774 mg/ℓ:

	Total COD_{eff}	= 2 142 mgCOD/ℓ
COD_{orgN}	(see Appendix 2)	= 164,5 mgCOD/ℓ

From Table 2, SCFA are:

HAc = 722 mg/ℓ; HPr = 389 mg/ℓ and HBr = 333 mg/ℓ

$$\text{i.e. } COD_{SCFA} = 722\text{x}1{,}067 + 389\text{x}1{,}512 + 333\text{x}1{,}816$$
$$= 1\,963 \text{ mgCOD}/\ell$$

and from Eq. 1.1

$$2\,142 = 1\,963 + 164{,}5 + COD_{\text{glucose not fermented}}$$

$$\text{i.e. } COD_{\text{glucose not fermented}} = 14{,}5 \text{ mgCOD}/\ell.$$

This amount can be taken as negligible, that is, all the glucose had been processed. For the other loadings (see Table 3), substantial amounts of glucose in the feed were not processed.

Appendix 2

Calculation of the COD associated with the dissolved organic nitrogen measured in the high pH_2 zone of a UASB system

Sam-Soon *et al.* (1987) hypothesised that the organic nitrogen observed in the UASB system treating an apple juice concentrate is due to amino acids released by the hydrogenotroph *M.* Strain *AZ*. The principal amino acids released by *M.* Strain *AZ* (Zehnder and Wuhrmann, 1977) are alanine, valine and glutamine acid in mass ratios 0,47:0,30:0,23 and mol ratios 0,56:0,28:0,16.

The COD associated with each of these amino acids is calculated as follows:

Take the loading rate of 166,44 gCOD/d with excess NH_3-N (Table 2):

Average mmol organic nitrogen released/ℓ		= 17,7/14
		= 1,26 mmol/ℓ
Component	: mmolxCOD mass equiv.	= mgCOD/ℓ
Alanine	: (0,56 x 1,26) x 96	= 67,74 mgCOD/ℓ
Valine	: (0,28 x 1,26) x 192	= 67,74 mgCOD/ℓ
Glutamic	: (0,16 x 1,26) x 144	= 29,03 mgCOD/ℓ
		164,5 mgCOD/ℓ

i.e. COD in the form of free amino acids is 164,5 mgCOD/ℓ.

Appendix 3

Determination of volatile mass yield

In Sam-Soon *et al.* (1987), page 77 under the section **"(3) Volatile mass yield"**, the volatile mass yield was incorrectly calculated. The correct calculation is as follows:

Feed flow rate	= 92 ℓ/d
Mass influent COD (92.2703)	= 248 676 mgCOD/d
Mass effluent COD (92.2175)	= 200 100 mgCOD/d
∴ Total COD removed	= 248 676 - 200 100
	= 48 576 mgCOD/d
Sludge concentration	= 28 860 mgVSS/ℓ
Sludge wasted per day	= 700 mℓ/d
VSS generated (28 860.0,7)	= 20 202 mgVSS/d
Hence biomass yield	= VSS generated/Total COD removed
	= 20 202/48 576
	= 0,42 mgVSS/mgCOD removed

Thus, the volatile mass yield is 0,42 mgVSS/mgCOD removed not 0,36 as calculated by Sam-Soon *et al.* (1987).