Hypothesis for pelletisation in the Upflow Anaerobic Sludge Bed reactor

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Abstract

Biological pellet formation in methane fermentation systems is likely only in plug flow type reactors e.g., the Upflow Anaerobic Sludge Bed (UASB) reactor, treating carbohydrate/protein type wastes. Under this flow regime regions of high and low hydrogen partial pressure can form. Pellet formation is mediated by *Methanobacterium* strain *AZ*, an organism that utilises hydrogen as the sole energy source and generates all amino acids except cysteine. It is hypothesised that under high H_2 partial pressure (substrate) conditions, with adequate supply of ammonia, a high intracellular ATP/ADP ratio is generated but the organism growth is limited by the availability of cysteine. The high ATP/ADP ratio induces an over-production of amino acids, the excess is secreted as an extracellular polypeptide which enmeshes the organism assembly to form biopellets. Augmentation of cysteine in the feed decreases both polymer production and pellet formation.

Introduction

In the Upflow Anaerobic Sludge Bed (UASB) reactor the waste water enters the reactor at the bottom and passes upward, in a plug flow fashion, through a dense bed of sludge and then through a less dense suspended sludge blanket overlying the bed. At the top of the reactor an internal settler separates the gas and the liquid phases, and provides a quiescent region for settlement of suspended settleable solids.

A distinctive feature of successful UASB systems is the very high loading rates the systems can cope with. At full-scale design loading rates per unit reactor volume of 10 to 15 kg COD/m^3.d at 30°C have been suggested (Lettinga *et al.*, 1983). These rates are from 2 to 3 times higher than those applied in successfully operating completely mixed anaerobic systems. Despite these high loading rates the performance efficiencies of the UASB systems are equal to those attainable in completely mixed systems.

Visual appearance of UASB bed sludges ranges from a relatively uniform "smooth" sludge (Boari *et al.*, 1984) to "flocculant" (Hamoda and Van den Berg, 1984) to granular "pelletised" sludge (Lettinga *et al.*, 1980; Ross, 1984). As to the type of sludge that will develop, it would appear that the flow regime and the type of substrate feed are important factors:

- High density sludges with good settling properties appear to be unique to UASB systems. Such sludges have *not* been positively associated with completely mixed systems.
- Pelletised bed sludges have been associated with soluble wastes consisting mainly of carbohydrates (Lettinga *et al.*, 1980; Ross, 1984).
- Uniform smooth bed sludges have been reported with olive oil processing wastes (Boari *et al.*, 1984).
- Bed sludges that are slow growing with poor quality pellets have been reported for a substrate feed consisting of a mixture of acetic and propionic acids (de Zeeuw and Lettinga, 1980).

The manifestation of a pelletised sludge is particularly intriguing, not only from the novelty of its appearance but also from the extremely favourable reports on the performance of UASB systems in which this form of sludge has developed. In UASB systems with pelletisation the sludge bed material exhibits good settleability, high density (30 to 80 kg/m^3) and excellent solid/liquid separation. In laboratory-scale systems, despite the high bed density, visually the liquid appears to move readily through the bed and the gases generated do not appear to disturb the sludge particles unduly.

With regard to the constitution of the pellets, Ross (1984) analysed pelletised sludge obtained in a UASB reactor treating a glucose/starch waste. He produced excellent micrographs of thin slices through pellets. These clearly show filamentous-like extracellular structures found to be composed of long-chain macropolymers of carbohydrate and protein.

Up to the present the research into UASB systems has tended to follow a black box approach – attention has been focussed principally on the final products formed in the system. Product formation *within* the sludge bed is more likely to provide information on the processes active up the bed. Such information may also provide a clue to the conditions that are required to give rise to pellet formation. The objectives of this investigation were to study the product formation within the sludge bed of a system generating pellets, interpret this information in terms of the anaerobic fermentation theory and propose an hypothesis explaining pellet formation.

Normal mechanisms of the fermentation process

The literature on anaerobic fermentation tends to divide into two categories, studies into the biochemistry and biochemical processes *per se*, and studies on systems (at laboratory, pilot and full scale) with the objective of developing criteria for design and operation. These two categories unfortunately have tended to develop relatively independently of each other. The principal reason for this is that systems investigated usually have been completely mixed and operated under constant flow and load; under these conditions the critical loading state will be controlled by only one or two processes so that an almost 'black box' approach to system development has sufficed. However, under transient loading conditions in time and space, partial separation of processes is brought about and attention needs to be focussed on individual processes in order to determine the critical, or rate determining one for any particular loading condition. In this regard a

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qualitative understanding of the processes and their roles in the overall fermentation process can provide powerful assistance to the *systems experimentalist*, to identify the processes via product formation. To assist this category of researcher (rather than the biochemist) it is thought worthwhile to give a short didactic overview of fermentation processes.

Fermentation of a carbohydrate, lipid or protein substrate to methane gas, takes place in four stages involving three groups of organisms: solubilisation and acidogenesis (by acidogenic organisms), acetogenesis of short-chain fatty acid (by acetogenic organisms) and methanogenesis (by methanogenic organisms) (McInerney *et al.*, 1979). Product formation pathways for various substrates are set out in outline in Fig. 1 and for carbohydrate in greater detail in Fig. 2.

Stage 1: Solubilisation

In this stage complex long-chain macromolecules such as carbohydrates, lipids and proteins are solubilised extracellularly by acidogenic organisms to short-chain compounds, sugars, long-chain fatty acids and amino acids, respectively.

Stage 2: Acidogenesis

Substrate molecules from Stage 1 (i.e. fatty acids, amino acids and sugars) are ingested by the acidogenic organisms and fermented intracellularly to volatile fatty acids (e.g. acetic, propionic and butyric acids), carbon dioxide and hydrogen gas. The biochemical pathways by which the substrate is fermented and the nature of the end product (i.e. the type of volatile fatty acid produced) will depend primarily on the type of substrate and the hydrogen partial pressure. For example, long-chain fatty acids usually are fermented via the fatty acid spiral either to acetic acid and hydrogen under low hydrogen partial pressures, or, to butyric and propionic acids under high hydrogen partial pressures (Fig. 1). Sugars usually are fermented via the Embden-Meyerhof pathway either to acetic acid, hydrogen and carbon dioxide under low hydrogen partial pressures, or to acetic acid, propionic acid, carbon dioxide and hydrogen under high hydrogen partial pressures (Fig. 2). Taking glucose as an example, the more detailed pathways under low and high hydrogen partial pressures are summarised in Figs. 3a and b respectively. Under both low and high hydrogen partial pressure glucose is fermented first to pyruvic acid, via the Embden-Meyerhof pathway (EMP), thereafter the pathways differ depending on the types of electron sink: under low hydrogen partial pressure, only protons (H^+) act as the terminal electron acceptor; under high hydrogen partial pressures both pyruvic acid and protons act as the electron acceptor. The main steps whereby the electron transfer takes place in the respective acidogenic stages are described below:

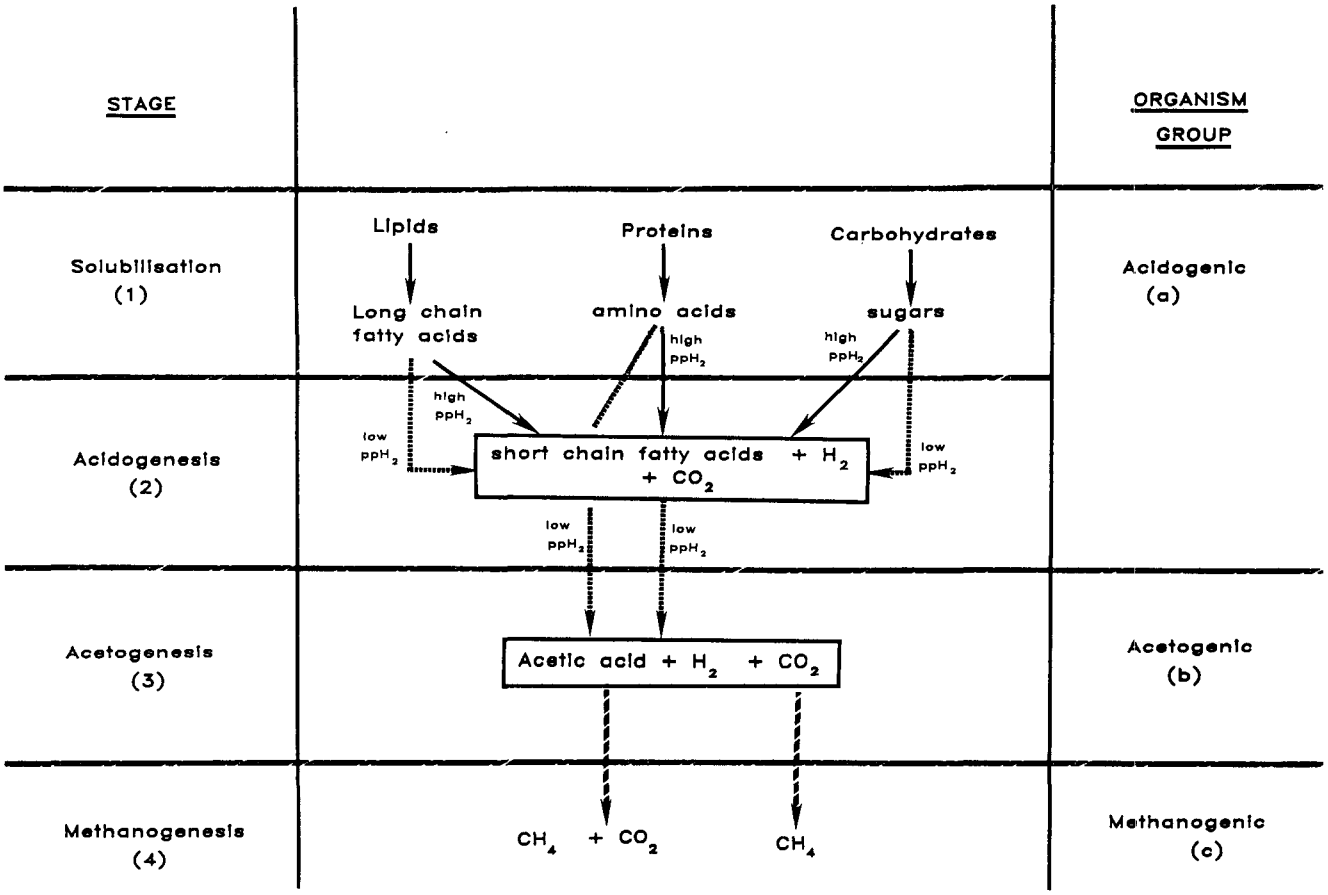

Figure 1
The four stages of the anaerobic methane fermentation process are effected by three groups of organisms. Note that for certain fatty acids, amino acids and sugars, depending on physiological conditions, the acidogenic and acetogenic phases may occur together, i.e. the substrate is converted directly to acetic acid, H_2 and CO_2.

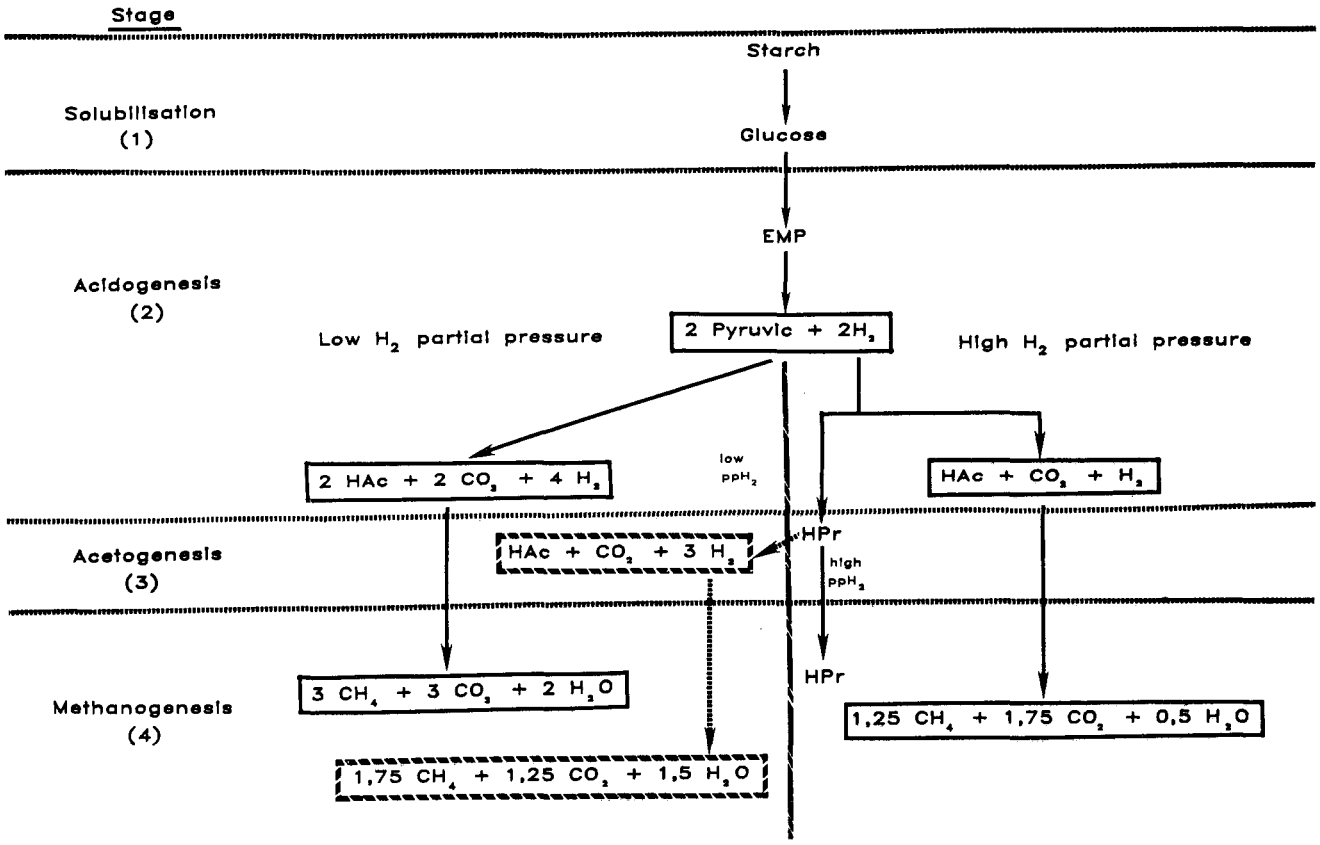

Figure 2
Methane fermentation of starch under high and low H_2 partial pressure conditions.

Step 1:

(This step is via the Embden-Meyerhof pathway). Glucose is fermented to pyruvic acid and hydrogen. The hydrogen is attached to an electron-carrying co-enzyme, NAD^+; and 2 moles ATP per mole glucose are conserved by the organisms, i.e.

$$C_6H_{12}O_6 \text{ (glucose)} + 2NAD^+ + 2ADP + 2P \rightarrow 2\ CH_3COCOOH \text{ (pyruvic acid)} + 2NADH + 2ATP \quad (1)$$

That is, *inter alia* one mole of glucose generates 2 moles of pyruvic acid.

Step 1 is common to both high and low H_2 partial pressures.

Step 1a:

The NADH formed in Step 1 needs to be dehydrogenated to maintain a high level of NAD^+ in order that the Embden-Meyerhof pathway remains operative (NAD^+ acts as the electron acceptor in this pathway). The dehydrogenation can take place in one of two ways depending on the hydrogen partial pressure:

Under low hydrogen partial pressures (less than about 10^{-6} atm), NADH is oxidised spontaneously to NAD^+ and hydrogen gas, i.e.

$$2NADH \rightarrow 2NAD^+ + 2H_2 \quad (2)$$

(Step 1a, Fig. 3a).

Under high hydrogen partial pressures, the forward reaction in Eq. (2) is no longer thermodynamically feasible. Consequently an alternative method for oxidising the NADH is needed. Usually this is effected by reducing pyruvic acid to propionic acid as follows (Wood, 1982):

$$CH_3COCOOH + 2NADH + ADP + P \rightarrow CH_3CH_2COOH + 2NAD^+ + ATP + H_2O \quad (3)$$

(Step 1a, Fig. 3b).

Step 1b:

Pyruvic acid is oxidised to short chain fatty acids. The oxidation step follows one of two pathways again depending on the hydrogen partial pressure.

Under low hydrogen partial pressures (Fig. 3a), the 2 moles

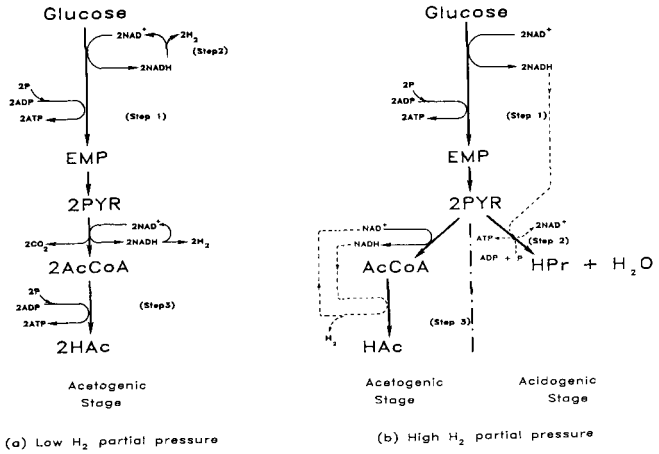

Figure 3
Acidogenic phase of glucose fermentation under low and high H_2 partial pressures to form acetic acid, propionic acid, H_2 gas and CO_2. (Abbreviations: EMP – Embden-Meyerhof pathway; PYR – pyruvic acid; AcCoA – acetyl coenzyme A; HPr – propionic acid; HAc – acetic acid).

pyruvic acid generated in Step 1, are oxidised to Acetyl-CoA and carbon dioxide, with NAD^+ acting as an electron acceptor, i.e.

$$2\,CH_3COCOOH + 2NAD^+ \rightarrow 2\,CH_3CoA + 2CO_2 + 2NADH \quad (4)$$

The 2 moles NADH formed [in Eq. (4)] are dehydrogenated by forming hydrogen gas, as in Eq. (2). The 2 moles acetyl-CoA formed [in Eq. (4)], are converted to 2 moles acetic acid with concomitant generation of 2 moles ATP, i.e.

$$2\,CH_3CoA + 2ADP + 2P \rightarrow 2\,CH_3COOH + 2ATP \quad (5)$$

The overall fermentation reaction of 1 mole glucose under low hydrogen partial pressure is obtained by adding Eqs. (1, 2, 4 and 5) to give:

$$C_6H_{12}O_6 + 2H_2O + 4ADP + 4P \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 + 4ATP \quad (6)$$

Thus one mole of glucose generates 2 moles of acetic acid, 2 moles of carbon dioxide, 4 moles of hydrogen and 4 moles of ATP.

Under high hydrogen partial pressures (Fig. 3b), already 1 mole of pyruvic acid has been converted to propionic acid (in order to oxidise NADH, Eq. (3) above), the single mole of pyruvic acid remaining is oxidised to acetyl CoA as in Eq. (4), i.e.

$$CH_3COCOOH + NAD^+ \rightarrow CH_3CoA + CO_2 + NADH \quad (7)$$

As noted earlier, under high hydrogen partial pressure, the NADH cannot be spontaneously oxidised to NAD^+ as the reaction is not thermodynamically favourable. However, the organism is capable of dehydrogenating the NADH by coupling this reaction with the thermodynamically favourable reaction in which acetyl-CoA forms acetic acid. No ATP is generated in this step [cf. the low hydrogen partial pressure reaction where 2 moles ATP are generated, Eq. (5)]. The coupled reaction is as follows:

$$CH_3CoA + NADH \rightarrow CH_3COOH + H_2 + NAD^+ \quad (8)$$

The overall acidogenic fermentation reaction of glucose under high hydrogen partial pressure is obtained by adding Eqs. (1, 3, 7 and 8), giving:

$$C_6H_{12}O_6 + 3ADP + 3P \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2 + 3ATP \quad (9)$$

That is, one mole of glucose generates 1 mole of propionic acid, 1 mole of acetic acid, 1 mole of hydrogen, 1 mole of carbon dioxide and 3 moles of ATP.

Comparing Eqs. (6 and 9) in the acidogenic stage, *under low hydrogen partial pressure*, 4 moles ATP are conserved whereas under *high hydrogen partial pressure* only 3 moles ATP are conserved. Furthermore under low hydrogen partial pressures 2 moles of acetic acid are formed whereas under high partial pressure one mole of propionic and one mole of acetic acid are formed.

Stage 3: Acetogenesis from short-chain fatty acids

Acetogenic organisms have an important intermediate role between acidogenesis and methanogenesis. Methanogenic organisms use formic acid, acetic acid (by cleavage), hydrogen, methanol and methylamines as substrate source to form methane; however short-chain fatty acids with more than 2 carbon atoms (i.e. $> C_2$) such as propionic and butyric acids cannot be fermented directly to methane. *Hydrogen-producing acetogenic* bacteria are capable of converting short-chain fatty acids longer than C_2, to acetic acid, carbon dioxide and hydrogen gas, *provided the hydrogen partial pressure is low*, below 2×10^{-3} atm and 9×10^{-3} atm for the degradation of butyric and propionic acids respectively (McInerney *et al.*, 1979). Under low hydrogen partial pressure, using propionic acid as an example,

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (10)$$

that is, propionic acid is converted to acetic acid.

Under high hydrogen partial pressure the forward reaction of Eq. (10) is thermodynamically unfavourable so that propionic acid remains unaltered in the system.

Stage 4: Methanogenesis

Methanogenic reactions take place optimally at pH near 7. Methanogenic organisms can utilise formic and acetic acids, hydrogen gas, methanol and methylamines as the electron donors. Taking acetate and hydrogen as examples, the following two reactions take place:

For acetate,

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (11)$$
(Acetoclastic methanogenic organisms)

and for hydrogen,

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad (12)$$

The following points are worth noting:

- The reaction described by Eq. (12) contributes to reducing the hydrogen partial pressure in the reactor; and
- both methanogenic reactions above take place irrespective of the hydrogen partial pressure, provided the pH remains near neutral.

With glucose as influent substrate, *under low hydrogen partial pressure* and with pH close to neutrality, in the acidogenic/acetogenic phase, 1 mole glucose produces 2 moles acetic acid and 4 moles hydrogen [Eq. (6)]; in the methanogenic phase the 2 moles acetic acid produce 2 moles methane [Eq. (11)], and the 4 moles hydrogen produce 1 mole methane [Eq. (12)], giving an overall production of 3 moles methane, see Fig. 2. *Under high hydrogen partial pressure* and with pH close to neutrality, the acidogenic/acetogenic phase reactions produce acetic acid plus propionic acid and hydrogen [Eqs. (3) and (7)]. The propionic acid is not degraded by the methanogenic organisms so that the fermentation products for glucose as substrate are as follows: 1 mole glucose gives rise to 1 mole acetic acid, 1 mole propionic acid and 1 mole hydrogen [Eq. (9) and Fig. 2]; the 1 mole acetic acid will give 1 mole methane, and the 1 mole hydrogen will give 0,25 moles methane, a total of 1,25 moles methane (cf. 3 moles methane when hydrogen partial pressure is low) and 1 mole propionic acid – that is, the methane production is reduced by 58 per cent relative to that when the hydrogen partial pressure is low.

Experimental

To obtain information on product formation along the line of flow, sampling ports were installed up the wall of an operating UASB reactor. The reactor consisted of a clear perspex tube 100 mm in diameter, 1 200 mm reactor liquid depth with a conical settler at the top (Fig. 4). The effective reactor volume was 9 ℓ. The substrate was apple juice concentrate containing principally sugars with negligible nitrogen, TKN/COD = 0,006 mgN/mgCOD. The concentrate was diluted to approximately 2 600 mgCOD/ℓ. To ensure adequate trace elements and nutrients for growth, solutions of these were made up, see Table 1, following in the main the recipe of Zehnder and Wurhmann (1977); 3 mℓ trace element solution and 50 mℓ nutrient solution per litre of feed were added.

Previously the UASB system receiving this substrate had developed a pelletised sludge (Dold *et al.*, 1987). The bed was retained but the initial volume of the bed was set at 3ℓ. Pertinent operating data are listed in Table 2. Details of experimental procedures have been reported elsewhere (Dold *et al.*, 1987).

At the start of the experiment no sludge was wasted from the bed in order to determine the influence on the bed mass. Initially the bed mass built up rapidly but gradually the build-up rate declined; when the bed mass had increased to 6 ℓ, to prevent direct loss of the bed material via the effluent, it was necessary to waste approximately 250 to 300 mℓ of bed material per day.

The pellet size varied up the reactor, being largest at the bottom, about 2 to 4 mm diameter, and smallest at the top, about 1 mm diameter. Measurements on the bed material gave the following information: mean bed density = 37 000 mgVSS/ℓ; COD/VSS = 1,23 mgCOD/mgVSS and TKN/COD = 0,09 mgN/mgCOD. Despite the turbulence of the gases passing through the bed, there was no observable mixing of the pellet

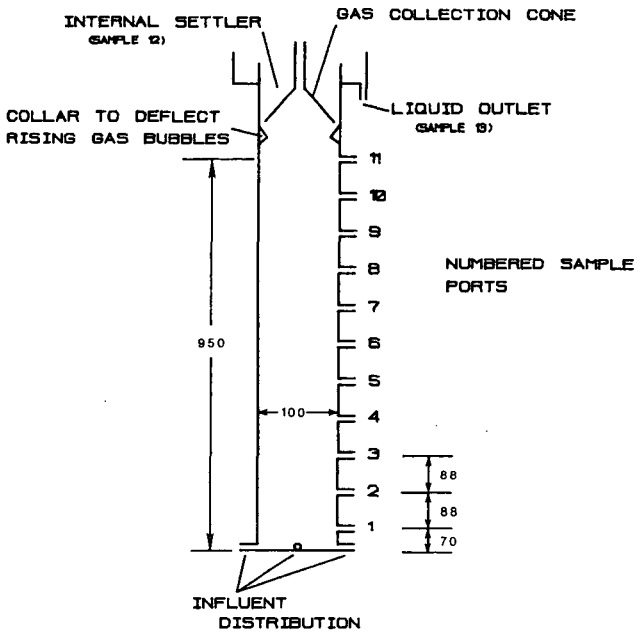

Figure 4
Schematic diagram of laboratory UASB reactor.

TABLE 1
TRACE ELEMENT AND NUTRIENT SOLUTIONS

Trace element solution	g/ℓ
H_3BO_3	0,05
$FeCl_2.2H_2O$	2,00
$ZnCl_2$	0,05
$MnSO_4$	0,5
$CuCl_2.2H_2O$	0,03
$(NH_4)_6Mo_7O_{24}.4H_2O$	0,05
$Aℓ.Cl_3.6H_2O$	0,05
$CoCl_2.6H_2O$	2,00
$MnCl_2$	0,25
$MgCl_2$	1,00
EDTA	0,05
KI	0,05
$NiCl_2.6H_2O$	0,25
HCL (Conc.)	1 mℓ
Nutrient solution	
NH_4Cl	5,00
K_2HPO_4	2,00

layers. As the bed mass increased, fine particles were ejected increasingly from the bed into the region above to form a suspended sludge blanket. This blanket increased in depth until it extended to the effluent discharge level, whereafter it continuously discharged with the effluent at approximately 118 mgVSS/ℓ which, at the influent flow rate of 92 ℓ/d, indicated a loss of 10 856 mgVSS/d. This mass loss was additional to the mass wasted directly from the bed (250 × 37 000/1 000 = 9 250 mgVSS/d), that is a total loss of 20 106 mgVSS/d, which implies a sludge age of about 37 000 × 6/(20 106) = 11 d.

Once the steady state had been attained, samples were taken up the line of flow and tested for: total soluble COD, volatile fatty acids, free and saline ammonia and Total Kjeldahl Nitrogen (hence

TABLE 2
OPERATING DATA FOR UASB PROCESS WITH APPLE CONCENTRATE AS INFLUENT

Influent COD concentration	2 600 mgCOD/ℓ
Volumetric flow rate	92 ℓ/d
Nominal velocity of flow in reactor	0,81 cm/min
Hydraulic retention time	2,35 h
Organic loading rate	26,6 kgCOD/m³ reactor/day
Operating temperature	30 °C
Influent total alkalinity	8 387 mg/ℓ as $CaCO_3$
Influent pH	8,25
Free and saline ammonia (NH_3-N)	65 mgN/ℓ

organic nitrogen by difference), total alkalinity (from the influent alkalinity and the induced alkalinity changes due to fatty acid and nitrogen conversions) and pH (measured in a special device that allowed the sample to be taken and the pH measured without loss of dissolved gases).

Profiles of the parameters measured up the bed are shown in Fig. 5 (a, b and c).

Profile analysis

From Fig. 5, the profile clearly shows 3 zones of behaviour:

- a lower active zone;
- an upper active zone; and
- an upper inactive zone.

The upper bound of the lower active zone is defined by the bed level at which the propionic acid concentration attains a maximum. The upper bound of the upper active zone is defined by the bed level where the propionic and other acids, COD and organic nitrogen attain stable minimum levels. Above this level stretches the upper inactive zone in which virtually no biokinetic growth takes place – the sludge bed in this zone is by and large surplus, serving as a buffer to accommodate any perturbations in the loading rate. The approximate bed volumes of the three zones were respectively 2; 3 and 1 ℓ.

In the lower active zone:

- propionic acid concentration increases to a maximum, to 156 mg/ℓ;
- acetic acid concentration also increases to a maximum but at a slower rate than the propionic acid, to 121 mg/ℓ;
- total soluble COD reduces to more than half its initial value, to 1 206 mg/ℓ;
- free and saline ammonia (NH_3-N) concentration drops sharply to a near minimum, to 27 mg/ℓ;
- organic nitrogen concentration increases sharply to a maximum, to 49 mg/ℓ;
- total alkalinity decreases to a minimum, to 8 181 mg/ℓ as $CaCO_3$; and
- pH declines from 8,10 to 7,07.

In the upper active zone:

- propionic acid concentration decreases to a minimum, to zero mg/ℓ;
- acetic acid concentration does likewise, to 6 mg/ℓ;
- total soluble COD reduces further, to a stable minimum value, to 240 mg/ℓ;
- free and saline ammonia (NH_3-N) concentration remains virtually constant, at 22 mg/ℓ;
- organic nitrogen concentration decreases to a stable minimum value of 20 mg/ℓ;
- total alkalinity increases to a stable value of 8 317 mg/ℓ as $CaCO_3$; and
- pH increases to a stable value of 7,38.

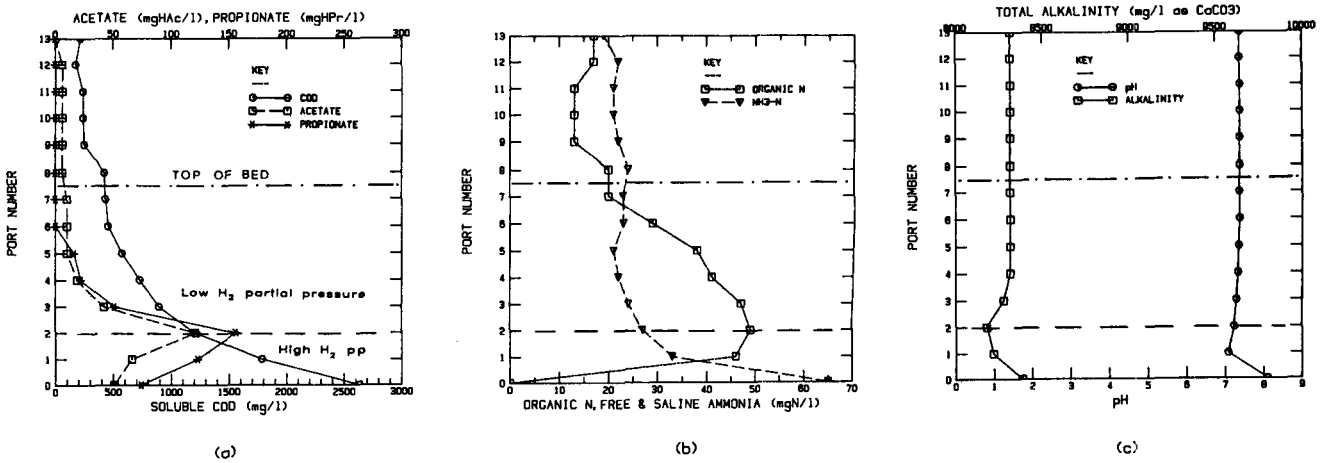

Figure 5
Concentration and pH profiles observed in the single reactor UASB system.

In the lower active zone, the profile of increasing propionic acid concentration implies that the hydrogen partial pressure is high (From our earlier description, a high hydrogen partial pressure gives rise to propionic and acetic acid generation). The increasing profile in the volatile fatty acids concentrations implies a progressive reduction in alkalinity and increase in acidity – these would cause the decline in the pH. The pH did not fall below 7,00, a value which does not inhibit methanogenesis. The decreasing profile of the total soluble COD concentration (which includes the COD of volatile fatty acids) indicates that methane is generated. An unexpected behaviour pattern is the increase in organic nitrogen, concomitant with the decline in free and saline ammonia.

In the upper active zone, the acetic and propionic acid concentrations commence to decrease. The decrease in propionic acid implies that the H_2 partial pressure has declined to such a low value that propionic acid is being converted to acetic acid by acetogenesis. The reduction in H_2 partial pressure must have commenced in the lower active zone, achieving the required minimum value at the point where the propionic acid concentration is a maximum. Consequently the propionic acid, generated in the lower active zone, is being converted to acetic acid and hydrogen in the upper active zone, and these in turn are converted to methane via methanogenesis. The decreases in propionic and acetic acids in the upper active zone reduce acidity and increase alkalinity; these in turn cause the pH to rise.

The behaviour, with regard to acid and methane production and pH described above, is consistent with our present understanding of anaerobic fermentation. However, the generation of organic nitrogen in the high H_2 partial pressure region cannot be explained in terms of normal anaerobic fermentation biochemistry.

Pellet growth and decay

With regard to the sludge bed, the observations are that:

- the pellet layers do not mix;
- the pellet sizes tend to decrease in an upward direction;
- the bed grows to a stable maximum mass; and
- there is a loss of pellet fragments from the bed.

This would indicate that the pellets grow in the lower active zone of the bed and undergo some form of break-up in the higher zones and are lost in the form of fines in the effluent. When the bed mass is small the rate of generation of pellets exceeds the rate of break-up. As the bed mass grows the rate of break-up will increase until eventually a stable mass is established in which the rate of generation equals the rate of loss from the sludge bed.

The validity of the above conclusions could be verified experimentally by separating the lower active zone of high H_2 partial pressure from the upper active and inactive zones of low H_2 partial pressure. This was done by building another UASB reactor of the same diameter as the existing reactor but having a volume of 3ℓ. Pelletised sludge was withdrawn from the bottom of the existing reactor up to the level defining the lower active zone and seeded into the new reactor, approximately 2ℓ. In this fashion a separate high H_2 partial pressure reactor was created. The influent feed entered this reactor and the overflow discharged to the existing reactor which now served as a low H_2 partial pressure reactor. The two-reactor system was operated in this series fashion with the same substrate feed rate as that previously to the single UASB reactor.

The high H_2 partial pressure reactor immediately showed a rapid growth rate of pellet mass. To maintain a constant bed mass of 2ℓ in this reactor, 700 mℓ (20 200 mgVSS/day) of pelletised sludge had to be removed daily, defining a sludge age of 2,0/0,7 = 2,9 days. The pellets generated were relatively uniform in size, about 2 to 3 mm in diameter. The effluent from this reactor contained virtually no fines.

In the low H_2 partial pressure reactor (initial bed volume 5ℓ), receiving the effluent from the high H_2 partial reactor (but not the sludge generated in the high H_2 partial pressure reactor), the mean pellet size continuously decreased; after 3 weeks operation, the pellet size had decreased from the initial 1 to 2 mm mean diameter to less than 0,2 mm. The sludge bed volume also declined by about 800 mℓ. Concomitantly the suspended sludge blanket above the bed showed a gradual increase in VSS concentration. During this period the effluent discharge remained relatively clear with virtually no suspended volatile solids present (whereas in the single reactor system, suspended solids were lost continuously via the effluent). The retention of the suspended solids probably was due to two effects, the larger volume of liquid above the sludge bed in the low H_2 partial pressure reactor and the reduced turbulence, due to the decrease in gas production, these two allowing a higher concentration to develop in the suspended blanket. Unfortunately no measurements were made on the change in concentration of the suspended bed with time so that no estimate is available on the rate of sludge loss from the pelletised bed.

The profiles through the series reactor system are shown in Fig. 6 (a, b and c). These are similar to those in the single reactor system (Fig. 5), except that in the series system a peak in the organic nitrogen is present at the bottom of the low H_2 partial pressure reactor. No explanation for this peak is as yet forthcoming.

Hypothesised mechanism of pelletisation

From the system behaviour presented in the previous section, a number of aspects were unusual and are summarised as follows:

- The net sludge production per unit mass of COD removed was exceptionally high. In the single reactor the net VSS production was 20 160 mgVSS/d for a mass of COD removed of $(2\,633 - 176) \times 92 = 266\,000$ mgCOD/d, i.e. a net yield of 0,09 mgVSS/mgCOD removed. In the high H_2 partial pressure reactor the net VSS production was 20 200 mgVSS/d and the COD removed 55 663 mgCOD/d giving a yield of 0,36 mgVSS/mgCOD removed. These respective yield values are 3 and 12 times higher than the yield normally expected in anaerobic systems, about 0,03 mgVSS/mgCOD removed (Ten Brummeler *et al.*, 1985).
- The growth of the sludge mass was confined to the high H_2 partial pressure region.
- The system produced soluble organic nitrogen in the high H_2 partial pressure region.

The generation of organic nitrogen was particularly perplexing. Either the organic nitrogen arose from the death of organisms or was generated in the growth process. It was reasoned that the

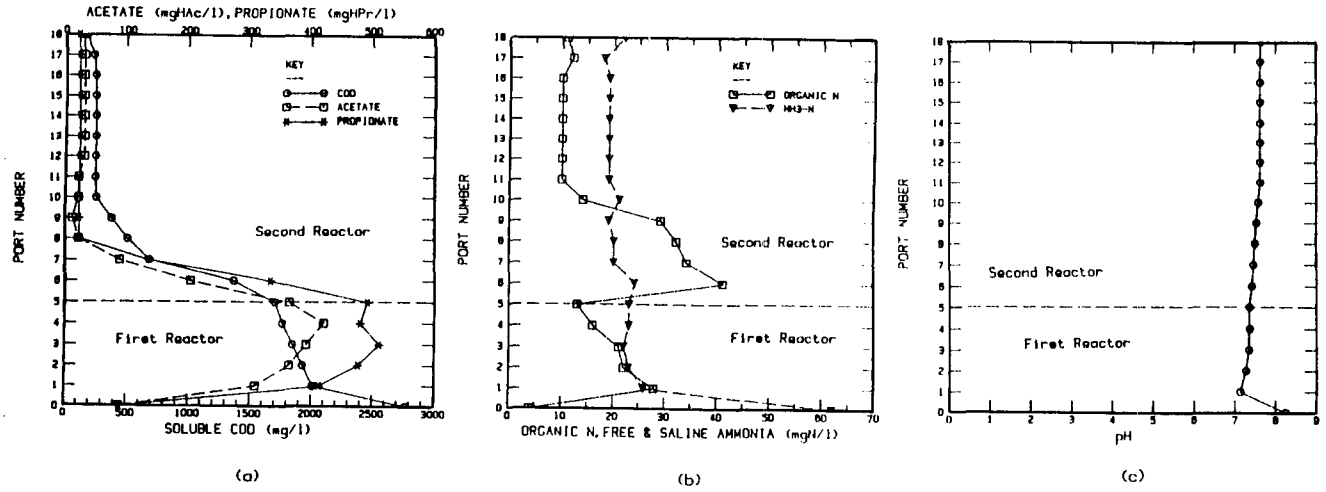

Figure 6
Concentration and pH profiles observed in the two-in-series reactor UASB system.

rapid mass increase in organic nitrogen associated with rapid apparent growth made death an unlikely cause; therefore attention was focussed on the proposition that organic nitrogen generation was a product in the growth process. From a literature survey, the work of Zehnder and Wuhrmann (1977) appeared to be directly relevant. They isolated a hydrogen utilising methano-organism, *Methanobacterium* strain *AZ* (*M.*strain *AZ*) from digested sewage sludge. Growth tests on *M.*strain *AZ* indicated *inter alia* that:

- the organism is a pH neutrophile;
- hydrogen serves as the sole electron-donating substrate and, carbon dioxide as the sole external source of electron acceptor;
- the organism produces its amino acid requirements very effectively, with the exception of the sulphur containing amino acid, cysteine – an external cysteine source is necessary for growth;
- the organism has a high specific growth rate, provided all nutrients are present;
- during the growth of the organism, exceptionally high concentrations of amino acids are secreted to the surrounding medium; and
- the organism grows in rosette-type clusters.

The growth characteristics of the *M.*strain *AZ* appear to provide a basis for the hypothesis on the formation of pelletised sludge in the UASB system:

> When the *M.*strain *AZ* is surrounded by excess substrate i.e. high H_2 partial pressure, the ATP/ADP ratio will be high. Simultaneously the high ATP level will stimulate amino acid production and cell growth. However, because *M.*strain *AZ* cannot manufacture the essential amino acid cysteine, cell synthesis will be limited by the rate of cysteine supply. If free and saline ammonia is present in excess there will be an over-production of the other amino acids; the organism reacts to this situation by either releasing these excess amino acids to the surrounding medium and/or by linking these in polypeptide chains which it stores extracellularly by extrusion from active sites. These polypeptide chains bind the species and other organisms into clusters forming a separate microbiological environment – the so-called biopellets.

Support for the hypothesis is to be found in the experimental observations on the lower active (high H_2 partial pressure) region, by examining:

(1) The COD/VSS and TKN/COD ratios of the pellets.

(2) The rate of disappearance of free and saline ammonia, coupled to the rate of generation of organic nitrogen.

(3) The yield of volatile solids.

(4) The effect of cysteine supplement on volatile solids yield.

(1) ***Ratios of COD/VSS and TKN/COD of pelletised sludge:*** Analysis of pellets generated in the UASB system described above, gives a COD/VSS ratio of 1,23 ± 0,06 mgCOD/mgVSS; this ratio is significantly lower than that usually observed in anaerobic systems, viz. 1,40 to 1,50 mgCOD/mgVSS (McCarty, 1972). This discrepancy can be accounted for if one accepts that a large fraction of the pellets consists of biopolymers. Microorganisms utilising a carbohydrate-type substrate can produce two types of extracellular biopolymer, a polypeptide type i.e. a sequence of peptide-bonded amino acids, or a polysaccharide type.

Zehnder and Wuhrmann (1977) found that the amino acids in the supernatant of their pure culture of *M.*strain *AZ* were principally alanine, valine and glutamic acid in the molar proportions of 0,56:0,28:0,16 respectively.

Assuming that the polypeptide consists of the above three amino acids in the given molar ratios, the COD/VSS ratio will be 1,21 mgCOD/mgVSS for the polypeptide and unity for the polysaccharide (see Appendix 1). For a "standard" protoplasm composition, the COD/VSS ratio is about 1,42 (McCarty, 1972). Accepting these COD/VSS ratios, then to obtain the observed ratio of 1,23 for a polypeptide polymer, about 90 per cent of the

pellet volatile solids should be polymer and 10 per cent protoplasm; for a polysaccharide polymer, about 40 per cent of the pellet volatile solids should be polymer and 60 per cent protoplasm (see Appendix 1). To determine which of the two polymers is generated, one needs to examine the TKN/COD ratio of the pellets. The observed ratio was 0,09 ± 0,003 mgN/mgCOD. For protoplasm the "standard" composition suggests a TKN/COD ratio of 0,086 mgN/mgCOD (McCarty, 1972); for a polysaccharide the TKN/COD ratio will be zero; for a polypeptide, assuming it is composed of alanine, valine and glutamic acid molecules in the molar proportion of 0,56:0,28:0,16 respectively, the TKN/COD ratio is 0,11 mgN/mgCOD (see Appendix 1). Applying these ratios to the mass proportions of polymer:biomass in the pellets calculated above (0,9:0,1 for the polypeptide, and 0,4:0,6 for the polysaccharide), the compounded TKN/COD ratios for the sludge mass should be as follows: for a polypeptide 0,11 mgN/mgCOD; and for the polysaccharide 0,04 mgN/mgCOD. Comparing these two values with the observed TKN/COD ratio of 0,09, it would appear that the most likely polymer is a polypeptide.

(2) ***Free and saline ammonia (NH_3-N) and organic nitrogen profiles:*** Referring to the free and saline ammonia concentration profile (Fig. 5), in the high H_2 partial pressure zone, the concentration decreased by 38 mgN/ℓ and the total soluble COD concentration decreased by 1 427 mgCOD/ℓ. If this mass of free and saline ammonia were utilised for protoplasm synthesis then accepting a TKN/COD for protoplasm = 0,086 mgN/mgCOD and COD/VSS = 1,42 mgCOD/mgVSS, the TKN/VSS ratio should be 0,086 × 1,42 i.e. 0,122 mg/mgVSS. Accepting a biomass yield of 0,03 mgVSS/mgCOD removed (Ten Brummeler *et al.*, 1985), the COD associated with the utilisation of 38 mgN/ℓ, would be (38/0,122)/0,03 i.e. 10 383 mgCOD/ℓ. However the observed COD utilised was only 1 427 mgCOD/ℓ; consequently the disappearance of the free and saline ammonia cannot be associated with protoplasmic mass generation only.

Referring to the organic nitrogen concentration profile (Fig. 5), in the high H_2 partial pressure region, there was a release of 49 mgN/ℓ of organic nitrogen. If one ascribes the generation of organic nitrogen to death of organisms, then in this zone there was a death rate which greatly exceeded the protoplasm growth rate. Thus neither growth nor death can explain the nitrogen behaviour. The alternative explanation which is more acceptable, is that the generation of the organic nitrogen is due to the *M.*strain *AZ* that secretes amino acids under high H_2 partial pressure, when there is a deficiency in cysteine and an adequate supply of ammonia nitrogen (Zehnder and Wuhrmann, 1977).

(3) ***Volatile mass yield:*** The average volatile mass yield in the high H_2 partial pressure region was calculated 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
Sludge concentration	= 28 860 mgVSS/ℓ
Sludge wastage per day	= 700 mℓ/d
VSS generated (28,86.700)	= 20 202 mgVSS/d
COD of free amino acids generated (see Appendix 1)	= 7 087 mgCOD/d
∴ Total COD removed	= [248 676 − (200 100 − 7 087)] = 55 663 mgCOD/d
Hence biomass yield	= VSS generated/Total COD removed = 20 202/55 663 = 0,36 mgVSS/mgCOD removed.

A yield value of 0,36 mgVSS/mgCOD removed is very much higher than the reported biomass yield value in anaerobic processes – Shea *et al.* (1968) and Ten Brummeler *et al.* (1985) reported yield values of about 0,03 mgVSS/mgCOD removed. Accepting the formation of polypeptide polymers, the high yield can be explained as follows:

> Assume that pyruvic acid is the central substance within the bacteria from which synthesis occurs (McCarty, 1972). The ATP requirement for synthesis of 100 mg protoplasm from pyruvic acid is approximately 12 mmols ATP (McCarty, 1972; assuming that the synthesis reaction is 60 per cent efficient); the ATP requirement for synthesis of 100 mg polypeptide polymer (alanine, valine and glutamic acid molecules) is about 1 mmol ATP. This indicates that extracellular polypeptide will have a yield value of about 12 times that for protoplasm. Accepting that about 90 per cent of the volatile solids generated is extracellular polypeptide (see (i) above), the yield will be (0,9 × 0,36) + (0,1 × 0,03) = 0,33 mgVSS/mgCOD; based on the assumptions and deductions developed so far.

(4) ***Effect of cysteine supplement on volatile solids yield:*** In terms of our hypothesis if cysteine is limited, the *M.*strain *AZ* can produce protoplasm only to the extent governed by the availability of cysteine; with restricted cysteine and adequate ammonia a large fraction of the hydrogen (COD uptake) will be converted to amino acids and generation of polypeptide. The ATP requirement for amino acids production is relatively low compared to protoplasm production so that the fraction of the hydrogen oxidised to methane for energy production will be relatively low; but the yield of sludge (principally polypeptide) will be high, i.e. methane gas production should be low. Should the feed be supplemented with cysteine, protoplasm production will increase and polypeptide production will decrease. The ATP requirement for protoplasm formation is about 12 times that for the same molar mass of polypeptide formation. Consequently a higher fraction of hydrogen (COD uptake) would need to be oxidised to methane to provide the energy required. Hence the sludge yield would decrease and the methane production would increase.

In order to test the effect of cysteine, the feed was supplemented with 12,2 mg cysteine per litre of feed. The sludge production before and after cysteine addition is shown in Fig. 7. Sludge production was reduced by 50 per cent (by volume), from 700 mℓ/d to 350 mℓ/d. The effect of the cysteine supplement was immediate because the sludge production determined 24 h after cysteine addition was the same as that subsequently, indicating no transition effect. The methane production unfortunately could not be checked due to breakdown of instrumentation.

It is possible that if a higher cysteine concentration had been supplied a further drop in sludge production would have been observed. It is likely however that even with a stoichiometrically adequate cysteine supply some polypeptide formation would persist for the following two reasons:

- The cysteine molecule needs to be translocated across the cytoplasmic membrane whereas the hydrogen can be expected to diffuse readily across. Consequently, internal to the

organism, a disequilibrium between the concentration of amino acids generated and the cysteine will be induced. These concentrations will be brought into balance by polypeptide formation.

- Internal to the pellet, the rate of transport of hydrogen can be expected to be much higher than for the large cysteine molecule, again leading to an imbalance and polypeptide generation.

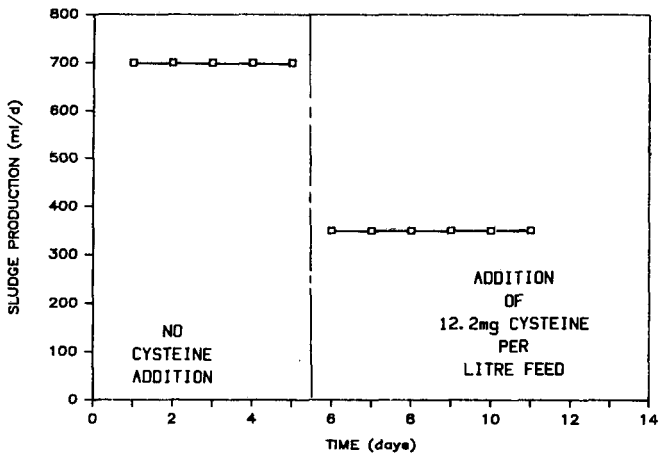

Figure 7
Effect of cysteine addition on pelletised sludge production in the high H_2 partial pressure reactor.

Implications

Criteria for pellet formation

Accepting the hypothesis for pelletisation, the ecological conditions under which pelletisation is likely to occur appear to be as follows:

- an environment with a high partial pressure of hydrogen;
- a nitrogen source, in the free and saline ammonia form, which is non-limiting;
- a limited source of cysteine either from the feed or becoming available from the action (e.g. death) of other organisms; and
- a near neutral pH.

The following situations can be identified under which one can expect, or not expect pelletisation:

- no pelletisation in systems where the influent substrate does not yield hydrogen in the fermentation processes, e.g. acetate as sole substrate;
- 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;
- no pelletisation in systems where the substrate yields hydrogen but in order to obtain complete conversion, operation requires a low H_2 partial pressure, e.g. carbohydrates and proteins in completely mixed reactors; and
- pelletisation in systems where the substrate yields hydrogen but the operation allows regions for high H_2 partial pressure build-up, e.g. carbohydrates and proteins in plug flow reactors.

We will now consider the evidence available on each of these:

- No experiments apparently have been reported where acetate is the sole carbon substrate source. With acetate as the sole substrate no hydrogen is released; without hydrogen the hydrogen-utilising methanogenic organisms including the *M.*strain *AZ*, necessarily must be absent.

  Very slow formation and poor quality pellets have been reported on a substrate feed consisting of a ***mixture*** of acetate and propionate (De Zeeuw and Lettinga, 1980). With such a substrate mixture the sole source of hydrogen is from the degradation of the propionate, but this conversion can take place only at low hydrogen partial pressures; the substrate (H_2) concentration being low, the ATP/ADP ratio of *M.*strain *AZ* also will be low and polypeptide production is unlikely. The pellet formation that De Zeeuw and Lettinga (1980) did observe probably can be accounted for by the fact that they substituted 10 per cent by COD of their feed with yeast extract (an amount well in excess of the trace nutrient requirements for organism growth from acetate and propionate), and this COD fraction probably was responsible for providing an extra hydrogen source, via acidogenesis, for pellet formation at the bottom of the reactor.

- Pelletisation has not been observed with olive oil processing wastes (Boari *et al.*, 1984). The acidogenic phase of the fermentation process readily produces hydrogen from carbohydrate under both low and high H_2 partial pressures. However, in fermentation of long-chain fatty acids, hydrogen release can take place only under low H_2 partial pressures, hence a high ATP/ADP ratio is unlikely to develop, so that one of the two conditions for polypeptide polymer formation is absent; it is likely therefore that even if excess free and saline ammonia is present, polymer formation will not take place.

- No pelletisation has been reported in completely mixed reactor systems. In a 'well balanced' completely mixed system, the pH will be maintained near neutral but the H_2 partial pressure will remain low due to hydrogen utilisation by the methanogenic organisms including *M.*strain *AZ*. With a low H_2 partial pressure the ATP/ADP ratio will be low; hence even if adequate free and saline ammonia is present, the probability of over-production of the amino acids will be small and polypeptide formation is not likely to occur.

  High H_2 partial pressure conditions can develop in a completely mixed system only under 'imbalance'. Imbalance in such a system usually is accompanied by low pH which, if allowed to persist, will eventually cause failure of the system. Even if the pH is buffered to near neutrality, from a practical point of view the system is likely to be looked upon as operating unsatisfactorily for reason that under high H_2 partial pressure, methane fermentation is reduced by 58 per cent with the balance of the COD in the effluent as propionic acid.

Consequently unless there is a deliberate objective to maintain the system in a high H_2 partial pressure state, the necessary conditions for pellet formation will exist in such systems only over a relatively short transition period.

- Pelletisation has been reported in UASB systems with carbohydrate substrate wastes (Lettinga *et al.*, 1980; Ross 1984); experience in this investigation supports this finding. In the acidogenic phase of the fermentation of carbohydrates, hydrogen is readily produced under both low and high H_2 partial pressure. Hence in the UASB system where a region of high H_2 partial pressure can exist, and in the presence of excess free and saline ammonia, polypeptide polymer formation should take place according to the hypothesis proposed here.

Alkalinity: An aspect that will have important implications on the success of a UASB system is the alkalinity requirement of the system. In a UASB system the phase separation into high H_2 and low H_2 partial pressures is essential in order to create the high H_2 partial pressure region for the growth of *M.*strain *AZ*. In the high H_2 partial pressure region there is a loss of alkalinity principally due to the generation of volatile fatty acids. In the system studied here the influent total alkalinity was 8 387 mg/ℓ as $CaCO_3$ (i.e. ALK/COD = 3,2 mgALK as $CaCO_3$/mgCOD influent). The total alkalinity declined to a minimum of 8 181 mg/ℓ at the point where the volatile faty acids peaked, that is, there was a consumption of 206 mg/ℓ as $CaCO_3$. However in the low H_2 partial pressure region virtually all the total alkalinity lost by the carbonate system was recovered to give an effluent alkalinity of 8 317 mg/ℓ as $CaCO_3$. The small net loss of alkalinity (70 mg/ℓ as $CaCO_3$) was that due to amino acid formation from the dissolved ammonia. The sole function of the alkalinity provision in the influent was to prevent undue pH decline in the high H_2 partial pressure region.

It is clear from the behaviour pattern described above that a critical factor in maintaining stability in such a system will be the pH. In the high H_2 partial pressure region, should the pH decline to too low a value, methanogenesis could be inhibited, inducing an even more acidic state, which will spread upward through the bed and eventually cause partial or complete failure of the system. Consequently, for successful operation of a UASB system, it is important that the buffering provided in the influent is sufficiently large to ensure that the pH profile does not show a decline below a minimum value of about pH 6,6. The higher the substrate concentration concomitantly the higher will be the alkalinity changes and hence the higher the alkalinity provision in the influent will need to be. Particularly with strong wastes, to reduce the alkalinity requirement, consideration should be given to recycle the effluent to the influent to reduce the effective substrate concentration, in this fashion reusing the alkalinity from the effluent.

In full-scale plants the alkalinity problem possibly is ameliorated to some extent by the intermixing effects due to the difficulty of ensuring perfect plug flow conditions. Such mixing effects however will result in a reduction in the rate of pellet formation. If the mixing is too efficient it is possible that pellet formation will cease due to "dilution" of the high H_2 partial pressure.

Conclusions

(1) There is strong evidence that pelletisation is due to the action of *Methanobacterium* strain *AZ*. This organism utilises H_2 as its sole energy source. It can produce all its amino acids except cysteine which needs to be supplied from an external source. With a cysteine deficiency, in an environment of high H_2 partial pressure with free and saline ammonia available, protoplasm synthesis is limited by the cysteine supply and a fraction of the excess amino acids produced is secreted as extracellular polypeptide; this polymer binds the organisms together to form pellets. It is possible that other anaerobic bacteria may have characteristics similar to the *M.*strain *AZ* and contribute to pellet formation.

(2) Pelletisation is unlikely in completely mixed systems because in such systems the H_2 partial pressure is always likely to be low if the objective is optimal methane fermentation.

(3) Pelletisation appears to be possible only in plug flow or semi plug flow systems because in these systems phase separation tends to occur. However, phase separation is a necessary but not sufficient requirement – the influent substrate is also a determining factor. Pelletisation is unlikely with the following substrates:

- Acetate or propionate as sole substrate because there is no acidogenic phase; the conversion of propionate to acetic acid and H_2 occurs only under low H_2 partial pressure and the acetic acid does not produce H_2 in methane fermentation.

- Edible oily wastes because the oil is broken down to volatile fatty acids and hydrogen only under low H_2 partial pressure conditions. Pelletisation is likely with a carbohydrate substrate in a plug flow system: H_2 is released during the conversion of a carbohydrate to volatile fatty acids; under high loading conditions if the H_2-utilising organisms cannot utilise the H_2 at the rate it is generated, a region of high H_2 partial pressure will form, thereby providing an environment for growth for *M.*strain *AZ*.

(4) The loss in alkalinity between influent and effluent in a UASB system treating a carbohydrate is very small. However, due to the generation of volatile fatty acids in the high H_2 partial pressure region, the pH in this region may drop significantly if the system is not buffered adequately. This may lead to failure of the system.

Appendix 1

Calculation of the relative masses of organisms and polypeptide of pellets with measured COD/VSS ratio = 1,23.

Assume (a) organisms have COD/VSS ratio = 1,42;

(b) polypeptide is composed of alanine, valine and glutamic acid; the principal amino acids released by *M.*strain *AZ* (Zehnder and Wuhrmann, 1977). The mole ratios are: alanine:valine:glutamic = 0,56: 0,28:0,16. Hence the mass fractions are alanine: valine:glutamic = 0,47:0,30:0,23

(c) the COD/VSS ratio of the amino acids is: alanine: 1,08, valine:1,64 and glutamic:0,98.

(d) the TKN/COD ratio of the amino acids is: alanine:0,146, valine:0,073 and glutamic:0,097

(i) COD/VSS ratio of polypeptide $= (0{,}47 \times 1{,}08) + (0{,}03 \times 1{,}64) + (0{,}98 \times 0{,}23)$
$= 1{,}21$

(ii) consider lg sludge to be composed of Xg organisms, and hence (1-X)g polypeptide, i.e.

lg sludge = Xg organisms + (1 − X)g polypeptide (a)

Substituting the COD/VSS values above into Eq. (a),
$1{,}23 = (X \times 1{,}42) + [(1 - X) \times 1{,}21]$
and solving for X,
$X = 0{,}10$

i.e. the sludge is composed of approximately 10 per cent by mass of organisms and 90 per cent by mass of polypeptide.

Similar calculations for polysaccharide polymer are as follows:

COD/VSS of polysaccharide = 1,0
$\therefore 1{,}23 = (X \times 1{,}42) + [(1 - X) \times 1{,}0]$
$X = 0{,}57$

i.e. the sludge is composed of approximately 57 per cent by mass of organisms and 43 per cent by mass of polysaccharide.

(iii) TKN/COD ratio of the sludge comprising organisms and polypeptide:

TKN/COD or organisms = 0,086 mgN/mgCOD (McCarty, 1972)
TKN/COD of polypeptide $= (0{,}47 \times 0{,}146) + (0{,}30 \times 0{,}073) + (0{,}23 \times 0{,}097)$
$= 0{,}113$ mgN/mgCOD

Using the mass fraction of organisms to polypeptide obtained in (ii) above,

TKN/COD of sludge $= (0{,}90 \times 0{,}113) + (0{,}10 \times 0{,}086)$
$= 0{,}11$ mgN/mgCOD

COD of amino acids released in the high H_2 partial pressure reactor

Average mmols organic nitrogen released = 8,2/14
= 0,59

Component : mmols × COD mass equivalent

Alanine : $(0{,}56 \times 0{,}59) \times 96 = 31{,}72$ mgCOD/ℓ
Valine : $(0{,}28 \times 0{,}59) \times 192 = 31{,}72$ mgCOD/ℓ
Glutamic : $(0{,}16 \times 0{,}59) \times 144 = 13{,}59$ mgCOD/ℓ
$77{,}03$ mgCOD/ℓ

∴ Total COD in the form of free amino acids : $77{,}03 \times 92$
= 7 087 mgCOD/ℓ

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