PELLETIZATION
IN
THE UPFLOW ANAEROBIC SLUDGE BED (UASB) REACTOR

by

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September 1989
SYNOPSIS

This investigation was prompted by a study into the feasibility of treating an apple juicing waste water in an upflow anaerobic sludge bed (UASB) reactor system. Past experience with this system suggested that a pelletized sludge would be produced due to the nature of the waste water. This indeed was observed but the system exhibited an unusual feature, a high removal of nitrogen far in excess of that normally found in normal anaerobic processes. This observation stimulated a far reaching investigation into, the behaviour of the pelletized sludge bed, the causes giving rise to pelletization, a biochemical model explaining pellet formation, verification of the biochemical model, criteria for pellet formation, pH control in the pelletized sludge bed, and a kinetic model for the UASB process.

Feasibility study
The apple juicing waste water was about 99 per cent soluble, consisted principally of sugars, was acidic (pH 4.5 to 5.5) and deficient in nitrogen and phosphorus. Trace elements and macro nutrients were added to give an influent COD:N:P ratio of approximately 100:4:1.

The UASB system performance was evaluated at two temperatures, 25 and 30°C. the maximum COD loadings achieved were 10 and 15 kgCOD/m³ reactor/d (i.e. 30 and 45 kgCOD/m³ sludge bed volume/d respectively) at 25 and 30°C respectively. The influent COD concentration, at both temperatures, was about 2500 mg/l.

Pelletization was observed at both temperatures. The pelletization process started when the loading was about 5 kgCOD/m³ reactor/d in both cases and once started, was very rapid – over a period of six to seven days roughly half the sludge bed was transformed into pellets.

A significant observation was that once pelletization commenced there was a substantial removal of free and saline ammonia (NH₃-N), about 8 to 10 times higher than that observed in completely mixed anaerobic systems.

Product formation
The high removal of NH₃-N by the pelletized sludge motivated an enquiry into the behaviour of the pelletized sludge bed. From visual observations the pellets showed
only slight movement with time, from which it was concluded that the flow in the bed approached a plug flow regime. Accordingly product formation was measured at discrete points along the axis of the reactor. The following parameters were measured: short chain fatty acids (SCFA), soluble COD, TKN, NH₃-N, alkalinity and pH. From the concentration profiles three zones of behaviour were identified:

- **A lower active zone** in which the concentrations of the SCFA's, propionate and acetate, increased to maxima, soluble COD reduced to about half its influent value, NH₃-N reduced to a minimum, organic nitrogen (orgN) increased to a maximum and alkalinity and pH declined to minimum values. From the biochemistry of fermentation processes the increasing concentration of propionate implied that this was a zone of high hydrogen partial pressure.

- **An upper active zone** in which the propionate and acetate concentrations decreased to minima, soluble COD decreased to a near minimum, NH₃-N remained near constant, orgN decreased to a minimum, alkalinity increased to near its value in the influent and pH increased to a stable value. The decreasing concentration of propionate indicated that this was a zone of low hydrogen partial pressure.

- **An upper inactive zone** in which no observable biokinetic activity was present.

The pellet size decreased from the bottom to the top of the sludge bed. Fine volatile solids, apparently from pellet break up, were continuously discharged from the top of the bed to the suspended sludge blanket above the bed. It was concluded that pellet growth took place in the bottom zone(s) of the sludge bed and pellet break up in the higher zones.

In order to obtain more information on the pellet formation-break up, a two-in-series UASB reactor system was set up with the first reactor having a bed volume equal to the lower active zone of a single UASB system. The first reactor immediately showed pellet generation with virtually no fines, whereas in the reactor containing the upper active and inactive zones, pellet break up took place with substantial fines production. The specific pellet yield in the first reactor was estimated to be about 0.4 mgVSS/mgCOD removed — a value close to that for aerobic growth. In addition to the high specific pellet production, the first reactor also exhibited features not observed in 'normal' anaerobic systems, viz:
• Low COD/VSS ratio for the pelletized sludge of 1.23 against "normal" of 1.42 mgCOD/mgVSS for anaerobic processes.

• High removal of NH$_3$-N.

• High production of orgN.

**Biochemical model**

The high production of orgN was particularly perplexing; either the orgN arose from the death of organisms or was generated in the growth process. Death was an unlikely cause because the rate of orgN production would have required an excessive death rate yet no inert debris was produced; the alternative was that the orgN was a product of the growth process.

From a literature survey, the characteristics of one species appeared to be directly relevant — a methanogen, *Methanobacterium* strain AZ (*M. Strain AZ*), now classified as *Methanobrevibacter arboriphilus*. Essentially the species utilizes hydrogen as sole energy source and can produce its amino acid requirements with the exception of the sulphur containing amino acid, cysteine — an external cysteine source is necessary for growth. In a hydrogen rich environment, with an adequate supply of NH$_3$-N and cysteine limitation, the species in pure culture secretes high concentrations of amino acids (orgN) to the surrounding medium. These characteristics provided a basis for an hypothesis on pellet formation:

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 releasing some of these excess amino acids to the surrounding medium and linking the balance of the excess amino acids 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.
Using the mass ratios of the three major amino acids released in pure culture studies, calculations indicated that the observed TKN/COD and COD/VSS ratios of the pellets could be satisfied only if about 90 per cent of the pellet mass consisted of polypeptide polymer.

**Hypothesis verification**

The hypothesis was tested against the following implications:

- *Pellet formation should be confined to the high hydrogen partial pressure region:* In a UASB system treating a carbohydrate waste water, a high hydrogen partial pressure (high \( \overline{p}H_2 \)) zone develops within the lower active zone due to the breakdown of carbohydrates to SCFA by acidogenesis. The high \( \overline{p}H_2 \) (\( > 10^{-4.1} \text{atm} \)) zone extends up to the level where the propionic acid concentration reaches a peak. The UASB reactor was divided into two in series reactors with the first reactor equal in volume to that constituting the high \( \overline{p}H_2 \) zone. In the high \( \overline{p}H_2 \) zone pellet growth was observed, together with a high generation of soluble organic nitrogen; in the low \( \overline{p}H_2 \) zone pellet break up occurred.

- *Addition of cysteine should reduce pellet formation:* In terms of the behaviour pattern of *M. Strain AZ* if cysteine is supplied in trace concentration polypeptide formation should decrease. This was tested by supplementing the influent to the high \( \overline{p}H_2 \) reactor with a trace concentration of cysteine - immediately (within 24h) there was a reduction in the specific pellet yield, of about 50 per cent.

- *Pellets are generated from the substrate:* In the literature, presence of a polymer matrix had been observed in the pellets, however, its origin(s) was/were not identified; one investigator ascribed its presence to polymer present in the influent, incorporated in the pellets by an agglutination process. To ascertain unambiguously the origin of the pellet polymer, a non-polymer defined carbohydrate substrate - glucose, was tested. Excellent pellet formation was observed in the UASB system.

- *Limitation of NH\(_3\)-N in influent should reduce pellet formation:* With glucose as substrate, a detailed enquiry was initiated into the biochemical reactions taking place in the lower active zone (because the biochemical fermentation pathways of glucose are well established). A UASB system with bed volume limited to constitute only the lower active (high \( \overline{p}H_2 \)) zone was set up. From mass balance
considerations the gross specific yield (organism + polymer) of the hydrogenotrophs was determined to be between 0.21 and 0.24 mgVSS/mgCOD (H\textsubscript{2}) or expressed differently 0.4 to 0.5 mgVSS/mgCOD removed. Reducing the NH\textsubscript{3}-N in the influent from an excess amount to slightly more than that required for cell synthesis, resulted in a decrease in the overall gross specific pellet yield from 0.52 to 0.11 mgVSS/mgCOD removed. From mass balance calculations it was concluded that at the lower yield of 0.11 mgVSS/mgCOD removed, virtually no VSS was generated by the hydrogenotrophs; this was supported by further mass balance calculations which indicated that there was no uptake of hydrogen by the hydrogenotroph *M. Strain AZ*. Their decreased activity was ascribed to the intracellular high ATP/ADP level within the species (due to high H\textsubscript{2} substrate concentration) which the species cannot decrease, through the generation of amino acids and polypeptides, when NH\textsubscript{3}-N is limiting; accordingly the H\textsubscript{2} will leave the high pH\textsubscript{2} as gas.

**Criteria for pellet formation and their verification**

From the hypothesis (and the verification tests above), conditions necessary for pellet formation could be set out:

- An environment with a high pH\textsubscript{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, and

- A near neutral pH.

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

1. Pelletization in systems where the substrate yields hydrogen and the operation allows a zone of high H\textsubscript{2} partial pressure build-up, e.g. carbohydrates and proteins in plug flow reactors. If the nitrogen source is less than about 0.0186 mg(NH\textsubscript{3}-N)/mg influent COD the mass of pellets generated per influent COD will be reduced accordingly.
(2) No pelletization in systems where the influent substrate does not yield hydrogen in the fermentation process, e.g. acetate as sole substrate.

(3) No pelletization 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 pelletization where the substrate can generate a high pH but some of the H₂ generated is preferentially utilized by other organisms such as sulphate-reducers.

(5) No pelletization in systems where the influent substrate can be broken down only under low H₂ partial pressure conditions, e.g. propionate and lipids.

With regard to (1) this study and others have demonstrated that pelletization takes place in a UASB system receiving carbohydrate substrates with excess nitrogen. With limited nitrogen this study showed that pellet production was also limited.

With regard to (2) the literature reports no pelletization in UASB systems with acetate only as substrate even if nitrogen is present in excess in the influent.

With regard to (3) there is no report on pelletization in completely mixed reactors.

With regard to (4) this was verified in a study on a UASB system with glucose as substrate and sulphate (SO₄²⁻) ions added to the influent feed. Pellet formation was reduced. Sulphate reducers appeared to utilize hydrogen preferentially thereby reducing pH₂ and hence limiting pelletization. As SO₄²⁻ concentration increased in the influent from trace to excess, decreases were observed in (1) NH₃-N uptake, (2) orgN generation, (3) pellet size, from 2-3 mm to 1-2 mm, (4) nett pellet yield, to 1/5 of the yield at trace SO₄²⁻ concentrations; the reduced yield, remained constant when the SO₄²⁻ supplementation increased above some fixed value.

With regard to (5) a UASB system was studied with oleic acid as substrate. Pelletization did not take place; the three characteristic zones did not develop in the sludge bed; both NH₃-N uptake and orgN generation were low and only acetate was detected. With oleic acid as substrate the sludge bed was well defined but of a
gelatinous nature.

**pH control**

Maintenance of a pH > 6.6 at all points in the profile is crucial to maintaining the UASB reactor efficiency. One aspect that became very clear in the study on UASB system behaviour was its high alkalinity requirement to ensure that the minimum pH, in the pH profile in the sludge bed did not decline below 6.6. By trial a minimum alkalinity requirement of about 1.6 mg alkalinity as CaCO₃/mg influent COD was found to be adequate. In full scale systems such an alkalinity requirement would be substantial and an important factor in operational costs.

The high alkalinity requirement prompted enquiry into operational procedures to reduce this requirement: In passing up the bed there is an alkalinity loss in the lower active zone but this alkalinity is recovered in the upper zones so that only a small nett alkalinity loss from influent to effluent is observed. Thus, in effect any alkalinity added in the influent is wasted in the effluent.

Conceptually the alkalinity in the effluent can be recovered partially by instituting a recycle from the effluent to the influent. In this way the alkalinity per influent COD is increased and accordingly alkalinity supplementation to the influent can be reduced. However no detailed information was available on the effect of a recycle on the pelletization and performance of a UASB system. Accordingly, a study was initiated to determine the effects of recycle on alkalinity requirements, maximum loadings and process performance. The following findings were obtained:

- The minimum alkalinity supplementation for a carbohydrate waste with zero alkalinity, in a flow through system, should not be less than about 1.2 mg alkalinity (as CaCO₃)/mg influent COD.

- With a recycle, alkalinity supplementation is reduced to a value given by $1.2 \cdot \frac{\text{flow}}{\text{flow} + \text{recycle flow}}$.

- Provided the COD loading rate is less than the peak, a recycle does not appear to influence adversely either pellet formation or system performance.

**Design**

From the information gathered on systems behaviour on flow-through systems and
systems with a recycle, the following design guidelines are suggested:

- UASB system will produce pelletized sludge only with substrates that contain a significant fraction of carbohydrates or proteinaceous materials. Where short chain fatty acids, acetate, propionate, butyrate and higher dominate, pelletization either will be poor or not occur at all. Substrate containing fats and oils will not give rise to pelletized systems.

- Tentatively the maximum loading a system without recycle can sustain should be determined with influent COD between 2000 and 5000 mg/l. If the influent waste water has a higher influent COD, a recycle should be imposed such that the COD concentration of the combined flow (base + recycle) is within the above range.

- Alkalinity supplementation should be calculated accepting that 1,2 mg alkalinity (as CaCO₃)/mg influent COD is required; if a recycle is imposed this alkalinity requirement can be reduced by multiplying by the fraction, (flow)/(flow + recycle flow).

- The design loading should be lower than the peak loading determined experimentally; about half the peak loading. For example with the apple juicing waste water at 30°C the peak loading was about 45 kgCOD/m³ sludge bed volume/d (i.e. 15 kgCOD/m³ reactor volume/d), giving an operating loading of ≈ 22 kgCOD/m³ sludge bed volume/d. Tentatively a minimum pelletized sludge volume provision in the reactor should be about 0,03 m³/kg COD influent.

**Kinetic model**

From the data on product formation in the pelletized bed it was possible to identify the key product and the processes associated with the various compounds produced. With glucose as substrate, 11 compounds and 12 processes were identified. Stoichiometric and kinetic constants for the biologically mediated processes (acidogenesis, acetogenesis, and methanogenesis from acetate and hydrogen) were obtained either from the literature or this study. The constants for only 3 processes had to be found by calibration, i.e. curve fitting.

Simulation of the system behaviour at COD loadings below the maximum gave good correlation for COD, SCFA, orgN and NH₃-N profiles for the flow through system.
For the system with a recycle the experimental response indicated better performance than the simulated response; this appears to be due to pellet debris in the recycle. The model accepts debris as inert but there are indications that the debris is biologically active — this aspect however is not included in the model.
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<td>Acetyl Coenzyme A</td>
</tr>
<tr>
<td>Ac^-</td>
<td>Acetate</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>b</td>
<td>Specific organism decay rate (/d)</td>
</tr>
<tr>
<td>b_A</td>
<td>Specific acidogen decay rate (/d)</td>
</tr>
<tr>
<td>b_AP</td>
<td>Specific acetogen decay rate (/d)</td>
</tr>
<tr>
<td>b_MA</td>
<td>Specific acetoclastic methanogen decay rate (/d)</td>
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<td>b_MH</td>
<td>Specific H₂-utilizing methanogen decay rate (/d)</td>
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<tr>
<td>Butyrl CoA</td>
<td>Butyrl Coenzyme A</td>
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<tr>
<td>CoA</td>
<td>Coenzyme A</td>
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<td>COD</td>
<td>Chemical oxygen demand (mgCOD/l)</td>
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<tr>
<td>COD_eff</td>
<td>Effluent COD (mgCOD/l)</td>
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<td>COD_glucose</td>
<td>Glucose COD (mgCOD/l)</td>
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<tr>
<td>COD_orgN</td>
<td>COD associated with soluble organic nitrogen (mgCOD/l)</td>
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<td>COD_SCFA</td>
<td>Short chain fatty acids COD (mgCOD/l)</td>
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<tr>
<td>EMP</td>
<td>Embden Meyerhof pathway</td>
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<td>GT</td>
<td>Glucose fermented (moles/d)</td>
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<tr>
<td>HAc</td>
<td>Undissociated acetic acid</td>
</tr>
<tr>
<td>HPr</td>
<td>Undissociated propionic acid</td>
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HBr  
Undissociated butyric acid

HRT  
Hydraulic retention time (h)

$[HAc]_{G1}$  
Undissociated acetic acid formed from glucose at high $\tilde{p}H_2$ (moles HAc/d)

$[HAc]_{G2}$  
Undissociated acetic acid formed from glucose at low $\tilde{p}H_2$ (moles HAc/d)

$[HAc]_{G3}$  
Undissociated acetic acid formed from glucose at both high and low $\tilde{p}H_2$ (moles HAc/d)

$[HAc]_{obs}$  
Undissociated acetic acid measured experimentally (moles HAc/d)

$[HAc]_{ox}$  
Undissociated acetic acid oxidized to methane (moles HAc/d)

$[HAc]_T$  
Total undissociated acetic acid generated from glucose fermentation (moles HAc/d)

$[HBr]_{G3}$  
Undissociated butyric acid formed from glucose under both low and high $\tilde{p}H_2$ (moles HBr/d)

$[HPr]_{G1}$  
Undissociated propionic acid formed from glucose at high $\tilde{p}H_2$ (moles HPr/d)

i  
Index referring to compounds in matrix

IAWPRC  
International Association for Water Pollution Research and Control

i$_{XBN}$  
Nitrogen content of biomass (mgN/ℓ)

i$_{XPN}$  
Nitrogen content of polymer (mgN/ℓ)

j  
Index referring to processes in matrix

$K_A$  
Growth rate half saturation coefficient for acidogens (mgCOD/ℓ)

$K_{AP}$  
Growth rate half saturation coefficient for acetogens (mgCOD/ℓ)

$K_{BP}$  
Specific rate for pellet breakup (/d)

$K_{EP}$  
Specific rate for adsorption/enmeshment of soluble organic nitrogen (/d)
<table>
<thead>
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<th>symbol</th>
<th>description</th>
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<tr>
<td>( K_{MA} )</td>
<td>Growth rate half saturation coefficient for acetoclastic methanogens (mgCOD/l)</td>
</tr>
<tr>
<td>( K_{MH} )</td>
<td>Growth rate half saturation coefficient for ( \text{H}_2 )-utilizing methanogens (mgCOD/l)</td>
</tr>
<tr>
<td>( K_{ND} )</td>
<td>Specific rate for ammonification of soluble organic nitrogen (/d)</td>
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<td>LCFA</td>
<td>Long chain fatty acids</td>
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<td>( M(N\text{H}<em>3\text{-N})</em>{\text{acid}} )</td>
<td>Mass of nitrogen incorporated in acidogenic mass per day (mgN/d)</td>
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<tr>
<td>( M(N\text{H}<em>3\text{-N})</em>{\text{hyd}} )</td>
<td>Mass of nitrogen incorporated in hydrogenotrophic mass per day (mgN/d)</td>
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<td>( M(N\text{H}<em>3\text{-N})</em>{\text{hyd.cell}} )</td>
<td>Expected mass of free and saline ammonia removal by hydrogenotrophs for cell synthesis (mgN/d)</td>
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<td>( M(N\text{H}<em>3\text{-N})</em>{\text{in,out}} )</td>
<td>Mass of free and saline ammonia per day in the influent, effluent respectively (mgN/d)</td>
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<tr>
<td>( M. \text{Strain AZ} )</td>
<td><em>Methanobacterium</em> strain AZ</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>( \text{NAD}^+ )</td>
<td>Nicotinamide adenine dinucleotide (oxidized form)</td>
</tr>
<tr>
<td>( \text{NADH} )</td>
<td>Nicotinamide adenine dinucleotide (reduced form)</td>
</tr>
<tr>
<td>( \text{NH}_3\text{-N} )</td>
<td>free and saline ammonia</td>
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<td>( \text{orgN} )</td>
<td>Soluble organic nitrogen</td>
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<td>( \text{pH}_2 )</td>
<td>Hydrogen partial pressure (atm)</td>
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<td>PYR</td>
<td>Pyruvic acid</td>
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<td>r</td>
<td>process rate</td>
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<td>Rem</td>
<td>COD removal efficiency (%)</td>
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<tr>
<td>( R_h )</td>
<td>Hydraulic retention time (h)</td>
</tr>
<tr>
<td>( R_s )</td>
<td>Sludge age (d)</td>
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<tr>
<td>( R_1, R_2, R_3 )</td>
<td>Alkyl groups</td>
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</tbody>
</table>
\[ S_A \] Undissociated acetic acid concentration (mgCOD/\ell)

\[ S_{CH_4} \] Methane generated (mgCOD/\ell)

\[ S_G \] Glucose concentration (mgCOD/\ell)

\[ S_H \] Hydrogen concentration (mgCOD/\ell)

\[ S_{I,D} \] Inert mass due to death of organism (mgCOD/\ell)

\[ S_{I,P} \] Soluble inert polymer mass (mgCOD/\ell)

\[ S_{ND} \] Soluble organic nitrogen (mgN/\ell)

\[ S_{NH} \] Soluble ammonia (mgN/\ell)

\[ S_P \] Undissociated propionic acid concentration (mgCOD/\ell)

\[ SCFA \] Short chain fatty acids

\[ TDS \] Total dissolved inorganic solids (mgTDS/\ell)

\[ TKN \] Total Kjeldahl nitrogen concentration (mgN/\ell)

\[ TSS \] Total suspended solids

\[ UASB \] Upflow anaerobic sludge bed

\[ VSS \] Volatile suspended solids

\[ X_{B,A} \] Acidogen mass (mgCOD/\ell)

\[ X_{B,AM} \] Acetoclastic methanogen mass (mgCOD/\ell)

\[ X_{B,AP} \] Acetogen mass (mgCOD/\ell)

\[ X_{B,P} \] Polymer mass (mgCOD/\ell)

\[ X_{B,MH} \] H\textsubscript{2}-utilizing methanogen mass (mgCOD/\ell)

\[ X_{N,B} \] Nitrogen content of biomass (mgN/\ell)

\[ X_{N,H} \] Nitrogen content of polymer (mgN/\ell)

\[ Y_A \] Specific yield of acidogens (mgCOD volatile mass/mgCOD)

\[ Y_{AP} \] Specific yield of acetogens (mgCOD volatile mass/mgCOD)

\[ Y_{H_2} \] Specific yield of H\textsubscript{2}-utilizing methanogens (mgVSS/mgCOD)
$Y_{MA}$  Specific yield of acetoclastic methanogens (mgCOD volatile mass/mgCOD)

$Y_{MH}$  Specific yield of $H_2$ (mgCOD volatile mass/mgCOD)

$Y_P$  Specific yield of polymer (mgCOD volatile mass/mgCOD)

$\dot{\mu}$  Maximum specific growth rate (/d)

$\dot{\mu}_A$  Acidogen maximum specific growth rate (/d)

$\dot{\mu}_{AP}$  Acetogen maximum specific growth rate (/d)

$\dot{\mu}_{MA}$  Acetoclastic methanogen maximum specific growth rate (/d)

$\dot{\mu}_{MH}$  $H_2$-utilizing methanogen maximum specific growth rate (/d)

$\Delta$  Symbol denotes removal

$\Delta$COD  COD removed from system per day (mgCOD/d)

$\Delta M(NH_3-N)$  Nitrogen removed from system per day (mgN/d)

$\Delta MX_{acid}$  Mass of acidogens generated per day (gCOD volatile solids/d)

$\Delta MX_{H_2}$  Mass of $H_2$-utilizers generated per day (gVSS/d)

$\Delta SO_4^-$  Sulphate removal (mgSO$_4^-$/d)

$^\circ C$  Temperature in degrees Celsius.
INTRODUCTION

Production of fruit juices is an important agricultural based industry in the Western Cape Province of South Africa. Of the many juices produced, apple juice constitutes the major fraction.

The location of the processing plants in most instances is near or within the apple growing areas. Treatment of the waste waters from these plants, located in the agricultural areas, usually is of an elementary nature, principally by anaerobic/aerobic stabilization pond systems. The effluent quality from these systems tends to be unpredictable and often very poor due to inadequate designs or low level of operational supervision. In some instances, for example, where the plants are located above water storage dams, pressure has been exerted on the plant management to discharge a treated effluent of more consistent and higher quality. As a consequence there have been requests from the industry to suggest improved system(s) of treatment appropriate to the situation within which the industry operates — systems that are simple to maintain and operate as technical supervision and interest in maintaining and operating the plant are likely to be minimal.

Characteristics of the wastewater from apple juice production, are as follows:

1. Principal constituents, sugars such as glucose, fructose and sucrose.
2. Upwards of 95 per cent soluble.
3. COD relatively low, fluctuating between 1500 and 4500 mg/l.
4. Waste water flow seasonal, starting late in the year and ending about July the following year.

Experience in the Western Cape would indicate that the most appropriate treatment method for agricultural wastes is one based on anaerobic digestion. Anaerobic digestion has a long history of application in the Western Cape, in the treatment of waste waters from agriculturally-based industries, principally from wine distilleries and glucose/starch manufacture. Both industries produce waste waters that are
virtually totally soluble. The wine industry in particular operates under circumstances similar to those in the fruit juice industries — seasonal operation with a relatively low level of operational control. Anaerobic systems that have been developed to deal with these wastes are the reversed-flow clarigester and the anaerobic contact systems. Mixing in the reversed-flow clarigester is from gasification and the mixing is not well defined but appears to tend towards a plug flow regime. Mixing in the contact process is by mechanical stirring and the mixing tends towards a completely mixed regime.

A reversed-flow clarigester had been installed to treat a glucose/starch waste water with an influent COD concentration of approximately 7000 mg/l. This system operated successfully for many years, since about 1962, under a very low level of supervision. The sludge generated in the system was of a granular nature with good settling properties.

Reversed-flow clarigesters had also been tested to treat wine distillery waste waters (average influent COD ≈ 20 000 mg/l). Although an explicit statement in the literature could not be found, it seems that the operation of the system was not successful. The system was modified into a contact-type anaerobic system: a mixer was installed in the digester compartment to improve contact between substrate and sludge; also a recycle stream of the mixed liquor from the upper part of the reactor to the bottom influent point. The performance of the modified system was satisfactory but the sludge generated was filamentous and settled poorly. To provide adequate settling, the clarigester design was replaced by a system consisting of a contact anaerobic reactor discharging to a separate secondary settling tank.

In evaluating the anaerobic contact system for application to fruit juicing waste waters, a significant difference between the apple juicing waste and the wine distillery waste is that the COD of wine distillery waste is approximately 20 000 mg/l. Experience on completely mixed anaerobic contact laboratory-scale units at the University of Cape Town, treating influent concentrations of less than 5000 mg/l, indicated that with respect to the flow, the settling tanks for low concentration wastes needed to be disproportionately larger with regard to the flow than for high concentration wastes in order to maintain a low concentration of VSS in the effluent. If this was not done the mass loss of sludge in the low influent COD waste system could become so high that the sludge age was reduced below that needed for stable operation. Indeed for the low strength wastes, for the same COD
mass loading per day, the VSS in the effluent needed to be less than that for the high strength wastes as the flow per COD is larger and the sludge loss accordingly higher. It was concluded that for the treatment of the low strength apple juice waste water, the completely mixed anaerobic type systems were likely to give rise to difficulties in operation.

An alternative system examined was the upflow anaerobic filter. Experimental investigations into upflow anaerobic filter systems at the University of Cape Town on laboratory and pilot scale systems treating wine distillery and glucose/starch waste waters, showed that the upflow filter system is feasible but has the following drawbacks: (1) It requires a relatively high recycle ratio from the effluent to the influent otherwise the pH at the bottom of the upflow reactor was reduced to such low values that the anaerobic process commenced to fail at the bottom, the failure zone spreading up through the reactor; alternatively high alkalinity dosage was required to limit the pH decline, and (2) the voids between the packing material tended to plug up causing channeling.

A third group of systems examined were the 'new' generation of anaerobic digestion systems such as the upflow anaerobic sludge bed (UASB) and the anaerobic film expanded bed (AAFEB) systems.

The UASB system has found extensive application in the Netherlands for the treatment of agricultural and industrial waste waters such as sugar beet, potato processing, corn starch, distillery and slaughter house. With certain types of waste waters a pelletized form of sludge has been reported which settles readily into a bed. Very high sludge concentration in the reactor has been observed, in excess of 100 kg/m³.

In the AAFEB system the biological mass is attached to a support medium (e.g. sand) and the medium/biological particles are kept in suspension by recycling the clear liquid above the bed to the influent.

Over the past decade there has been an increasing interest in the UASB and AAFEB systems. These have found application principally in the treatment of medium to high strength industrial and agricultural wastes, of an essentially soluble nature. The UASB and AAFEB systems appear to provide a solution for the problem with the reversed-flow clarigester and anaerobic contact processes, namely, retention of
the sludge within the system without having to resort to low sludge concentrations necessitating large reactor volumes and settlers. With these two systems high density and good settling properties of the sludges have allowed high throughput of the waste waters. Hydraulic retention time as short as 1.5 to 2 hours for low strength influents of about 1500 mgCOD/l has been reported.

In South Africa the UASB and AAFEB systems per se have received relatively little or no attention either at laboratory, pilot or full scale level. Although the reversed-flow clarigester without mechanical mixing probably is very similar in operation as that in the UASB system, however, its success in treating glucose/starch waste and its failure to treat the wine distillery waste indicated that the UASB system may not necessarily be successful in treating the apple juicing waste. In order to assist making a choice as to which of the two systems merited detailed investigation, an exploratory study at laboratory scale was inaugurated into each system, following the directives as set out in the literature.

Great difficulties were experienced in starting up the AAFEB system; after 6 months the system still did not operate successfully. The most likely reason was the lack of experience in the design, choice of support material and operation. In contrast, with the UASB system, start up and successful operation were readily achieved. The UASB system also appeared to be much simpler to operate than the AAFEB system. It was concluded therefore that the UASB system had the highest expectation for successful implementation and operation in the treatment of the apple juicing waste water. Accordingly it was decided to proceed with a feasibility study of the UASB system for the treatment of this waste water.

The feasibility study encompassed the start-up, system performances, maximum loadings, interaction of waste COD concentration and hydraulic retention time, effect of temperature and sensitivity to shock loadings (Chapter 3).

From the feasibility study it became apparent that a major limitation on the system performance was the development of a low pH region in the bottom of the reactor. This led to a general enquiry into the biochemical processes taking place in the sludge bed. Product formation along the line of flow in the reactor system was analyzed in terms of established normal anaerobic fermentation theory (Chapter 4). This in turn led to an enquiry into the processes giving rise to the phenomenon of pelletization and an hypothesis on the pelletization phenomenon evolved (Chapter 5).
This hypothesis stated that pelletization arose from the generation of polypeptides by a specific hydrogen-utilizing methanogen under the following conditions: (1) a high hydrogen partial pressure, (2) excess free and saline ammonia, (3) deficiency of the amino acid cysteine, and (4) near neutral pH. The hypothesis predicted that no or limited pelletization would be observed when: (1) the hydrogen generated is preferentially removed by organisms such as sulphate reducers, (2) a waste ferments only under low hydrogen partial pressure e.g. lipids, (3) a waste does not give rise to generation of hydrogen e.g. acetic acid, (4) there is an inadequate concentration of free and saline ammonia in the influent, and (5) an adequate supply of cysteine is available.

To verify the hypothesis the system was studied using a defined substrate glucose (Chapter 6). Glucose was selected because its biochemical pathways and product formation in the fermentation process are well established. From this study it was possible to quantify the various products formed, in particular the hydrogen flux, and to calculate the hydrogen utilizing methanogen yield and the mass of polypeptide generated. To check some of the hypothesis predictions, studies were undertaken to ascertain the effects of (1) NH₃-N limitation on pellet formation, (Chapter 6), (2) SO₄²⁻ ions, (3) lipid substrate, and (4) cysteine supplementation (Chapter 7).

The study on product formation indicated the importance of alkalinity in the control of pH in the UASB system, also that the system required higher alkalinities for successful operation than the other anaerobic systems (viz. anaerobic contact process). Alkalinity provision could impose a significant operational cost, accordingly attention was directed to ways and means to reduce the alkalinity requirement, in particular where high influent COD had to be treated. Recycling from the effluent to the influent was investigated (Chapter 8). From this investigation it was found that the alkalinity supplementation could be reduced substantially by instituting a recycle. A recycle also was shown to be a necessity when treating waste waters with high influent COD strengths.

Finally, a mathematical model was developed that describes the stoichiometry and kinetic behaviour of the various processes operating in a UASB system that produces a pelletized sludge mass (Chapter 8).
CHAPTER 2

LITERATURE REVIEW

1. ORIGIN

The upflow anaerobic sludge bed (UASB) reactor was developed in the Netherlands in the early 70's as a means to treat waste waters from sugar beet and potato processing industries. The UASB concept was developed from two upflow reactor systems, the (1) reversed-flow clarigester and (2) upflow anaerobic filter (Mosey, 1981).

The reversed-flow clarigester was developed in South Africa in the early 1960's for treating mainly industrial waste waters. Hemens et al. (1962) were searching for an economical and effective method of treating a glucose/starch effluent and selected an anaerobic activated sludge process. Needing a full-scale plant at short notice, they decided to use an existing type of treatment plant, a Dorr-Oliver clarigester.

The Dorr-Oliver clarigester was designed for the treatment of raw sewage. It consisted of a settling tank built on top of an aerobic digester. The settling tank had a conical bottom with a central opening. The raw sewage was discharged downwards also at the centre. The supernatant (settled sewage) discharged via a peripheral overflow weir. The sludge settling to the conical bottom was moved to the central opening by means of a scraper mechanism and discharged to the digester compartment below.

Hemens et al. transformed the clarigester into an anaerobic activated sludge plant by removing the original raw sewage discharge point (in the top settling compartment) and introducing the inlet feed point at the base of the bottom compartment. Thus the flow direction in the system was reversed and created an upflow anaerobic digester; the original primary settling tank now became a secondary settling tank separating the anaerobic reactor sludge from the mixed liquor and discharging the treated water. No mechanical mixing was provided.

Hemens et al. found that the modified system was viable for the glucose/starch waste water with influent COD of 10 000 mg/l at temperature 20-25°C. At a loading of 3 kgCOD/m³/d the COD removal was about 80 per cent. The system developed a
good settling sludge and granular sludge developed at the bottom of the reactor but no particular importance was attached to this phenomenon (Ross, 1989).

The reversed-flow *clari-gester* was later used to treat wine distillery waste waters (Stander et al., 1968) at a temperature of 30°C. For influent COD of 22 400 mg/l, a COD removal of 97,5 per cent was achieved at a loading of 3,2 kgCOD/m³/d. However the sludge developed on wine distillery waste waters had poor settling properties due to its "diffuse and filamentous nature" (Mosey, 1981).

At about the same time (mid 60's) McCarty in the USA was investigating ways of retaining biomass in an anaerobic system such that low strength waste waters may be treated without having recourse to long hydraulic retention time (as in the anaerobic conventional process) or sludge recycling (as in the anaerobic contact process). Young and McCarty (1967) developed the upflow anaerobic filter reactor. In the anaerobic filter reactor the reactor is packed with pebble gravel or some other packing material. The waste water enters and is distributed across the bottom of the filter bed, the flow is upward through the bed and the filter is submerged completely. Biological solids grow attached to the packing material (pebble gravel) and are trapped within the interstices. The packing material also serves to separate the gas generated from the liquid and provide quiescent areas for settling of suspended growth.

Young and McCarty (1967) tested the system at 25°C with synthetic wastes such as mixtures of SCFA (acetic and propionic acids) and a protein-carbohydrate mixture having COD concentrations between 1500 to 6000 mg/l. They found that:

1) Relatively low strength waste waters (1500-6000 mgCOD/l) could be treated effectively without solid recycling. For the protein-carbohydrate waste the process achieved COD removal efficiencies between 90 to 63 per cent at organic loadings of 0,5 to 4 kg/m³ reactor/d. With the SCFA mixture the COD removal ranged from 98 to 68 per cent over the same loading range.

2) For the same loadings, at high loadings (≈ 4 kg/m³ reactor/d) COD removal efficiency was improved for the higher influent COD concentrations (for both substrate mixtures)

3) Biological solids accumulated in the lower region of the filter and most of the
influent COD was removed within this region.

4) Biological solids were retained efficiently inside the filter bed; total solids accumulated were between 37 300 to 171 000 mgVSS. The average effluent suspended solids concentrations were between 7 and 90 mgVSS/ℓ.

5) With protein/carbohydrate wastes, flocculated solids were suspended in the voids together with granules of diameter approximately 3.1mm. They attributed the formation of granules to a rolling action induced by the rising gas bubbles.

Other investigators (van den Berg and Lentz, 1979; Young and Dahab, 1982) found that most of the biological solids were present in suspended form in the void spaces with a small amount attached to the surface of the packing material. They concluded that the biological growth on the packing material provides a polishing action.

The anaerobic filter has been found suitable for soluble waste waters. With waste waters that contain suspended solids, the suspended materials settle readily and accumulate in the voids of the packing material causing channeling of the influent feed (Van den Berg and Kennedy, 1983). Consequently solid wasting is necessary to prevent plugging of the packing.

Although it is not clear from the literature it would appear that at the beginning of the 70's Lettinga et al. at the University of Wageningen, the Netherlands, evolved the UASB system by combining the reversed-flow clarigester and the anaerobic filter: They took cognizance of the experience with the anaerobic filter that the biological mass accumulated in the lower region of the filter where most of the influent COD was removed. Accordingly they made provision for an unpacked lower region in their system where the biological mass could accumulate (Mosey, 1981). They removed the overlying support material progressively until none was present in the system. To separate the gas, liquid and solids, an internal gas/liquid/solid separator was introduced in the upper part of the system, the solid particles settling back into the digesting zone. There was no mechanical mixing in the digesting zone. This system has been called the upflow anaerobic sludge bed (or blanket) system or UASB system.

According to de Zeeuw (1988), formation of a granular sludge in the UASB system
was reported in 1974 by Lettinga and associates: At laboratory scale UASB system, operated on sugar beet waste water, developed a well settling flocculant sludge; the system operated efficiently up to a loading of 10 kgCOD/m³/d. However when the same waste water was treated in a pilot scale UASB system, a granular sludge bed developed and the system again operated efficiently but up to a maximum loading of 30 kgCOD/m³/d.

Development of granular sludges was subsequently observed in UASB systems treating waste waters from sugar refinery, potato/starch processing and distillery effluents (Hulshoff Pol et al., 1983; Lettinga et al., 1979). Certain waste waters tested did not develop a granular sludge: Olive oil processing waste water gave rise to a smooth uniform sludge bed and slaughterhouse waste water to a flocculant sludge bed (Boari et al., 1984; Sayed, 1982). These UASB systems appeared to operate efficiently but the loadings were very much lower than those achieved when granular sludge developed. Furthermore Hamada and Van den Berg (1984) reported that with non-granular sludges part of the sludge bed could be lost should there be a hydraulic surge through the system.

2. SUBSTRATE FEED:
The phenomena of granular sludge formation (or pelletization) appears to be associated with certain wastes. Pelletization has been observed in UASB systems (laboratory, pilot and full-scale) mainly with medium strength soluble waste waters consisting of carbohydrates, alcohols (methanol and ethanol) or proteins; poor pelletization appears to be associated with short chain fatty acids (SCFA) substrates:

**Carbohydrates:** Good pelletization with waste water has been observed with sugar beet, potato, corn and potato starch processings (Hulshoff Pol et al., 1983); maize starch (Ross, 1984) and distillery wastes (Hulshoff Pol et al., 1983). Wu et al. (1987) obtained "granulation" (i.e. pelletization) in two laboratory scale UASB reactors treating a glucose molasses solution (1000-3500 mgCOD/ℓ) and citrate waste water (20 000-36 000 mgCOD/ℓ); the authors made the following observations:

(1) With glucose molasses as substrate, granules appeared in 33 days, within a week after their appearance the reactor was filled with granules, of maximum diameter 3 to 4mm.

(2) With citrate waste water as substrate, granules appeared after 60 days;
within a week the reactor filled with granules, of diameter ranging from 0.2 to 2.0 mm.

From these two observations, Wu et al. concluded that granulation took place more rapidly with glucose molasses than with citrate waste water.

**Alcohols:** On a laboratory scale UASB reactor Lettinga et al. (1979) observed that with pure methanol as substrate pale yellow granules developed (size 0.5 to 4 mm). These granules were formed within a few months after start-up. They reported a biomass yield of between 0.11-0.14 g sludge/g COD removed, a yield very much higher than that expected in anaerobic processes.

Wiegant et al. (1985) operated a UASB at thermophilic temperature (55°C) seeded with thermophilic sludge cultivated on sucrose. They observed pelletization, with granules of 1 to 3 mm. With vinasse (high strength waste water of alcohol distilleries) as substrate feed, the size of the sludge granules decreased significantly, from 1-3 mm to 0.5 mm. They concluded that compounds present in the waste water might be detrimental to pellet formation.

Dolfing (1987) in an endeavour to develop a laboratory system to grow granular sludge under defined conditions, investigated various mixtures of organic compounds as substrate to UASB reactors. The organic compounds were: SCFA (acetate, propionate and butyrate), lactate, valerate, sucrose and ethanol. The influent COD ranged from 2000 to 6500 mg COD/l depending on the loading rate. In all the experiments a substantial amount of yeast extract (425 mg/l) was added to the feed. Dolfing concluded that

1. With the various substrate mixtures but with a principal fraction of SCFA, pellet growth was poor and filamentous growth was dominant.
2. Ethanol as sole substrate feed gave rise to good growth of granular methanogenic sludge.
3. Compared to the other substrates, the growth yield with ethanol was significantly the highest.
4. Sludge granules grown on ethanol consisted of rod and coc-shaped bacteria;
with the mixtures of substrates, filamentous organism growth was dominant.

**Proteins:** Schulze *et al.* (1988) using gelatine (protein) as substrate found that:

(1) Goods granules (mean diameter 1-2mm) were formed when the influent gelatine concentration was 5g/l; smaller granules (0.5-1mm in diameter) were formed when the gelatine concentration was 15g/l.

(2) At the lower gelatine influent concentration the biomass concentration was 41.5 g/l compared to 37.3 g/l at higher gelatine concentration.

(3) In both experiments pelletization was observed within 3 weeks.

(4) When the influent feed concentration was 5 g/l, the maximum ammonium concentration in the effluent was measured at 1000 mg/l i.e. 200 mg (NH₃-N)/g gelatine; when the influent feed was 15 g/l gelatine, the effluent ammonium concentration was 2700 mg/l, i.e. 180 mg (NH₃-N)/g gelatine and the removal efficiency was 73 and 68 per cent respectively. They concluded that the high NH₃-N concentrations generated did not appear to have a significant effect on process performance.

**Short chain fatty acids (SCFA):** de Zeeuw and Lettinga (1980), Hulshoff Pol *et al.* (1982), ten Brummeler *et al.* (1985), and Dolfing (1987) (all these authors were from the same research institution) tested mixtures of SCFA such as acetic and propionic acids as substrate. This type of substrate was of general interest because many industrial waste waters contain significant concentrations of SCFA, or, these can form rapidly from the dissolved biodegradable fraction in the waste.

All the experiments made use of mixtures of acetic and propionic acids (total COD ~ 3000 mg/l), supplemented with yeast extract (~ 250 mg/l). Generally they observed that the granules formed from SCFA mixtures were weak and tended to break apart after vigorous shaking. They concluded that pelletization can be obtained by using only SCFA as substrate.

Hulshoff Pol *et al.* (1984) investigated mixtures of SCFA and sucrose (carbohydrate); they found:
(1) With substrate containing mainly SCFA (90 per cent SCFA and 10 per cent sucrose), granules that were formed had poor settling quality and were filamentous in nature. Biomass concentration, at the start of the experiment, was 12.8 gVSS/ℓ and subsequently decreased to 6.3 gVSS/ℓ at the termination of the experiment. Bed washout was observed during the experiment. The biomass yield was ± 0.028 gVSS/g COD removed; this yield is comparable with that obtained in normal anaerobic processes (0.03 gVSS/g COD removed).

(2) With substrate containing mainly sucrose (90 per cent sucrose and 10 per cent SCFA), good settling granules were obtained. These consisted of filaments, cocs and duplococs. The bed volatile mass concentration increased from 12.5 to 19.6 gVSS/ℓ in about 55 days. No bed sludge washout was observed even at the highest loading. Sludge yield was estimated at 0.093 gVSS/gCOD removed; this yield is more than 4 times that expected in normal anaerobic processes.

(3) After 130 days operation the bed mass concentrations for the two influent substrates were approximately 9 gVSS/ℓ (for the 10 per cent sucrose + 90 per cent SCFA substrate) and 40 gVSS/ℓ (for the 90 per cent sucrose + 10 per cent SCFA substrate).

(4) The time taken for the appearance of granules on the different substrates was:

95% sucrose + 5% SCFA < 10% sucrose + 90% SCFA < 100% SCFA.

Hulshoff Pol et al. findings are of considerable importance as they demonstrated positively the difference between sugar type and SCFA type substrates on pellet formation. SCFA substrates produced less pellets of poorer structural integrity with different microbial populations than sugar type substrates. The findings of Hulshoff Pol et al. are supported by the following reports:

(1) Lettinga et al. (1983) reported that the development of granular sludge proceeded at a faster rate with unsoured (no SCFA present) sugar solutions then with a SCFA solutions of the same COD and nutrient content.
(2) Wiegant and Lettinga (1985) observed that granular sludge formed on sucrose substrate, disintegrated when maintained on a SCFA solution for more than 6 months. They speculated that this phenomena may have been due to the decay of the sugar fermenting organisms which they assumed were responsible for the structure of the granules.

**Lipids:** Pelletization has not been reported in UASB systems with lipids as substrate feed. Boari et al. (1984) using the UASB system with olive oil (lipid) processing waste water as substrate did not observe pellet formation; they reported that a sludge bed formed but it was uniform and smooth in consistency with good settleability.

**Conclusions**

From the work reported on the influence of substrate type on pelletization, the following conclusions can be made:

(1) Carbohydrates, alcohol and protein waste waters give rise to well formed high density pellets.

(2) SCFA do not appear to promote pelletization or promote pelletization only to a limited degree; pellets that formed are ill defined, filamentous and with low density.

(3) With carbohydrates a net sludge yield of 3 times that associated with normal anaerobic processes is obtained, of about 0.093 mgVSS/mgCOD removed. With SCFA a yield very near that associated with normal anaerobic processes is obtained, of about 0.03 mgVSS/mgCOD removed.

(4) In UASB systems treating lipids, a sludge bed is formed but of a smooth consistency, i.e. no pelletization is observed.

3. **MICROBIOLOGICAL POPULATIONS IN PELLETIZED SLUDGE**

In the literature on pelletization in UASB systems the general consensus appears to be that pelletization occurs in the methanogenic phase, either mediated by specific methanogenic organisms or as a result of extracellular polymer excreted by these organisms:
de Zeeuw and Lettinga (1980) investigated two UASB systems fed on a mixture of SCFA as substrate. One system was supplemented with 30 mg Ca\(^{2+}\)/l (calcium-poor reactor) and the other with 300 mg Ca\(^{2+}\)/l (calcium-rich reactor). They observed that:

1. Filamentous bacteria resembling *Methanothrix* were predominant in the calcium-rich reactor with growth taking place on the external surface of the flocs. They also indicated that these filamentous bacteria were absent from the seed sludge.

2. Increasing number of *Methanosarcina* spp. type organism were observed in both reactors after an extended period (50 days) of operation.

3. Microscopic examinations of the granules in the calcium-rich reactor showed that the main part consisted of rod-shaped bacteria. Furthermore in the surrounding solution of the disintegrated granules, a large number of *Methanosarcina* spp. type organisms were present.

4. Growth of filamentous bacteria (acetate degrading methanogens) created a bulking anaerobic sludge resulting in low VSS concentrations in the system.

From the above observations de Zeeuw and Lettinga speculated that pelletization may have been initiated by *Methanosarcina* spp. as these organisms can form spongy clumps that could serve as support for other bacteria.

Hulshoff Pol et al. (1982) identified two types of granules according to the type of organisms present:

1. 'rod-granules': these mainly consist of rod-shaped bacteria

2. 'filamentous granules': these mainly consist of filamentous bacteria.

However Hulshoff Pol et al. observed that the predominant bacterium in both types of granules showed much resemblance with *Methanothrix soehngenii*.

Hulshoff Pol (1984) examined the type of granule formed and its associated methanogenic flora using a mixture of SCFA and a carbohydrate. He observed the
following:

(1) 90 per cent SCFA-COD + 10 per cent sucrose-COD as substrate: The granules formed were poor settling and filamentous in nature and most of the granules were formed by attachment of the filamentous bacteria, presumably *Methanothrix*, to biologically inert particles originating from the seed sludge.

(2) 5 per cent SCFA-COD and 95 per cent sucrose COD as substrate: The granules formed initially consisted of long filaments and small amount of cocs and duplococs. However during the experiment a shift in composition of the granules was observed; the number of filaments decreased whereas the number of mobile rods increased. Furthermore the granules were not formed by attachment on inert support particles.

(3) 10 per cent SCFA-COD and 90 per cent sucrose-COD: Microscopical observation of the sludge showed that autofluorescing H₂ consuming methanogens predominated with long filaments resembling *Methanothrix* present in small numbers.

Ross (1984) examined the granules formed in a full scale clarigeser treating a maize processing waste by scanning electron microscopy. He commented that whereas in flocculant anaerobic sludges filamentous organisms extended outside the flocs, in granular sludges no filaments extended beyond the surface of the granules. Microscopic examination of a crushed granule by wet and stained preparations showed mainly three morphological types of bacteria:

(i) long rods (5-15 μm) with flat ends, non-motile and gram-positive.

(ii) Cocci (2,5 μm diameter) often in small bunches of two to three bacteria; non-motile; gram-positive.

(iii) Short rods (2,5 μm long); very motile; gram-negative.

Ross also observed that appreciable polypeptide chains were present; these appear to bind the organisms together in the granules.

Riera (1985) reported that granules formed on sugar cane molasses waste water as
substrate, consisted of sarcinae and rods with the latter predominating.

Alibhai and Forster (1986) examined several types of granules grown on various substrates, Table (2.1), using scanning electron microscopy. By comparing the morphology of various known methanogenic species, the types of bacteria were identified as listed in Table (2.1). They concluded that the presence of Methanothrix in all the granules, indicated that this organism has an important role in the formation of granules.

Table 2.1: Sludge characteristics as determined by scanning electron microscopy (after Alibhai and Forster, 1986).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reactor size</th>
<th>Physical structure</th>
<th>Biological Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td>Full-scale</td>
<td>Stratified</td>
<td>Core: Loosely packed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inner: Methanothrix soehngenii</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Inner: Methanobrevibacter spp.; M. soehngenii</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surface: Polymer/inert particle matrix Methanobrevibacter spp. Methanothrix soehngenii</td>
</tr>
<tr>
<td>Sugar beet/potato</td>
<td>Full-scale</td>
<td>Homogeneous</td>
<td>Inner: Methanococcus; Methanosarcina</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surface: Polymer/inert particle matrix Methanospirillum hungatei M. soehngenii</td>
</tr>
<tr>
<td>SCFA</td>
<td>Lab-scale</td>
<td>Homogeneous</td>
<td>Inner: Methanothrix soehngenii</td>
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<td></td>
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<td>Surface: Polymer/inert particle matrix Methanospirillum hungatei M. soehngenii</td>
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<tr>
<td>SCFA</td>
<td>Lab-scale</td>
<td>Homogeneous</td>
<td>Inner: Methanothrix soehngenii</td>
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<td></td>
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<td>Surface: Inert particles Methanothrix soehngenii</td>
</tr>
<tr>
<td>Maize starch</td>
<td>Full-scale</td>
<td>Stratified</td>
<td>Core: Calcium carbonate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inner: Methanothrix soehngenii</td>
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<td></td>
<td>Inner: Methanobrevibacter spp. M. soehngenii</td>
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<td></td>
<td></td>
<td></td>
<td>Surface: Polymer/inert particle matrix Methanothrix soehngenii</td>
</tr>
<tr>
<td>Spent fermentation liquor</td>
<td>Lab-scale</td>
<td></td>
<td>Methanobacterium bryantii or Methanospirillum hungatei</td>
</tr>
</tbody>
</table>
Dolfing (1987), in an attempt to elucidate the mechanism of pelletization, investigated the methanogenic microflora of the granules formed on different substrates. He observed that:

(1) With ethanol as substrate: the sludge granules formed consisted of rod and coc-shaped bacteria and filamentous bacteria were absent.

(2) With mixtures of SCFA: the sludge granules formed were fluffy and consisted mainly of filamentous bacteria resembling *Methanothrix soehngenii*. This organism grew in long intertwined filaments which appeared to serve as a matrix, enclosing micro-colonies of other groups of bacteria.

From these observations Dolfing implicated the presence of *M.soehngenii*-like organisms in the conglomerates that developed: Fluffy pellets were formed when *M.soehngenii* was abundant and grew as filaments whereas good pellets were formed when *M.soehngenii* occurred as single organisms or in short chains. However the author did not identify the conditions that would promote growth as single organisms or in short chains.

Wu et al. (1987), using fluorescent microscopy, observed that in granular sludges formed from glucose-molasses and citrate waste water substrates, the microflora consisted of rods and filaments with few sarcina and cocci. Using morphology the authors inferred that these organisms might belong to the *methanobacterium*, *methanosarcina* and *methanococcus* respectively. Scanning electron micrographs of the granular sludges showed that on the surface of the granules the microorganisms were present as long filamentous rods or short rods of 2-5 cells. These organisms resembled *Methanothrix soehngenii*. Methanaosarcina-type organisms were also observed on the surface of the granular sludges.

Thiele et al. (1988) examined the organisms in flocs formed on a whey processing substrate using electron microscopy. They observed large clumps of a sarcina which they presumed were *Methanosarcina barkeri*; long filaments were occasionally found within the flocs. Using UV-epi-fluorescence microscopy a presumptive homogenous distribution of a fluorescent rod was predominant; by size and shape similar to *Methanobacterium formicum*.

From the above description of the methanogenic microflora in anaerobic digesters it
is evident that in situ identification of the microflora is difficult and only presumptive naming could be done on the basis of structural characters as shape and cell envelope structure. Consequently the interpretation of micrographs has been limited to species which are recognized easily such as *Methanosarcina* sp. cells and *Methanothrix* sp. filaments, whereas other dominant genera are described only as rods, cocci, etc. Hence it would appear that present methods for identification of the various populations in an anaerobic digester are inadequate. Indeed Kobayashi *et al.* (1988) argued that conventional techniques such as phase-contrast, fluorescence and electron microscopy are useful only for tentative identification of the bacterial population. Furthermore Dubourguier *et al.* (1988) intimated that adaptation of classical methods (for example the agar roll tube technique and the deep agar method) to evaluate the number of bacteria in a sample from an anaerobic system have had limited success. These methods destroy the interactions between the species in the sample thereby giving rise to distortions in evaluating the community structure.

Recently a new method, the immunology technique (Archer, 1984, Conway de Macario and Macario, 1982, 1986) has been developed that allows rapid and accurate identification and quantification of methanogenic organisms. This method does not require isolation and culture of organisms. Macario and Conway de Macario (1988) using this technique for quantitative analysis of the methanogenic flora in anaerobic digesters identified a considerable diversity of methanogens, much larger than previously reported. The authors analyzed samples from 14 different digesters and found that two hydrogenotrophic methanogen predominated, *Methanobacterium formicicum* JF in 13 digesters and *Methanobrevibacter arboriphilus* AZ in 12 digesters. The authors also observed 14 strains of 11 species of methanogens in the digesters.

Dubourguier *et al.* (1985) have shown that *Methanobrevibacter* sp., *Methanospirillum* sp and *Methanothrix* sp. were the main methanogenic bacteria present in granular sludges. Dubourguier *et al.* (1988) reported that the dominant acetoclastic and hydrogenotrophic methanogens were *Methanothrix soehngenii* and *Methanobrevibacter arboriphilus* respectively in a UASB system fed with a mixed defined substrate consisting of glucose, SCFA, valerate and lactate. Furthermore the authors indicated that the microbiological analysis on the above defined mixed substrate, were similar to those reported in UASB reactors treating waste waters from starch and sugar industries. Archer (1988) reported that in propionate-degrading
enrichments, the major hydrogenotrophic methanogens were *Methanobrevibacter arboriphilus*.

4. POLYMER FORMATION:
The nature or role of extracellular polymers in the stabilization of granular sludge has been the subject of much discussion recently. Although extracellular polymer substances (ECP) of biological origin generated from lytic products, or biological excretions, usually are observed in natural systems, as yet no precise mechanism in either their formation or their role in bacterial aggregation has been postulated. Harris and Mitchell (1973) described several types of extracellular polymer substances of biological origin:

(1) Complex polysaccharides and polyamino acids. These are secreted or exposed at the bacterial surface and occur under varying physiological conditions especially during the declining growth and death phases.

(2) Polymeric material such as nucleic acids and proteins. These are excreted or introduced to the surrounding medium by cellular lysis and have been shown to play a significant role in bacterial aggregation.

Harris and Mitchell (1973) concluded that certain substrates and the relative concentrations of nutrients both influenced the composition and concentration of extracellular polymers. They further noted that these polymers are nonionic or anionic in the neutral pH range.

Ross (1984) was the first to propose that extracellular polymer (ECP) played a dominant role in the granulation process. He produced scanning electron micrographs of the granular sludge which showed the presence of ECP as fibrous strands protruding from the bacterial surface. He obtained a yield of ECP from the granular sludge, of about 4 percent of the suspended solids on a dry mass basis. Furthermore he observed that a fraction of both the soluble polymer in the glucose/starch feed, and the ECP which was bound to the pellet sludge surface, had the same molecular mass, namely $2 \times 10^6$. From these observations he concluded that pelletization in anaerobic sludges was similar to an agglutination reaction induced by polymers which can be present in the feed substrate or synthesized by the polymerization of simpler precursor molecules as a result of the metabolism of the feed substrate.
Dolfing et al. (1985) produced transmission electron micrograph of granular methanogenic sludge (grown on sugar containing waste waters) showing large amounts of extracellular material. Furthermore since the extracellular polysaccharidic fraction of granules was only 1 to 2 per cent, they concluded that it is likely that more than one type of extracellular polymer, excreted by more than one group of organisms, contributed to the stability of the matrix in which the bacteria are embedded. In contrast Grotenhuis et al. (1988) observed that in granules grown on propionate only 0.1 to 0.3 per cent of the carbohydrates were extracellular material.

Alibhai and Forster (1986) using scanning electron microscopy on pellet sections also observed the presence of a polymer matrix at the outer surface but provided no explanation how this could have been formed. They noted that the stability of the granules depended on the presence of this polymer matrix. Furthermore they observed that a sludge having a polymeric matrix at the surface exhibited the greatest structural stability during storage, and in those sludges where no such matrix was observed structural degeneration was more rapid.

Mahoney et al. (1986) produced electron micrographs of granular sludge cells in which the presence of extracellular polymeric substances were clearly visible in the intercellular spaces.

Harada et al. (1988) found that methane producing sludges fed on carbohydrate substrates (mixture of starch and sucrose, molasses and sucrose) produced pellets that were more stable and larger in diameter than those produced in sludges fed on SCFA, and speculated that this was due to extracellular material synthesized by acidogenic bacteria necessarily present in the fermentation of carbohydrate type substrate to SCFA.

Guiot et al. (1988) using a carbohydrate (sucrose) as substrate feed, with a nitrogen deficient medium observed that the total carbohydrate content of the biomass was higher than that generally found in biological masses (total carbohydrate/COD ratio of sludge was 16 per cent instead of the 10 to 12 per cent). They attributed this increase to be due to the formation of polymeric material but were of the opinion that this did not prime the granulation process and suggested that the polymeric material most likely was intracellular and hence could not participate in the aggregation process.
5. EFFECTS OF CALCIUM IONS:

The role of Ca\(^{2+}\) ions in promoting sludge pelletization in the UASB process has not been clearly elucidated up to the present. This is due mainly to contradictory results obtained by various investigators. Some showed that Ca\(^{2+}\) ions exert a positive effect on the flocculating ability of anaerobic sludge and enhanced the rate of sludge pelletization whereas others could find no evidence that Ca\(^{2+}\) ions induced pelletization. In their investigations de Zeeuw and Lettinga (1980) operated two UASB systems treating a SCFA mixture; one system was supplemented with only 30 mgCa\(^{2+}\)/l influent and the other with 300 mgCa\(^{2+}\)/l influent. They observed that in the calcium poor reactor (Ca\(^{2+}\) \(\sim\) 30 mgCa\(^{2+}\)/l), formation of grey-white granules (0.5-1.0 mm in size) with a VSS content of 90 per cent proceeded rapidly; these granules formed a granular bed in the lower part of the reactor. In contrast in the calcium-rich reactor ( Ca\(^{2+}\) \(\sim\) 300 mgCa\(^{2+}\)/l) a fine sludge bed was formed with only a few granules which were more or less dispersed throughout the height of the bed, and the bed material showed a tendency to float; they attributed this behaviour to the low concentration of phosphate of 0.5-0.6 mgP/l (cf. with 5 mgP/l in the calcium poor reactor) and concluded that although the presence of Ca\(^{2+}\) in the feed improved the sludge retention of the system, the Ca\(^{2+}\) concentration should be limited within 5-10 mgCa\(^{2+}\)/l — higher concentrations might limit the concentration of dissolved phosphate (nutrient) due to the formation of Ca\(_3\)(PO\(_4\))\(_2\).

In contrast to the observations above several investigators (de Zeeuw and Lettinga, 1980; Hulshoff Pol et al., 1982; Cail and Barford, 1985; and Mahoney et al., 1987) concluded that high concentrations, in the range 40-150 mgCa\(^{2+}\)/l, appeared to stimulate pelletization and increase settleability. However Hulshoff Pol et al. (1982) did point out that yet higher Ca\(^{2+}\) concentration (> 500 mgCa\(^{2+}\)/l) resulted in the formation of CaCO\(_3\) and Ca\(_3\)(PO\(_4\))\(_2\) precipitates — formation of a large number of CaCO\(_3\) crystals acted as a nuclei for bacterial attachment which, they concluded, stimulate a more dispersed growth and consequently an increased washout of the sludge.

Hulshoff Pol et al. (1983) showed that although Ca\(^{2+}\) ions improved sludge settleability and specific activity, the granulation process proceeded irrespective of high concentrations of Ca\(^{2+}\) (450 mg/l) or low concentrations of Ca\(^{2+}\) (150 mg/l). At both Ca\(^{2+}\) concentrations, granules contained increased sarcina- and coc-types. Cail and Barford (1985) reported a similar behaviour: In a reactor fed with 150 mgCa\(^{2+}\)/l more sarcina cells were selectively retained than in reactor with no Ca\(^{2+}\). but in the
latter a more diverse microbial population, consisting of cysts and macro-cysts, was present.

Schröder and de Haast (1987) using deproteinated cheese whey (11000 mgCOD/l) as substrate observed that in a UASB system fed with (1) 360 mgCa\(^{2+}\)/l, flocculation occurred with no definite pelletization, with the flocs consisting mainly of filamentous organisms, (2) 720 mgCa\(^{2+}\)/l, excellent pellet formation occurred with pellets consisting mainly of cocci and short rods. They concluded that calcium concentration enhanced pellet formation and influenced microbial composition of sludge particles.

Guiot et al. (1988) operated a UASB system fed with sucrose (5000 mg sucrose/l) as substrate and Ca\(^{2+}\) concentration of 80 mg/l. They found that Ca\(^{2+}\) has no significant effect on granulation at low substrate loading rate (less than 0.5 gCOD/gVSS/d) but at higher loading (> 0.5 gCOD/gVSS/d) pelletization proceeded rapidly. To explain the possible role of Ca\(^{2+}\) ions in enhancing pelletization they proposed that since Gram-positive and gram-negative bacteria have negatively charged surfaces (carboxyl and phosphate groups) bivalent cations such as Ca\(^{2+}\) could bridge negatively charged groups on the bacterial surfaces. However they found no evidence that Ca\(^{2+}\) can induce granulation of freely suspended and poorly active sludge.

Grotenhuis et al. (1988) observed that with propionate as substrate, with a high concentration of calcium, granules formed and these showed a steady increase in Ca\(^{2+}\) content — chemical analysis indicated that calcium was present mainly as precipitates of calcium phosphates. On addition of the Ca\(^{2+}\)-chelant, EGTA, in the ratio EGTA/Ca\(^{2+}\) = 0.9 about 70 per cent of the calcium was extracted from the granules and disintegration of the granules was observed. In contrast pellets from full scale reactors treating a variety of waste waters (from papermill to carbohydrate type wastes) when dosed with EGTA, the pellets remained intact even though 50 per cent of the calcium was removed, however, pellet strength decreased by up to 91 per cent. To explain this difference in behaviour, Grotenhuis et al. suggested that since calcium may be a constituent of extracellular polysaccharides and/or proteins which are present as sticking materials in granular sludge (see extracellular polymer section), and since for a sugar containing waste water, up to 2 per cent of the carbohydrates was extracellular material (compared to only 0.1 to 0.3 per cent for the propionate-grown granules), removal of calcium from the sticking materials in
the carbohydrate-grown pellets only leads to a weakening of the pellet structure, but removal of inorganic calcium phosphate precipitates from propionate derived granules would cause a disintegration of the granules.

Conclusion
Amid the confusion of conflicting evidence, perhaps the following conclusion on the effect of calcium on pelletization (or granulation) is reasonable:

Biological pellet formation appears to be principally dependent on the substrate feed. The presence of calcium may enhance the stability of the pellets that are formed. If the substrate cannot induce pellet formation calcium might assist, by precipitation, in some form of granulation but this form of aggregation might not sort strictly under biological pellet formation per se.

6. BIOCHEMISTRY:
Fermentation of a carbohydrate, lipid or protein substrate to methane gas, takes place in four stages involving three groups of organisms: solubilization and acidogenesis (by acidogenic organisms), acetogenesis (by acetogenic organisms) and methanogenesis (by methanogenic organisms) (McInerney, Bryant and Stafford, 1979). Product formation pathways for various substrates are set out in outline in Fig 2.1 and for glucose, a carbohydrate, in greater detail in Fig 2.2 (a, b and c).

Stage 1: Solubilization
In this stage complex long chain macromolecules such as carbohydrates, lipids and proteins are solubilized extracellularly by acidogenic organisms to short-chain compounds, sugars, 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 short chain fatty acids (SCFA) (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 SCFA produced) will depend primarily on the type of substrate and the hydrogen partial pressure (pH₂). For example, fatty acids usually are fermented via the fatty acid spiral either to acetic acid and hydrogen under low pH₂, or, to butyric and propionic acids under high pH₂, see Fig 2.1. Sugars usually are fermented via the Embden-Meyerhof pathway to SCFA (such
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, $\text{H}_2$ and $\text{CO}_2$. 

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<td>Methanogenic (c)</td>
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**Fig 2.1:** 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, $\text{H}_2$ and $\text{CO}_2$. 

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**Acetogenes (3):**

Short chain fatty acids + $\text{H}_2$ (propionic & butyric acids) 

Acetic acid + $\text{H}_2$ + $\text{CO}_2$ 

$\text{CH}_4$ + $\text{CO}_2$ 

$\text{CH}_4$
Acidogenic phase of glucose fermentation under low and high $H_2$ partial pressures to form acetic acid, butyric acid, propionic acid, $H_2$ gas and $CO_2$.

Abbreviations: EMP - Embden Myerhof pathway; PYR - pyruvic acid; AcCoA - acetyl coenzyme A; NAD$^+$ - nicotiamide adenine dinucleotide (oxidized form); NADH - nicotiamide adenine dinucleotide (reduced form); ButyrlCoA - butyl coenzyme A; HAc - acetic acid; HBr - butyric acid; HPr - propionic acid.
as acetate, butyrate and propionate), hydrogen and carbon dioxide. Cohen et al. (1979), for example, showed that, during acid fermentation of glucose, the fermentation products consisted of acetate, propionate, butyrate, hydrogen and CO₂, and that these represented 96 per cent of the soluble products. The relative fractions of the various SCFA however are dependent upon the pH in the medium, see below. At low pH, glucose is fermented to acetic acid, butyric acid, hydrogen and carbon dioxide and at high pH, glucose is fermented to acetic acid, propionic acid, butyric acid, hydrogen and carbon dioxide.

The detailed fermentation pathways for glucose under low and high pH, are shown in Fig 2.2(a, b and c). Under both low and high pH glucose is fermented first to pyruvic acid and intracellular generated electrons (attached to the electron accepting coenzyme NAD⁺, forming NADH) via the Embden-Meyerhof Pathway (EMP), see Fig 2.2; thereafter the pathways differ depending on the electron sink utilized to dehydrogenate the NADH: under low pH, only intracellular protons (H⁺) act as the terminal electron acceptor (i.e. H⁺ is the electron sink), under high pH, pyruvic, acetyl-CoA and protons act as electron acceptors. The sequences whereby the electron transfer takes place in the respective acidogenic stages are described below:

**Sequence 1:** (Embden-Meyerhof pathway, both high and low pH)
Glucose is fermented to pyruvic acid and hydrogen via the Embden-Meyerhof pathway (EMP). The hydrogen is attached to the electron-carrying co-enzyme, NAD⁺, and 2 moles ATP per mole glucose are conserved by the organisms, i.e.

\[
C₆H₁₂O₆ \text{(glucose)} + 2 \text{NAD}⁺ + 2 \text{ADP} + 2 \text{P} \rightarrow 2 \text{CH₃COCOOH (pyruvic acid)} + 2 \text{NADH} + 2 \text{ATP} \quad (2.1)
\]

That is, one mole of glucose generates 2 moles of pyruvic acid. Sequence 1 is common to both low and high pH; Fig 2.2(a, b and c).

**Sequence 2:** (Dehydrogenation)
The NADH formed in sequence 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). Dehydrogenation can take place in one of three ways depending on pH:

**Low $\dot{\text{pH}}_2$:**

Under low $\dot{\text{pH}}_2$ ($\dot{\text{pH}}_2$ of less than $10^{-4}$ atm, calculated from data of Lehninger, 1972) (see Fig 2.2a), oxidation of NADH is a downhill reaction i.e. NADH is oxidized spontaneously to NAD$^+$ and hydrogen gas, i.e.

$$2 \text{NADH} \rightarrow 2 \text{NAD}^+ + 2 \text{H}_2 \quad (2.2)$$

The NAD$^+$ thus regenerated acts as electron acceptor for further oxidation of glucose to pyruvic acid via the EMP (sequence 1). The two moles pyruvic acid generated in sequence 1, Eq (2.1) are oxidized further to acetyl CoA and carbon dioxide (Fig 2.2a):

$$2 \text{CH}_3\text{COCOOH} + 2 \text{NAD}^+ \rightarrow 2 \text{CH}_3\text{CoA} + 2 \text{CO}_2 + 2 \text{NADH} \quad (2.3)$$

The 2 moles NADH formed from this step again are dehydrogenated spontaneously to form hydrogen gas, as in Eq (2.2); the two moles of acetyl-CoA are converted to 2 moles acetic acid with concomitant generation of 2 moles ATP, i.e.

$$2 \text{CH}_3\text{CoA} + 2 \text{ADP} + 2\text{P} \rightarrow 2 \text{CH}_3\text{COOH} + 2 \text{ATP} \quad (2.4)$$

Hence under low $\dot{\text{pH}}_2$ the overall fermentation of 1 mole glucose is:

$$\text{C}_6\text{H}_{12}\text{O}_6 + 2 \text{H}_2\text{O} + 4 \text{ADP} + 4 \text{P} \rightarrow 2 \text{CH}_3\text{COCOOH} + 2 \text{CO}_2 + 4 \text{H}_2 + 4 \text{ATP} \quad (2.5)$$

The overall reaction depicted by Eq (2.5) is only slightly downhill; a small change in $\dot{\text{pH}}_2$, for example, will cause the reaction to become thermodynamically unfavourable. Noting from Eq (2.5) that $\text{H}_2$ is released, the rate of $\text{H}_2$ removal must be at least as fast as $\text{H}_2$ generation in order to maintain the low $\dot{\text{pH}}_2$ condition required for acetate generation only. In the production of $\text{H}_2$ if the $\dot{\text{pH}}_2$ increases only slightly the reaction could become thermodynamically unfeasible until the $\text{H}_2$ is removed i.e. $\dot{\text{pH}}_2$ declines again. It would seem that in intermediate state the acidogens utilize an alternative pathway (Fig 2.2b) in which butyric acid is produced. Experimental investigations have shown that during acidogenic fermentation of glucose under low $\dot{\text{pH}}_2$ conditions acetate was not the only SCFA generated, butyrate also is generated (Thauer et al., 1977; Jones and Woods, 1986):
In the fermentation of 1 mole glucose to acetate as the only SCFA, hydrogen and carbon dioxide, 4 moles ATP are generated; this implies an 85 per cent energy conversion approximately (Thauer et al., 1977). This high percentage energy conversion implies that the reaction (glucose to acetate) is only slightly downhill (≈ -2 Kcal/mol glucose oxidized). The conversion of glucose to butyric acid is energetically strongly downhill at the expense that only 3 moles ATP is produced per mol of glucose oxidized (cf 4 moles ATP in acetic acid production) and only 2 moles H$_2$ is produced (cf 4 moles H$_2$ in acetic acid production). The possible overall pathway for butyric acid production is shown in Fig 2.2b. The overall reaction of butyric acid formation is as follows:

$$C_6H_{12}O_6 + 3\text{ ADP} + 3\text{ P} \rightarrow CH_3CH_2CH_2COOH + 2\text{ CO}_2 + 2\text{ H}_2 + 3\text{ ATP}$$  \hspace{1cm} (2.6)$$

In summary the classification of butyrate formation in a low pH$_2$ system arises from the experimental difficulty of effecting glucose oxidation to acetate only (with generation of H$_2$ and CO$_2$) in pure culture system in the absence of H$_2$ utilizing bacteria to maintain a low pH$_2$.

**High pH$_2$:**

Under high pH$_2$ (see Fig 2.2c), the forward reaction in Eq (2.2) is no longer thermodynamically feasible. Consequently an alternative method for oxidizing the NADH generated in the EMP is needed. This is effected in two ways, which may take place simultaneously or alternatively (by the same or different groups of acidogens):

Alternative 1 – The two moles of acetyl-CoA is reduced to butyric acid, see Fig 2.2b, (Wolin 1974, Thauer et al., 1977)

$$2\text{ Ac-CoA} + 2\text{ NADH} + \text{ ADP} + \text{ P} \rightarrow \text{ CH}_3\text{CH}_2\text{CH}_2\text{COOH}$$

$$+ 2\text{ NAD}^+ + 2\text{ H}_2 + \text{ ATP}$$  \hspace{1cm} (2.7)$$

This reaction is thermodynamically favourable but at the cost of producing a less oxidized SCFA and only one ATP (cf. 2 ATP produced when the 2 moles acetyl-CoA is converted to acetic acid under low pH$_2$, Eq (2.4).

Alternative 2 – One of the 2 moles of pyruvic acid, Eq (2.1), is reduced to propionic
acid so as to oxidize the NADH produced from EMP, (Wood, 1982), see Fig 2.2c:

$$\text{CH}_3\text{COCOOH} + 2\text{NADH} + \text{ADP} + \text{P} \rightarrow \text{CH}_3\text{CH}_2\text{COOH} + 2\text{NAD}^+ + \text{ATP} + \text{H}_2\text{O} \quad (2.8)$$

The remaining mole of pyruvic acid is oxidized to acetyl-CoA as in Eq (2.4), i.e.

$$\text{CH}_3\text{COCOOH} + \text{NAD}^+ \rightarrow \text{CH}_3\text{CoA} + \text{CO}_2 + \text{NADH} \quad (2.9)$$

The NADH cannot be spontaneously oxidized to NAD$^+$ as the reaction is not thermodynamically favourable at high pH. However, the organism can achieve dehydrogenation of the NADH by coupling this reaction with the thermodynamically favourable reaction in which acetyl-CoA is converted to acetic acid, but at the cost that no ATP is generated [cf the low pH reaction where 2 moles ATP are generated, Eq (2.4)]. The coupled reaction is as follows:

$$\text{CH}_3 - \text{CoA} + \text{NADH} \rightarrow \text{CH}_3\text{COOH} + \text{H}_2 + \text{NAD}^+ \quad (2.10)$$

Hence for alternative 2 under high pH, the overall fermentation of glucose becomes:

$$\text{C}_6\text{H}_{12}\text{O}_6 + 3\text{ADP} + 3\text{P} \rightarrow \text{CH}_3\text{CH}_2\text{COOH} + \text{CH}_3\text{COOH} + \text{CO}_2 + \text{H}_2 + 3\text{ATP} \quad (2.11)$$
in which only 3 ATP are produced.

**Stage 3: Acetogenesis from short chain fatty acids**

Acetogenic organisms have an important intermediate role between acidogenesis and methanogenesis. Methanogenic organisms use as substrate source, formic acid, acetic acid (by cleavage), hydrogen, methanol and methylamines to form methane; however short chain fatty acids with more than 2 carbon atoms (i.e. > C2) (such as propionic and butyric acids) cannot be fermented directly to methane (McInerney *et al.*, 1979). However, hydrogen-producing acetogenic bacteria are capable of converting short chain fatty acids longer than C2, to acetic acid, carbon dioxide and hydrogen gas, *provided the hydrogen partial pressure is low*, below $10^{-2.7}$ atm and $10^{-4.1}$ atm for the degradation of butyric and propionic acids respectively (McInerney *et al.*, 1979).

Propionic acid is oxidized as follows (for pH < $10^{-4.1}$ atm):

$$\text{C}_3\text{H}_6\text{O}_3 + 3\text{ADP} + 3\text{P} \rightarrow \text{CH}_3\text{CH}_2\text{COOH} + \text{CO}_2 + \text{H}_2 + 3\text{ATP}$$

is used.
2.25

\[ CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3 H_2 \]  
(2.12)

and butyric acid is oxidized as follows for \( pH_2 < 10^{-2.7} \) atm:

\[ CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2 CH_3COOH + 2 H_2 \]  
(2.13)

Under high \( pH_2 \) (i.e. \( > 10^{-2.7} \) atm) the forward reaction of both Eqs (2.12) and (2.13) is thermodynamically unfavourable so that propionic and butyric acids remain unaltered in the system.

**Stage 4: Methanogenesis**

For a carbohydrate type substrate the two main sources for methane production are:

1. Hydrogen oxidation and
2. Acetate cleavage. Methane can also be formed from formic acid, methanol and methylamines by specific groups of methanogens. However their production tends to be relatively insignificant in the fermentation of carbohydrates and hence will not form part of the discussion.

The methanogens can be classified into three groups according to their energy source:

1. **Hydrogenogrophs** (\( H_2 \)-utilizing methanogens): These methanogens can utilize hydrogen only as their energy source. Examples of mesophilic hydrogenotrophs are *Methanobrevibacter* sp. and *Methanobacterium* sp.

2. **Acetoclastic methanogens**: These methanogens can utilize acetate only as their energy source, an example of mesophilic acetoclastic methanogen is *Methanothrix* sp.

3. **Hydrogen/acetate utilizing methanogens**: These methanogens can utilize both acetate and hydrogen as energy source; for example *Methanosarcina* sp.

Methane formation takes place according to the following reactions:

For acetate,
\[ \text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^- \]  \hspace{1cm} (2.14)

For hydrogen,

\[ 4 \text{H}_2 + \text{HCO}_3^- \rightarrow \text{CH}_4 + 3 \text{H}_2\text{O} \] \hspace{1cm} (2.15)

Variations in \( \hat{\text{p}}\text{H}_2 \) do not appear to affect either the hydrogenotrophs or the acetoclastic methanogens. However for the \( \text{H}_2/\text{acetate} \) utilizing methanogens, in particular \textit{Methanosarcina} sp, there are reports that high \( \hat{\text{p}}\text{H}_2 \) results in the catabolic repression of acetate cleavage. For example Smith and Mah (1978) observed that \textit{Methanosarcina} strain 227 utilizes hydrogen (with \( \text{CO}_2 \) as carbon source) preferentially over acetate in mixtures of these substrates resulting in the inhibition of acetate cleavage; when hydrogen concentration was reduced to a low level by utilization, acetate cleavage took place. In other \textit{Methanosarcina} sp., such as \textit{Methanosarcina barkeri}, rapid inhibition of acetate cleavage also occurred in the presence of \( \text{H}_2/\text{CO}_2 \) (Baresi \textit{et al.}, 1978 and Ferguson and Mah, 1983).

7. **ROLE OF HYDROGEN IN ANAEROBIC FERMENTATION**

Profiles on anaerobic filter treating a protein-carbohydrate substrate produced by Young and McCarty (1967) all exhibited sharp increases in SCFA in the lower section of the filter, followed by rapid declines in the upper section of the filter. They did not identify the individual SCFA species. The rise in SCFA concentration was associated with a decline of the \( \hat{\text{p}}\text{H} \) and the fall in SCFA with a recovery of the \( \text{pH} \). In terms of the biochemistry discussed in the previous section the accumulation of SCFA can be attributed to a rise in the \( \hat{\text{p}}\text{H}_2 \) because most of the influent \text{COD} was removed in the lower section and a protein-carbohydrate substrate releases hydrogen during fermentation. A high \( \hat{\text{p}}\text{H}_2 \) we have seen, favours the generation of SCFA (such as propionic and butyric acids) other than acetic acid. The decline in SCFA concentration can be ascribed to the oxidation of these SCFA to acetate by acetogens and the rapid conversion of the acetate to methane by the acetoclastic methanogens. The fact that SCFA declined implies that the \( \hat{\text{p}}\text{H}_2 \) had declined below \( 10^{-4.1} \) atm because conversion of propionate and butyrate can take place only under low \( \hat{\text{p}}\text{H}_2 \).

The behaviour above occurred at the bottom of the reactor over a relatively short distance so that hydrogen generation and utilization must have taken place within this region. In this region Young and McCarty noted the presence of sludge in
granular form in the voids of the packing material. This leads one to suggest that perhaps the aggregation of sludge into granules in the lower region of the UASB system arises from some advantage to be gained by the different species of organisms involved in anaerobic fermentation. Various studies in the literature support this conclusion:

(1) Based on reported mean residence time for hydrogen in a mature digester (i.e. a digester where acetate and propionate concentrations are between $10^{-4}$ and $10^{-3}$ moles/l with $\bar{p}H_2 < 10^{-4}$ atm), Gujer and Zehnder (1983) calculated that on average the sites of $H_2$ generation and uptake are less than 76 µm apart and concluded that $H_2$ producing organism and $H_2$ utilizing organisms must form a close association. McCarty and Smith (1986) calculated that, for a system to which a load of $10$ kgCOD/m$^3$/d is applied, if all $H_2$ generated are removed, then the distance between $H_2$ producing and $H_2$ utilizing organism is estimated to be about 11 µm. If the load is increased to $40$ kgCOD/m$^3$/d then the distance would have to be about 5 µm for removal of all $H_2$ generated. They concluded that a process design that encourages different species to live in proximity to one another, were more favourable for high rates of organic conversion. This is supported by the observation that in conventional anaerobic systems (i.e. completely mixed systems) the maximum loadings achieved are $< 5$ kgCOD/m$^3$/d for maximum process efficiency whereas in UASB systems the maximum loadings can be 40 kgCOD/m$^3$/d or even higher. This is so because in completely mixed system the organism population is dispersed uniformly in the system and the $\bar{p}H_2$ is distributed uniformly whereas in UASB system (plug flow reactor) the organism population is packed closely in the granules and hydrogen transfer between $H_2$ producing and $H_2$ utilizing organisms is effected more rapidly.

(2) Tomei et al. (1985) observed that in an enrichment culture from a river sediment, inoculated into a complex medium containing pyruvate, yeast extract and casamino acids, bacteria formed clumps and they noted the presence of large amounts of extracellular polymer. They identified a butyrate degrading spore former in juxtaposition with a methanobrevibacter-type fluorescent rod. From this observation they hypothesized that the formation of clumps is an important evolutionary development enhancing inter species $H_2$ transfer. Tomei et al. also observed that the $H_2$-utilizer Methanospirillum sp. grew as free organisms in the
surrounding liquid whereas *Methanobacterium* sp. and *Methanobrevibacter* sp. tended to grow in clumps; from this they argued that if the pH$_2$ in the clumps were higher than in the surrounding liquid (as postulated by Boone, 1984) then growth within the clumps would favour bacteria with lower affinity for hydrogen but higher maximum growth rates than those with higher affinity but lower growth rates. They concluded that formation of clumps may be species specific.

(3) Thiele *et al.* (1988) showed conclusively that organisms living in flocs are more efficient than those living as free flora for anaerobic fermentation. With lactose as substrate they found that:

(i) Lactose was metabolized rapidly by both flocs and free flora into mainly ethanol and acetate, but with flocs lower levels of butyrate and propionate were present than with free flora.

(ii) Free flora showed a much higher and transient H$_2$ production (> 2 x 10$^{-2}$ atm) whereas flocs produced lower, and more stable, H$_2$ levels (< 9 x 10$^{-3}$ atm).

(iii) Flocs were more active at conversion of ethanol to acetate and methane than free flora.

(iv) Both total and specific ethanol oxidation activity and hydrogen consumption activity was very much higher in the floc than in the free flora — flocs displayed 87 per cent of total H$_2$ consuming methanogenic activity and 76 per cent of total ethanol-consuming activity.

From above observations Thiele *et al.* concluded that flocs functioned to compartmentalize preferentially both syntrophic ethanol metabolism and methanogenic hydrogen consumption within a digester ecosystem.

From the work reviewed above it would appear that in flocs or granules syntrophicity is mainly between H$_2$-producing acetogens and H$_2$-utilizing methanogens. The reason for this would be that the acetogens can derive their energy for growth from degradation of SCFA with carbon chains $>$ C$_2$ (e.g.
propionate and butyrate) only if pH$_2$ is sufficiently low for the reaction to be thermodynamically feasible. This hypothesis finds support in the observations of Dubourguier et al. (1988); they identified microcolonies of *syntrophobacter* (propionate degrading acetogens) and *Methanobrevibacter* sp. in granular sludge. However aggregation may be beneficial also to the acidogenic organisms: Teixiera de Mattos et al. (1984a, 1984b) found that the growth of *Klebsiella aerogenes* decreased and the rate of glucose consumption reduced when the glucose concentration increased to above 20 g/L. However when they sparged the culture with nitrogen gas, the residual glucose level decreased with a concomitant increase in cell yield. They concluded that metabolic gases (hydrogen and/or carbon dioxide), in particular carbon dioxide was the cause of the growth inhibition. A similar behaviour was observed by Crabbendam et al. (1985) with growth of *Clostridium butyricum* on glucose. In the pellet both H$_2$ and CO$_2$ was removed by the H$_2$ utilizing methanogens and in this fashion the reduced partial pressures (or concentrations) would also benefit the acidogens.
CHAPTER 3

FEASIBILITY STUDY

1. INTRODUCTION

The Upflow Anaerobic Sludge Bed (UASB) system has found application in the treatment of medium to high strength industrial and agricultural waste waters. All these systems to date have been operated at temperatures in the optimum range between 30 to 35°C. With one exception a feature of these systems has been the development of a highly settleable pelletized sludge. The systems producing pelletized sludge all treated carbohydrate type waste waters. The exception was a UASB system treating olive oil wastes; this system did not produce a pelletized bed but instead a good settling sludge bed of smooth consistency was obtained.

The waste water from apple juicing factory contains a high percentage of fructose and it was decided accordingly to investigate whether treatment of apple juicing waste water in a UASB system was feasible. Furthermore, because control of the system at the apple juicing factory was accepted to be inadequate it was decided to investigate whether it was feasible to operate the system at temperatures lower than the optimum range of 30 to 35°C. The lower temperature selected was 25°C. The effluent from the plant was about 30°C; with temperature losses it was estimated that the treatment plant was likely to operate at about 25°C.

The feasibility study on the UASB system treating apple juice waste devolved into the following tasks, to:

1. Evaluate the performance of the system at 25°C and 30°C.

2. Ascertain if acceptable organic loadings can be achieved at 25°C.

3. Investigate the interaction of waste COD concentration, flow rate and hydraulic retention time in the low to medium influent concentration range (1000 - 5000 mg/l).

4. Ascertain if pellet formation would take place in (2) and (3) above.
2. EXPERIMENTAL SET-UP
Two identical laboratory-scale UASB reactors were set up: The reactors are constructed from transparent perspex cylinders (Figs 3.1 and 3.2). The inside diameter of the reactor is 100 mm and the height 1200 mm, giving a total effective reactor volume of 9.0 litres. The bottom of the reactor is flat with four inlet ports spaced around the circumference discharging in a horizontal direction. At the top of the reactor is the gas/liquid/solid separator. Gas collection is by means of a hollow inverted cone; rising gas bubbles are deflected into the cone by a collar around the inside wall of the reactor below the cone. The gas passes from the cone, along a gas line, through a liquid trap to prevent carry over of liquid in the gas line. The volume of the effluent gas is measured by a wet gas meter (model No. DM3A, Alexander Wright, London). Effluent discharge is via an annular space between the gas collection cone and the reactor wall to enter a small solid/liquid separator (volume approximately was 500 ml). Clarified liquid flows over a launder to the collection vessel while solids, which settle out, are returned into the reactor by gravity. Eleven sampling ports are evenly spaced along the length of the reactor. Temperature in the reactors is maintained at 25 or 30° C (± 0.2° C) by an on/off control of a heating tape wrapped around the length of the reactor.

The feed for the reactor was made up each day to the required concentration and pumped to the reactor from a refrigerated vessel at 4°C, using a variable speed peristaltic pump. The feed was distributed through the four inlet ports at the bottom of the reactor wall to provide an even flow in the reactor and minimize channeling.

During the start-up period when the influent flow rate was very low, to positively prevent channeling and improve feed distribution, an axially-mounted metal plate slow stirrer (7 revs/min) was positioned 100 mm above the reactor base to mix the lower section of the reactor contents.

3. WASTE WATER CHARACTERISTICS
The influent feed stock was of two types:

(1) Apple juice concentrate; and
(2) Batches of the apple processing waste water from the factory.

The characteristics of these wastes are listed in Table 3.1.
Fig 3.1: Schematic diagram of the laboratory-scale UASB reactor showing the numbered sampling ports.

Fig 3.2: Photograph of the two laboratory-scale UASB reactors.
### Table 3.1: Apple waste water characteristics

<table>
<thead>
<tr>
<th></th>
<th>COD (mg/ℓ)</th>
<th>TKN (mgN/ℓ)</th>
<th>P (mgP/ℓ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple concentrate source</td>
<td>699700</td>
<td>4340</td>
<td>670</td>
</tr>
<tr>
<td>Apple waste water</td>
<td>1500—4000</td>
<td>92—116</td>
<td>11—27</td>
</tr>
</tbody>
</table>

### Table 3.2: Trace element and nutrient solutions.

<table>
<thead>
<tr>
<th>Trace element solution</th>
<th>g/ℓ</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_3\text{BO}_3$</td>
<td>0,05</td>
</tr>
<tr>
<td>$\text{FeCl}_2\cdot2\text{H}_2\text{O}$</td>
<td>2,00</td>
</tr>
<tr>
<td>$\text{ZnCl}_2$</td>
<td>0,05</td>
</tr>
<tr>
<td>$\text{MnSO}_4$</td>
<td>0,5</td>
</tr>
<tr>
<td>$\text{CuCl}_2\cdot2\text{H}_2\text{O}$</td>
<td>0,03</td>
</tr>
<tr>
<td>$\left(\text{NH}_4\right)_6\text{Mo}<em>7\text{O}</em>{24}\cdot4\text{H}_2\text{O}$</td>
<td>0,05</td>
</tr>
<tr>
<td>$\text{AlCl}_3\cdot6\text{H}_2\text{O}$</td>
<td>0,05</td>
</tr>
<tr>
<td>$\text{CoCl}_2\cdot6\text{H}_2\text{O}$</td>
<td>2,00</td>
</tr>
<tr>
<td>$\text{MnCl}_2$</td>
<td>0,25</td>
</tr>
<tr>
<td>$\text{MgCl}_2$</td>
<td>1,00</td>
</tr>
<tr>
<td>EDTA</td>
<td>0,05</td>
</tr>
<tr>
<td>KI</td>
<td>0,05</td>
</tr>
<tr>
<td>$\text{NiCl}_2\cdot6\text{H}_2\text{O}$</td>
<td>0,25</td>
</tr>
<tr>
<td>HCL (Conc.)</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient solution</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{NH}_4\text{Cl}$</td>
<td>5,00</td>
</tr>
<tr>
<td>$\text{K}_2\text{HPO}_4$</td>
<td>2,00</td>
</tr>
</tbody>
</table>
Based on filtration, the COD of the apple waste water and apple concentrate was about 99 per cent soluble. Both the waste water and the concentrate were deficient in the principal inorganic nutrients i.e. nitrogen and phosphorus. These were supplemented by chemical addition, by adding 50 mg NH$_4$Cl and 10 mg K$_2$HPO$_4$ per 1000 mgCOD to give an influent COD:N:P ratio of approximately 100:4:1. In addition to N and P, a trace metal solution as suggested by Zehnder and Wurhmann (1977) for enrichment cultures of methanogenic bacteria, was added to the feed. Detailed composition of this trace solution is given in Table 3.2.

The pH of the batches of waste water and the diluted apple concentrate used as substrate feed generally were in the region of pH 4.6; i.e. highly acidic with little buffering capacity. To prevent the pH in the process from falling below the levels that inhibit methanogenesis, alkalinity was added to the influent. Addition of 1.6 mg alkalinity as CaCO$_3$ per mgCOD caused the pH above the sludge bed in the reactor to stabilize around pH 7.

Initially the substrate utilized was "synthetic" in that it was made up by diluting the apple concentrate with tap water to the required influent concentration as dictated by the selected organic loading and hydraulic retention time. Once the laboratory units had been acclimatized using apple concentrate, apple juicing waste water from the plant was substituted.

The waste water was collected at the processing plant at Grabouw, Cape Province, in 50ℓ plastic drums, in batches of 600ℓ and stored at the laboratory at 4°C. The concentration of these batches fluctuate widely over the course of the production season, the COD ranging from 1500 to 4000 mgCOD/ℓ. In this study concentrations of 1250, 2500 and 5000 mgCOD/ℓ were selected to check the behaviour of the system under different concentrations and loadings, these were achieved either by dilution with tap water or supplementation with apple concentrate.

4. ANALYTICAL METHODS
Reactor performance was assessed by daily or occasional measurements of the following parameters:

1. COD
2. Gas production
3. pH
4. short chain fatty acids (SCFA)
5. Alkalinity
6. TKN
7. NH₃-N
8. P
9. Total Dissolved Solids (TDS)

Daily measurements were: COD, gas production, pH, SCFA and Alkalinity. Determinations of the COD were in accordance with "Standard Methods" (1985). Reactor pH was measured with a Radiometer type 80 pH meter. Gas production was measured by means of a wet gas meter (model No. DM3A, Alexander Wright, London). Short chain fatty acids were determined by gas chromatography using a Packard 418 equipped with a glass column (30' x 4 OD x 4mm ID glass) containing 60/80 Carbopack C/0,35 Carbowax 29 M/0,1% H₃PO₄ (Supelco, Inc). The column temperature was 120°C with a nitrogen carrier gas flow rate of 20 ml/min and a flame ionization detector. To prevent some peak tailing (ghosting), the column was acidified with a 1% solution of formic acid in deionized water (injecting 5 µl into the column 10 times) before use. For measurement of SCFA, the sample was filtered through 0,45µ and acidified with 3% H₃PO₄ such that the sample contained 0,3% H₃PO₄ (i.e. 1 ml 3% H₃PO₄ to 9 ml sample). SCFA measured were acetic, propionic, isobutyric, n-butyric, 2 methyl butyric, 3 methyl butyric and n-valeric. The SCFA were identified and quantified by comparison of retention times and peak heights with those of known standards.

Alkalinity measurement deserves special mention. The alkalinity measured was the H₂CO₃/NH₄⁺/H₂PO₄⁻/Ac⁻/Pr⁻ alkalinity, that is, it included alkalinity contribution of the mixed weak acid/base systems in addition to the carbonate system. The determination was done using the modified Gran titration for mixtures of weak acid/base systems as described by Loewenthal et al. (1989). TDS was measured to establish the ionic strength of the effluent, required in the alkalinity determination.

Occasional measurements were: TKN, NH₃-N and P. Each time the loading was increased these parameters were measured in the effluent to determine whether these nutrients were present in sufficient concentration in the reactors for biological growth. These tests were done according to Standard Methods (1985).
5. **START-UP**

Different seeding sludges were used to start up the two systems. For the unit to be operated at 30°C, sludge from a clarigester treating glucose/starch waste at 30°C was used. This clarigester had, on occasion, produced a sludge that showed evidence of pellet formation; the sludge from the clarigester was fine and smooth in appearance. For the unit at 25°C, the seeding sludge was obtained from the first anaerobic pond treating the waste flow from the apple juicing factory; this sludge was coarse and contained some sand grains. The mean temperature in the pond during the production season was approximately 25°C.

The two reactors were inoculated with 3l of the respective sludges, filling the reactors to a depth of about 400 mm. The sludges settled to form a well defined sludge bed which remained so even after the slow stirrer was switched on.

To acclimatize the seeding sludges diluted apple concentrate was fed initially to each of the units, at a COD concentration of 500 mg/l and a flow rate of 8 l/d (i.e. loading 0,4 kgCOD/m³ reactor volume/d). Thereafter the load was increased step by step by maintaining the feed rate constant at 8l/d and increasing the feed concentration, until an influent COD of 2500 mg/l had been attained (i.e. 2 kgCOD/m³/d). Throughout this period the slow stirrer was kept switched on. To check the necessity for the stirrers, on occasion these were switched off, but channeling developed for the reason that the gas production was so low that the sludge compacted at the bottom, and the compaction was uneven.

Subsequent to each change of loading, the COD removal efficiency and gas production were monitored. When COD removal reached 90 per cent, or higher, it was assumed that the organism response had stabilized and the next increment of loading was applied. On average the systems were fully stabilized to the load increment within one week.

When the loading of 2 kgCOD/m³/d (i.e. flow rate: 8l/d, COD concentration = 2500 mg/l) had been attained with the COD removal efficiency at 90 per cent or greater, it was accepted that the sludge biomasses were fully acclimatized to the waste; as at this highest loading the gas production had become appreciable and the stirrers were no longer necessary. The stirrers were removed from the reactors, thereby changing the systems from a semi-stirred process to a UASB process.
6. FEASIBILITY STUDY

6.1 Operation

Once the sludge masses, at 25 and 30°C, had adapted to the influent (as described previously) the feasibility study commenced. The average COD of the waste flow from the factory was estimated at approximately 2500 mg/l and accordingly this concentration was selected to test the system responses. The influent was made up from the actual apple waste water. The flow rate at the start of the study was 8 l/d (loading = 2.2 kgCOD/m³/d). The load was increased every 7 days by 1.5 kgCOD/m³/d i.e. on average a rate of load increase of 0.21 kgCOD/m³ every day, until the system showed signs of failure.

Figures 3.3 and 3.4 show a record of the COD loadings versus time for the 25°C and 30°C systems respectively over the period of the study.

In the early low loading stages the sludge masses in the reactors were in excess of the masses expected to be generated at steady state. The mass of sludge per se therefore was not of immediate concern but the changes in the nature and appearance of the sludges, in particular the possibility of sludge pelletization, was of importance. Accordingly the sludges were closely monitored visually. For each load the following parameters were measured:

1. In the influent: Unfiltered COD, SCFA and pH.
2. In the effluent: filtered COD, SCFA, pH and VSS concentration (as mgCOD/l).
3. Gas production.
4. Sludge characteristics.

6.2 System response

In Figs 3.5 and 3.6 the COD removal vs loading and in Figs 3.7 and 3.8 the biogas production versus loading are shown for the 25°C and 30°C systems respectively. SCFA concentrations in the effluent are not plotted as these were virtually non-detectable until the maximum loads were approached, see Table 3.3.

COD removal was calculated from the influent COD and filtered effluent COD. In
Fig 3.3: COD loadings versus time (temperature = 25°C).

Fig 3.4: COD loadings versus time (temperature = 30°C).
Fig 3.5: Effect of organic loadings on organic removal efficiency; fixed influent COD, variable flow rate (temperature = 25°C; influent COD = 2500 mg/l).

Fig 3.6: Effect of organic loadings on organic removal efficiency; fixed influent COD, variable flow rate (temperature = 30°C; influent COD = 2500 mg/l).
Fig 3.7: Biogas production versus organic loading (temperature = 25°C).

Fig 3.8: Biogas production versus organic loading (temperature = 30°C).
Table 3.3: Steady state operating results for UASB reactors at 25°C and 30°C with an influent COD concentration of 2500 mg/l.

<table>
<thead>
<tr>
<th>COD loading (kgCOD/m³/d)</th>
<th>Rem (%)</th>
<th>Rₜ (h)</th>
<th>25°C Reactor</th>
<th>30°C Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>COD (mg/l)</td>
<td>pH</td>
</tr>
<tr>
<td>1,4</td>
<td>89</td>
<td>24,0</td>
<td>126</td>
<td>6,82</td>
</tr>
<tr>
<td>2,0</td>
<td>93</td>
<td>20,0</td>
<td>126</td>
<td>6,78</td>
</tr>
<tr>
<td>2,5</td>
<td>94</td>
<td>18,8</td>
<td>121</td>
<td>6,82</td>
</tr>
<tr>
<td>5,0</td>
<td>96</td>
<td>12,6</td>
<td>101</td>
<td>7,12</td>
</tr>
<tr>
<td>6,0</td>
<td>96</td>
<td>8,9</td>
<td>78</td>
<td>7,18</td>
</tr>
<tr>
<td>7,5</td>
<td>91</td>
<td>9,0</td>
<td>176</td>
<td>7,22</td>
</tr>
<tr>
<td>10,0</td>
<td>86</td>
<td>6,4</td>
<td>349</td>
<td>7,18</td>
</tr>
<tr>
<td>12,0</td>
<td>76</td>
<td>5,5</td>
<td>654</td>
<td>7,15</td>
</tr>
<tr>
<td>12,5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13,5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16,0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rem: COD removal efficiency (%)
Rₜ: hydraulic retention time (hours)
COD: filtered effluent COD concentration (mg/l)
HAc: effluent acetic acid concentration (mg/l)
HPr: effluent propionic acid concentration (mg/l)
nd: non-detectable (< 5 mg/l)
pH: pH measured from the settler.
order to have a "standard" reference, organic loadings were calculated in terms of the mass of substrate COD fed per day per unit total reactor volume (kgCOD/m³/d); these values would be conservative as the sludge beds occupied only one third to half the reactor volumes. A summary of the COD removal efficiencies, effluent filtered COD's, SCFA concentrations and pH, is given in Table 3.3.

6.3 Short chain fatty acids (SCFA) concentrations
Of the SCFA, when present in the effluent, only acetic and propionic acids were detected at the organic loadings applied. Under stable conditions, at temperatures of 25°C and 30°C, the concentrations of acetate and propionate essentially were non-detectable (< 5 mg/l) above the sludge bed for loadings of less than 7.5 and 12.5 kgCOD/m³/d respectively. However as the loading was increased further (and the hydraulic time decreased) for the fixed influent concentration, so the SCFA concentrations increased, Table 3.3.

6.4 Failure
From the results in Table 3.3 and the plots in Figs 3.5 and 3.6 it is apparent that the systems commenced to show signs of failure at loadings of approximately 10 and 15 kgCOD/m³/d, i.e. 30 and 45 kgCOD/m³ sludge bed volume/d, (COD : 2500 mg/l, flow rates 36 l/d and 60 l/d) at 25 and 30°C respectively. These results conform to the pattern of COD removal versus loadings observed in many studies on anaerobic digestion; that is, at low loadings the COD removal is high and remains so until above some maximum loading there is a rapid fall-off and failure of the system is approached. The presence of propionate in the effluent, at the loadings close to maximum applied, serves as a good indicator that the systems were becoming stressed and hence tending towards instability.

6.5 Biogas production
Total gas productions were measured and adjusted to standard temperature and pressure (STP). Biogas production versus time, in the 25°C and 30°C systems, are shown in Figs 3.9 and 3.10 respectively. With gas production a feature observed was the immediate response of the biogas production with increase or decrease in the

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4In this study the short chain fatty acids systems are referred to either as the SCFA system or as the salt of the SCFA, in both no quantitative description is implied as the relative concentration of acid and salt formed would imply pH specification. For example the propionic acid system and propionate system are used interchangeably so that the statements propionic acid concentration and propionate concentration both refer to the sum of the propionic acid and its dissociated salt species.
Fig 3.9: Biogas production versus time (temperature = 25°C).

Fig 3.10: Biogas production versus time (temperature = 30°C).
organic loadings. This is illustrated in Fig 3.10, at day 47: a sudden decrease in loading from 2.3 to 1.6 kgCOD/m³/d caused biogas production to drop immediately from 9 to 7 litres per day. When a day later the loading was increased to 3.7 kgCOD/m³/d, the gas production increased to 11.9 l/d. The reactor at 25°C showed similar behaviour.

Figures 3.7 and 3.8 show the biogas production vs COD loadings for the 25°C and 30°C systems respectively. The gas yields per kgCOD applied for the 25°C and 30°C systems were 0.36 and 0.41 m³/kgCOD applied respectively. These were fairly constant throughout the study.

Although the volumetric gas measurements are important for assessment of system response to biological conversion of the organic load, the gas quality is a better indication of the state of the system and also provides an indication of the energy content of the biogas. However, no facilities were available at that time to determine the CO₂/CH₄ ratio.

6.6 Biomass changes
The sludge in the reactor at 30°C initially was very fine, whereas in the reactor at 25°C initially the sludge was coarse with some sand present in it. As the organic loadings were increased, the sludge beds expanded and channeling and gas hold-up decreased. At organic loadings of approximately 3 kgCOD/m³/d, a higher fraction of the sludge, in the form of sludge debris, in both reactors was discharged from the beds to suspended sludge blankets above the beds and in time the sludge blankets built up to the gas/liquid separator. These observations applied over the first ten weeks of operation when the loadings were below 5 kgCOD/m³/d. However, over this period a gradual change in the sludge consistencies, from very fine to a more ‘grainy’ flocculated form took place. After this period there was a rapid change in the sludge morphology and rudimentary pellets became visible. At about 5 kgCOD/m³/d pelletization of the sludge became prominent in both UASB systems. (Flow rate approximately 18 l/d).

The process of pelletization, once started, was very rapid. Over a period of six to seven days roughly half the sludge bed was transformed into pellets, eventually pellets occupied a volume of about three litres of reactor, equal to the initial volume of sludge present at the start-up of the reactors. Flocculant sludge was still present in the upper portion of the sludge bed but it had good settling properties.
In the 30°C system the first pellets that formed ranged in size from approximately 0.5 to 2 mm in diameter to fine particles with different settling properties. In the 25°C system the pellets varied in size from 0.5 to 4 mm; the core of the pellets consisted of sand grains which were present in the sludge during start-up — it would appear that the sand grains acted as nuclei and enhanced the granulation process.

The organic loading appeared to influence the size of the pellets; as the loading increased, the pellet's size also increased. After eight months' operation (at loadings of about 28 kgCOD/m³/d) some of the pellets at the base of the reactors at 30°C had grown to 5 mm in diameter, the size of the pellets decreasing gradually with reactor bed height. The pellets were roughly spherical with well-defined surfaces. Almost no fine sludge particles were observed in the spaces between the pellets in the sludge bed. The pellets showed no propensity to attach to each other. The pellets were greyish-white in colour with a soft spongy consistency and broke up readily if handled; however, even with intense gasification the pellets did not appear to break up at depth in the beds; break up appeared to be confined to the upper layers of the beds.

The volatile suspended solids (VSS) and total suspended solids (TSS) for the pellets in the reactors at 25°C and 30°C were 31.74 gVSS/l and 44.44 gTSS/l, and 36 gVSS/l and 40 gTSS/l respectively. For the 25°C system the VSS/TSS was 0.71; this low value must be attributed to the presence of sand grains in the pellets. For the 30°C system in which the seeding sludge did not contain sand particles, the VSS/TSS ratio was 0.90.

6.7 Effluent solids concentration
The concentration of VSS in the effluent (expressed as mgCOD/l) were obtained from the difference between unfiltered and filtered effluent COD values. The VSS in the effluent stemmed principally from the fine debris particles in the sludge blanket above the sludge bed but some fine pellets also were buoyed into the sludge blanket. In this experimental study the magnitude of the effluent VSS concentration has little practical relevance because the solids/liquid separator was undersized compared with a separator in a full-scale plant. However it is worth noting the trends in the effluent VSS of Table 3.4. For both the 25°C and 30°C systems the effluent VSS concentrations showed approximately the same increase with decreasing hydraulic retention time for both the 25°C and 30°C systems. The increase was due principally to two factors: (1) With decreasing retention time (and increasing upflow
Table 3.4: Effect of Hydraulic Retention Time (HRT) on Effluent Solids Concentration (VSS measured as mgCOD/l) with an Influent COD of 2500 mg/l (difference between unfiltered and filtered effluent COD).

<table>
<thead>
<tr>
<th>HRT (d)</th>
<th>Loading rate (kgCOD/m³/d)</th>
<th>Effluent solids concentration (mgCOD/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25°C</td>
</tr>
<tr>
<td>24</td>
<td>2,5</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>5,0</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>7,5</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>10,5</td>
<td>113</td>
</tr>
</tbody>
</table>

Table 3.5: Response of 30°C system to a shock load.

<table>
<thead>
<tr>
<th>Days after shock</th>
<th>Loadings (kgCOD/m³/d)</th>
<th>COD removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20 to 0</td>
<td>~ 8</td>
<td>~ 93</td>
</tr>
<tr>
<td>0</td>
<td>14,5</td>
<td>86,7</td>
</tr>
<tr>
<td>1</td>
<td>9,7</td>
<td>86,1</td>
</tr>
<tr>
<td>2</td>
<td>11,4</td>
<td>83,1</td>
</tr>
<tr>
<td>3</td>
<td>11,5</td>
<td>82,0</td>
</tr>
<tr>
<td>4</td>
<td>10,3</td>
<td>84,9</td>
</tr>
<tr>
<td>5</td>
<td>9,2</td>
<td>88,4</td>
</tr>
<tr>
<td>6</td>
<td>9,2</td>
<td>88,3</td>
</tr>
</tbody>
</table>
velocity) a larger quantity of fine particles were "pushed" out of the reactor, (2) the decrease in retention time was accompanied by an increase in load, increased gas production and a larger degree of turbulence.

6.8 Sensitivity to shock loads
A general impression from the study is that the UASB system is reasonably robust to shock loadings. The data in Table 3.5 for the 30°C system illustrate the ability of the system to accommodate shock loadings. Prior to the shock the reactor had been loaded at a rate of 8 kgCOD/m³/d for approximately three weeks. A shock loading was then applied, almost doubling the load to 14.5 kgCOD/m³/d for 24 hours, by increasing the COD concentration but keeping the flow rate constant. The load was then reduced to approximately 10 kgCOD/m³/d. From Table 3.5 the reduction in COD removal efficiency caused by the shock load was almost negligible.

6.9 Temperature sensitivity
The comparative treatment capacity at 25°C and 30°C appears to be in accord with the reported temperature sensitivity of mesophilic anaerobic processes. Henze and Harremoës (1983), in reviewing data on the temperature dependency, found that in the range 10°C to 30°C the relationship between the process rate, $r$, at temperatures $T_1$ and $T_2$ may be expressed as:

$$\frac{r_{T_1}}{r_{T_2}} = e^{K(T_1-T_2)} \quad (3.1)$$

where $K = 0.10$/degC.

For the 25°C and 30°C systems,

$$\frac{r_{25}}{r_{30}} = r_{30} \cdot e^{0.1(25-30)}$$

$$= 0.61 \cdot r_{30}$$

If the maximum loading capacity is accepted as being proportional to the rate $r$ then the capacity at 25°C should be 61 per cent of that at 30°C. The experimental maximum capacity at 25°C was approximately $(10/15) = 67$ per cent of that at 30°C.
7. **INFLUENT COD CONCENTRATION/FLOW INTERACTION**

The feasibility study had accepted that the mean influent COD concentration as the average concentration expected at the plant i.e. 2500 mgCOD/l with the incremental load of 1.5 kgCOD/m^3/d applied every week the maximum loadings the UASB system at 25°C and 30°C were determined. It was now important to establish if the maximum loading is influenced by the influent COD concentration. As no background information was available on this aspect it was decided to approach the problem in an incremental way. In the first experiment increasing the influent concentration from 2500 to 5000 mg/l was to be tested. For comparative purposes the volume of the sludge bed was maintained at 3l, as before. Increase in COD load was to be in increments, commencing at 2500 mgCOD/l. To start the experiment in both the 25°C and 30°C systems the flow rates were reduced to half the maximum values, from 36 to 18 l/d (25°C) and 60 to 30 l/d (30°C) respectively. Thus with the initial influent COD concentration at 2500 mg/l, the load was reduced to half the maximum loading achieved previously. The loading was now increased in steps by increasing the COD concentration by 500 mgCOD/ℓ every 9 days and keeping the flow rate constant, hence, approximately 5 steps were required to increase the COD concentration from 2500 to 5000 mg/l, on average giving over the period a load increase per day of 0.22 kgCOD/m^3, approximately the same as before. When the concentration reached 5000 mg/l the loadings on the systems were again the same as the maximum with influent COD's of 2500 mg/l. The COD removal vs loadings of the systems are shown in Figs 3.11 and 3.12 for the 25°C and 30°C respectively. The SCFA concentrations at the various loadings are given in Table 3.6. Comparing COD removal plots and the effluent SCFA in Table 3.6 with the corresponding ones obtained with a constant COD concentration of 2500 mg/l, the responses are closely similar for each temperature up to the maximum loadings of 10 and 15 kgCOD/m^3/d at 25°C and 30°C respectively. At the maximum loadings the systems again showed signs of failure. It would seem that the influent COD concentration did not influence the maximum loadings.

To check the conclusion above, the performance of the 30°C UASB system only was tested with the influent COD concentration reduced to 1250 mg/l. The initial flow rate was retained at 60l/d giving a starting load of half the maximum attained previously. The organic loading was then increased in steps by increasing the influent flow rate (10l every 4 days) to give a load increment of 1.38 kgCOD/m^3/d every 6 days or over the period an average increase every day of 0.23 kgCOD/m^3. The COD removal versus loadings is shown in Fig 3.13 and the steady state
Fig 3.11: Effect of organic loadings on organic removal efficiency; fixed influent flow rate; variable influent COD (temperature = 25°C; influent COD = 2500-5000 mg/l).

Fig 3.12: Effect of organic loadings on organic removal efficiency; fixed influent flow rate; variable influent COD (temperature = 30°C; influent COD = 2500-5000 mg/l).
Table 3.6: Steady state operating results for UASB reactors at 25°C and 30°C with influent COD concentrations of 2500-5000 mg/l.

<table>
<thead>
<tr>
<th>Loading kgCOD/m²/d</th>
<th>Rem %</th>
<th>COD mg/l</th>
<th>pH</th>
<th>HAc mgHAc/l</th>
<th>HPr mgHPr/l</th>
<th>Rem %</th>
<th>COD mg/l</th>
<th>pH</th>
<th>HAc mgHAc/l</th>
<th>HPr mgHPr/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>90.5</td>
<td>283</td>
<td>7.22</td>
<td>93</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.2</td>
<td>84.0</td>
<td>569</td>
<td>7.20</td>
<td>177</td>
<td>84</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.8</td>
<td>68.0</td>
<td>1318</td>
<td>7.16</td>
<td>106</td>
<td>216</td>
<td>91.1</td>
<td>257</td>
<td>7.28</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>9.4</td>
<td>60.0</td>
<td>1830</td>
<td>7.18</td>
<td>372</td>
<td>196</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.7</td>
<td>51.7</td>
<td>2416</td>
<td>7.19</td>
<td>489</td>
<td>236</td>
<td>92.7</td>
<td>261</td>
<td>7.23</td>
<td>43</td>
<td>19</td>
</tr>
<tr>
<td>12.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>93.5</td>
<td>260</td>
<td>7.30</td>
<td>36</td>
<td>63</td>
</tr>
<tr>
<td>14.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>90.7</td>
<td>426</td>
<td>7.18</td>
<td>164</td>
<td>63</td>
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<tr>
<td>15.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>64.3</td>
<td>1778</td>
<td>7.28</td>
<td>131</td>
<td>558</td>
</tr>
</tbody>
</table>

Rem : COD removal efficiency  
COD : filtered effluent COD concentration  
HAc : Effluent acetic acid concentration  
HPr : Effluent propionic acid concentration  
pH : pH measured from the settler
Fig 3.13: Effect of organic loadings on organic removal efficiency; fixed influent COD; variable flow rate (temperature = 30°C; influent COD = 1250 mg/l).

Table 3.7: Steady state operating results for UASB reactors at 30°C with influent COD concentration of 1250 mg/l.

<table>
<thead>
<tr>
<th>Loading kgCOD/m³/d</th>
<th>Rem %</th>
<th>COD mg/l</th>
<th>pH</th>
<th>HAc mgHAc/l</th>
<th>HPr mgHPr/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.8</td>
<td>88.6</td>
<td>165</td>
<td>7.36</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>13.0</td>
<td>88.4</td>
<td>154</td>
<td>7.35</td>
<td>20</td>
<td>11</td>
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<tr>
<td>16.0</td>
<td>89.7</td>
<td>144</td>
<td>7.35</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>21.7</td>
<td>90.9</td>
<td>112</td>
<td>7.25</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>23.6</td>
<td>84.5</td>
<td>191</td>
<td>7.42</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>26.5</td>
<td>81.8</td>
<td>227</td>
<td>7.39</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>30.6</td>
<td>77.7</td>
<td>278</td>
<td>7.32</td>
<td>70</td>
<td>50</td>
</tr>
</tbody>
</table>

Rem : COD removal efficiency
COD : filtered effluent COD concentration
HAc : Effluent acetic acid concentration
HPr : Effluent propionic acid concentration
pH : pH measured from the settler
operating results are shown in Table 3.7. Up to a loading of 22 kgCOD/m³/d the COD removal was 90 per cent. The maximum loading applied was 30.6 kgCOD/m³/d (flow rate: 220 l/d) but the COD removal declined to about 78 per cent. The system was kept in operation at the maximum loading for three weeks and it maintained a stable state over this period. Thus with an influent COD of 1250 mg/l the maximum loading on the system had increased to about twice that attained at influent COD’s of 2500 and 5000 mg/l. Furthermore at a COD concentration of 1250 mg/l the COD removal was virtually independent of the loading up to 22 kgCOD/m³/d and also of the hydraulic time (HRT) for HRT as low as 1 hr (see Fig 3.14).

The disproportionately higher maximum loading at 1250 mgCOD/l influent, compared to those attained at 2500 and 5000 mgCOD/l, raised the question as to whether the maximum load perhaps was influenced by the rate of load increase. To check this the loading was reduced to approximately half the maximum (from 29 to 15 kgCOD/m³/d) by reducing the flow rate from 220 to 60 l/d and increasing the COD concentration from 1250 to 2500 mg/l. The sludge bed volume was still at 3l. The loading of 15 kgCOD/m³/d was now approximately the same as the maximum loading previously attained at 2500 mgCOD/l influent. It was now observed that at this loading the system showed no sign of failure. The loading was increased at an incremental loading of 0.5 kgCOD/m³/d every 4 days (0.125 kgCOD/m³ every day on average) compared to the previous incremental load increase of 0.21 kgCOD/m³ every day on average at influent COD of 2500 mg/l. With this reduced rate of incremental loading, over a period of 3 months, stability was maintained even though the maximum loading applied had increased to 28 kgCOD/m³/d. (Flow rate: 92 l/d, HRT: 2.3 h). At this maximum loading the COD removal remained at 95 per cent, with no sign of failure.

The experiment was repeated at influent COD concentration of 5000 mg/l. The loading was increased from 13.5 kgCOD/m³/d at increments of 0.5 kgCOD/m³/d every 3 days (0.167 kgCOD/m³ every day on average). At this rate the load on the system was increased to 28 kgCOD/m³/d over a period of about four months. Again at this maximum loading the removal was maintained at about 95 per cent, the system operated in a stable state with no sign of failure.

From the two experiments above it would appear that provided the rate of increase in load is kept sufficiently low, the same high loading, with stable operation, can be
Fig 3.14: Effect of hydraulic retention time (HRT) on organic removal efficiency; (temperature = 30°C; influent COD = 1250 mg/l).
achieved irrespective of the influent concentration. It would appear, therefore, that provided the increase in loading is applied at a sufficiently slow rate, the maximum loading and the efficiency of COD removal at any loading below the maximum, is not influenced by either the influent COD concentration or the flow rate (or equivalently the hydraulic retention time).

8. CONCLUSIONS
The following specific conclusions may be itemized from the results obtained in the this feasibility study:

1. Apple juicing waste water is amenable to treatment by the UASB system at temperatures less than optimal (25°C and 30°C).

2. The COD of the waste water, based on filtration, was 99 per cent soluble with short chain fatty acids (acetic and propionic acids) comprising approximately 5 per cent of the COD influent. The remainder is made up principally of sugars.

3. The waste water is deficient in the elements nitrogen and phosphorus. These elements must be supplemented through chemical addition in order to achieve complete anaerobic treatment.

4. The waste water is acidic with little buffering capacity. The pH is in the range 4.5 to 5.5. Alkalinity addition, to increase the buffer capacity, must be added to the waste prior to treatment. Addition of 1.6 mg alkalinity as CaCO₃ per mgCOD influent was sufficient to maintain the pH in the UASB reactor above the sludge bed close to 7.

5. The maximum loadings attainable is dependent on temperature. Under the same condition of loading increments (average 0.21 kgCOD/m³ every day) the maximum loadings at 30°C and 25°C were 15 and 10 kgCOD/m³/d respectively (i.e. 45 and 30 kgCOD/m³ sludge bed volume/d respectively), i.e. they were in the ratio of 1.5:1.

6. At 30°C the maximum loading attainable was shown to be dependent on the rate of increase of the load. If the loading is increased by too large increments or too short time increments the system will fail at loadings well below the maximum possible.
7. If the incremental load increase is sufficiently low (average 0.125 to 0.167 kgCOD/m³ every day) then for loading up to the maximum applied (28 kgCOD/m³/d) the COD removal appeared to be virtually independent of both concentration and hydraulic retention time. At maximum loading of 28 kgCOD/m³/d, at influent COD of 2500 to 5000 mg/l, the COD removal was above 90 per cent. At influent COD of 1250 mg/l, the COD removal was 90 per cent up to loading of 22 kgCOD/m³/d. At COD loadings below the maximum the COD removal was independent of loading.

8. Pelletization of the sludge occurred with the apple juicing waste water and was observed in both reactors at 25°C and 30°C. The size of the pellets ranged from 5 mm at the bottom of the bed to 0.5 mm at the top; as the loading increased the pellet sizes also increased.
CHAPTER 4

PRODUCT FORMATION

1. INTRODUCTION

Chapter Three reported on the study to determine whether apple juicing waste could be treated in a UASB reactor. The study in effect followed the procedures normally applied with completely mixed systems, by monitoring only the influent COD, TKN, pH and alkalinity, the reactor VSS and the effluent COD, VSS, short chain fatty acids (SCFA), alkalinity and pH. Alkalinity was added to the influent to raise the pH of the feed from 5.5 to about 7.5 thereby assuming that the effluent pH would be about 7 and presuming that the pH in the reactor also would be in the neutral range.

Visual observations of the pelletized bed indicated that there was very little intermixing between the upper and lower layers of the bed, that is, the movement of the fluid through the reactor very likely followed a plug flow or near plug flow mixing regime. If this was so then the possibility existed for partial separation of the dominant phases in anaerobic fermentation, principally acidogenesis in the lower zone of the sludge bed, with acetogenesis and methanogenesis in the upper zone of the bed. Such a phase separation could be traced by product formation such as SCFA, pH changes, along the line of flow.

By studying the product formation it was thought that a more detailed picture would emerge of the bed processes and their interactions. There is a precedent for this in past work, by considering a completely mixed anaerobic system operating under steady state and then subjected to a step increase in loading:

In a completely mixed anaerobic system operating at steady state under some selected sludge age, a normal feature is that the hydrogen partial pressure is maintained at a low level and the pH near neutrality. Acidogenesis converts the substrate, for example a carbohydrate, to mainly acetic acid, hydrogen and carbon dioxide; butyric acid also can be produced in small concentration. Methanogenesis produces methane from acetic acid and hydrogen. By utilizing the hydrogen, the methanogenic organisms are principally responsible for keeping the hydrogen partial pressure at a low level. Because the acetic acid is virtually completely converted to methane, the overall acidity and alkalinity changes are
small, and the pH will tend to remain stable.

When there is a step change increase in the loading on the system above, then a higher food/microorganism ratio or equivalently a lower sludge age is imposed. At this higher loading, if the hydrogen and acetate utilizing methanogens convert the hydrogen and acetate only partially, the hydrogen partial pressure and acetate concentration will increase. This can have two effects: (1) The pH will tend to decline, the magnitude of the decline depending on the excess mass of acids generated and the buffering capacity of the system, and (2) the hydrogen partial pressure ($\text{pH}_2$) will increase; if this increase is sufficiently high ($\text{pH}_2 > 10^{-4}$ atm) a shift in product formation takes place, from principally acetate (with butyrate in minor concentration) to acetate and propionate as the principal acids (and butyrate as a minor acid).

If the pH remains near neutrality both the hydrogen and acetate utilizing methanogens will continue to produce methane. However propionate and butyrate cannot be utilized by the methanogens, hence methane production is reduced and the effluent now contains propionate and butyrate. Consequently even though the load has increased, the rate of sludge production may not do likewise. In any event recovery to a low $\text{pH}_2$ state is likely to be slow; recovery will be speeded by not wasting sludge for a period until the sludge mass has built up to that consistent with the steady state sludge age previously operative but now under the higher loading. However it bears repeating that, provided the pH is maintained near neutrality the system can operate in a quasi-stable state, but at a reduced efficiency.

The pH may decline with a step increase in loading; this would happen if the buffering capacity of the system is insufficient to counter the effects of increased propionic, butyric and acetic acid concentrations. With decline of the pH to below about pH 6.6, the methanogenic organisms are increasingly inhibited, causing even higher concentrations of acetic, propionic and butyric acids, thereby further tending to depress the pH. Eventually the methanogenic reactions stop completely and the process fails.

From the description above the imposition of a step increase in loading to a completely mixed system gives rise to a transient state during which the relative magnitudes of products formed change with time subsequent to the step change until
a new steady state is established. An analogous transient state in space is introduced by having a plug flow regime even though the state at any level in the bed may remain steady in time. In the literature there appears to have been no enquiry into the existence of such a space transient in the sludge bed of a UASB system—only end products from the system have been widely reported.

Accordingly it was decided to:

(1) Investigate product formation and pH changes along the line of flow in a UASB system treating the apple juice waste water.

(2) Determine whether the UASB system behaves as a plug flow system in which there is a measure of separation of the fermentation processes.

(3) Identify the various biochemical processes taking place in the bed from the product formation.

2. MATERIALS AND METHODS

In this experiment the laboratory scale UASB reactor, described in Chapter 3, was used.

The last experiment, described in Chapter 3, dealt with the determination of the maximum stable loading using the apple juice waste water as substrate. The maximum loading imposed was: influent concentration 2500 mg/l, flow rate 92 l/d (i.e. loading 28 kgCOD/m³ reactor/d). Under this loading the effluent quality indicated a 95 per cent COD removal with virtually no short chain fatty acids (SCFA) and a pH around 7.2—the system operated efficiently and was in a stable state.

In this experiment the same loading was retained but the substrate was changed from apple juice waste water to diluted apple concentrate. The amount of alkalinity added to the substrate feed was 3.2 mg alkalinity as CaCO₃ per mgCOD. Trace element solution (3 ml), and NH₃-N and P nutrient solution (50 ml) were added per litre of feed. Detailed composition of these solutions are given in Table 3.2, Chapter 3. Operating details are summarized in Table 4.1.

At the start of the experiment the volume of pelletized sludge was three litres. The
### Table 4.1: Operating data for UASB process with apple concentrate as influent.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent COD concentration</td>
<td>2 600 mgCOD/l</td>
</tr>
<tr>
<td>Volumetric flow rate</td>
<td>92 l/d</td>
</tr>
<tr>
<td>Nominal velocity of flow in reactor</td>
<td>0.81 cm/min</td>
</tr>
<tr>
<td>Hydraulic retention time</td>
<td>2.35 h</td>
</tr>
<tr>
<td>Organic loading</td>
<td>26.6 kgCOD/m³ reactor/day</td>
</tr>
<tr>
<td>Operating temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Influent total alkalinity</td>
<td>8 387 mg/l as CaCO₃</td>
</tr>
<tr>
<td>Influent pH</td>
<td>8.25</td>
</tr>
<tr>
<td>Free and saline ammonia (NH₃-N)</td>
<td>65 mgN/l</td>
</tr>
</tbody>
</table>
bed volume was allowed to build up to the 6l level and thereafter maintained at this level by wasting the excess volume of bed material each day.

The system was operated for about a month within which time the bed had increased to the set volume of 6l. Each day the soluble COD, TKN, NH$_3$-N and pH were measured on the influent and effluent. Effluent VSS and gas production were also measured daily. The effluent VSS was determined by the difference between the unfiltered and filtered COD. Daily wastage of the pelletized bed, to maintain a 6l volume, was analyzed for VSS, COD and TKN.

With the sludge volume at 6l, when the effluent parameters indicated steady state, samples were taken at fourteen different ports, eleven of these from ports along the length of the reactor, starting from the uppermost port; a further three samples taken from the influent and effluent buckets and from the settler. The sampling ports are shown in Fig 3.1, Chapter 3. The filtered samples were analyzed for:

1. COD
2. Free and saline ammonia
3. Total Kjeldahl nitrogen (hence organic nitrogen from the difference between TKN and NH$_3$-N).
4. The H$_2$CO$_3$/NH$_4^+$/$H_2$PO$_4^-$/$Ac^{-}$/Pr$^-$ alkalinity as described in Chapter 3.
5. Short chain fatty acids, acetate, propionate, butyrate etc. as described in Chapter 3.
6. pH.

Samples from the eleven sampling ports down the reactor were taken by means of a special device that allowed the pH to be measured without loss of dissolved gases. The sampling device is shown schematically in Fig 4.1. After pH had been measured the sample was decanted carefully leaving the pelletized sludge. When all the samples had been decanted the pelletized sludge was returned to the reactor. Each of the decanted samples was filtered through Whatman's No.42 filter paper and tested for 'soluble' COD, NH$_3$-N and TKN. Part of the filtrate was filtered further
Fig 4.1: Sampling vessel.
through 0.45µm filter and analyzed for short chain fatty acids.

3. RESULTS
3.1 Reactor profiles

Profiles of the parameters soluble COD, propionate, acetate, NH$_3$-N, organic nitrogen, alkalinity and pH measured along the line of flow in the reactor are shown in Figs 4.2(a, b and c). From Fig 4.2 the profile indicates 3 zones of behaviour designated as follows:

(1) a lower active zone
(2) an upper active zone, and
(3) 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 ammonia 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. Approximately the bed volumes of the three zones were respectively 2, 3 and 1 £.

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;
• alkalinity decreases to a minimum, to 8 181 mg/l as CaCO$_3$;
• pH declines from 8.10 to 7.07.

In the upper active zone:

• propionic acid concentration decreases to a minimum, to zero mg/l;
Concentration and pH profiles observed in single UASB system with apple concentrate as influent (COD concentration = 2600 mg/l, flow rate = 92 l/d).

Fig. 4.2:
• 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 to 20 mg/l;
• alkalinity increases to a stable value of 8317 mg/l as CaCO$_3$;
• pH increases to a stable value of 7.38.

In the lower active zone, the profile of increasing propionic acid concentration implies that the hydrogen partial pressure (pH$_2$) is high (from the earlier description, a high hydrogen partial pressure gives rise to propionic and acetic acid generation). The increasing profile in the short chain fatty acid 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 6.6, a value which does not inhibit methanogenesis. The decreasing profile of the total soluble COD concentration (which includes the COD of short chain 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 pH$_2$ has declined to such a low value (pH$_2$ < 10$^{-4.1}$ atm) that propionic acid is being converted to acetic acid by acetogenesis. The reduction in pH$_2$ 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 increases 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 the present understanding of anaerobic fermentation. However, the generation of organic nitrogen in the high pH$_2$ region cannot be explained in terms of normal anaerobic fermentation biochemistry.

3.2 Pellet growth and decay:
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 6l, 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 2-5mm diameter, and smallest at the top, about 1mm 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 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.

3.3 Sludge age for the system:
Estimation of the sludge age of a UASB system is not as straight forward as for a completely mixed aerobic system. In the latter system, using the method of hydraulic control of sludge age, a fixed volume of mixed liquor is wasted every day, whereupon the sludge age ($R_s$) is defined as:

$$R_s = \frac{\text{volume of reactor}}{\text{volume wasted}/d} \quad (4.1)$$

This estimation presumes no VSS in the effluent. Usually in aerobic systems the loss of VSS in the effluent is so small that it can be neglected.

In a completely mixed anaerobic system a more acceptable estimation is:

$$R_s = \frac{\text{mass of sludge in reactor}}{\text{mass of sludge wasted}/d + \text{mass of sludge in effluent}/d} \quad (4.2)$$

In a plug flow anaerobic system the estimation given above is subject to one major difficulty: The density of the sludge bed increases with bed depth so that the mass of sludge usually is not known precisely except if the whole sludge bed is drained and
sampled.

In order to obtain a reliable estimate of \( R_s \) the whole bed was drained and sampled. The mean bed density was 37 000 mgVSS/\( \ell \).

A number of determinations were made on the mass of sludge wasted per day and the mass of fine particles in the effluent. The sludge age was calculated as follows:

\[
\begin{align*}
\text{Mass of sludge wasted} & = 250 \cdot \frac{37000}{1000} \\
& = 9250 \text{ mgVSS/d} \\
\text{Mass of fine particles in the effluent} & = 118.92 \\
& = 10856 \text{ mgVSS/d} \\
\text{i.e. Total mass of sludge wasted} & = 20106 \text{ mgVSS/d}.
\end{align*}
\]

Now the sludge age can be calculated from Eq (4.2):

\[
\begin{align*}
\text{i.e. } R_s & = \frac{37000 \cdot 6}{20106} \\
& = 11 \text{ days}.
\end{align*}
\]

3.4 **Volatile mass yield**

In the single reactor the net VSS production was 20 160 mgVSS/d. The mass of COD removed was:

\[
(2633-176) \cdot 92
\]

\[
= 226044 \text{ mgCOD/d}
\]

Hence the VSS yield

\[
\begin{align*}
& = \frac{\text{VSS produced/day}}{\text{mass of COD removed/d}} \\
& = \frac{20106}{226044} \\
& = 0.09 \frac{\text{mgVSS}}{\text{mg COD removed}}
\end{align*}
\]
This VSS yield value is close to the sludge yield value of 0.093 mgVSS/mgCOD removed reported by Hulshoff Pol et al. (1984) for a UASB system using mainly sucrose as substrate.

4. TWO-IN-SERIES REACTOR SYSTEM

In the previous section, the observations with regard to the sludge bed were, (1) the pellet layers do not mix, (2) the pellet sizes tend to decrease in an upward direction, (3) the bed grows to a stable maximum mass and (4) there is a loss of pellet fragments from the bed. These observations led to the conclusion 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. In order to test the conclusions made above on pellet growth and decay an experiment was set up as follows:

The lower active zone of high H$_2$ partial pressure (high pH$_2$) was separated from the upper active and inactive zones of low H$_2$ partial pressure by operating a two-in-series reactor system. A UASB reactor of the same diameter as the existing reactor, but having a volume of 3l, was placed upstream, and operated in series, with the existing UASB reactor. Pelletized sludge was withdrawn from the existing reactor up to the level defining the lower active zone and seeded into the new reactor, approximately 2l. In this fashion the 3l reactor should behave like a high pH$_2$ reactor. The influent feed entered this rector and the overflow was fed to the existing reactor via a peristaltic pump. The existing reactor now served as a low pH$_2$ reactor. The general layout of the two-in-series reactor system is shown in Fig 4.3. Using the same substrate (diluted apple concentrate), the system was operated under the same loading (i.e. COD concentration: 2600 mg/l; flow rate: 92 l/d) as that applied to the single UASB reactor. The same parameters were tested as before.

4.1 Behaviour of the high pH$_2$ reactor

The high pH$_2$ reactor immediately showed a rapid growth rate of pellet mass. To maintain a constant bed volume of 2l in this reactor, 700 ml of pelletized sludge had to be removed daily. The effluent contained virtually no fines (no VSS). Tests on the excess sludge mass and the whole sludge bed indicated that the bed density was uniform. Accordingly the sludge age could be formulated as follows:
Fig. 4.3: Schematic diagram of the laboratory-scale two-in-series UASB system.
4.14

\[ R_s = \frac{\text{Volume of sludge in reactor}}{\text{Volume of excess sludge formed/d}} \] (4.3)

\[ R_s = \frac{2,0}{0,7} = 2,9 \text{ days.} \]

The pellets generated were relatively uniform in size, about 2-3 mm in diameter.

The volatile specific mass yield in the high \( \text{pH}_2 \) zone can be calculated as follows:

\[ \text{Volatile specific yield} = \frac{(\text{Vol of sludge wasted})(\text{VSS of sludge})}{(\text{COD}_{\text{in}} - \text{COD}_{\text{out}}) \cdot \text{flow rate}} \]

\[ = \frac{0,7 \cdot 28860}{(2703 - 2175) \cdot 92} \]

\[ = 0,42 \text{ mgVSS/mgCOD removed.} \]

This specific yield value is about 5 times greater than that observed with the single UASB reactor.

4.2 Behaviour of the low \( \text{pH}_2 \) reactor

In the low \( \text{pH}_2 \) reactor (initial bed volume 4L), receiving the effluent from the high \( \text{pH}_2 \) reactor (but not the sludge generated in the high \( \text{pH}_2 \) reactor), the mean pellet size continuously decreased; after 3 weeks operation, the pellet size had decreased from the initial 1-2 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 from the low \( \text{pH}_2 \) reactor 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 \( \text{pH}_2 \) reactor, and the reduced turbulence due to the decrease in gas production, these two allowing a higher concentration to develop in the suspended blanket.
4.3 Two-in-series reactor concentration profiles

The concentration profiles for the two in-series reactor system are shown in Fig 4.4 (a, b and c). From Fig 4.4 in the first reactor (high pH_2 reactor):

- propionic and acetic acids increase to maximum values of 511 and 480 mg/ℓ respectively within the sludge bed;
- soluble COD reduces from 2703 to 2175 mg/ℓ only;
- free and saline ammonia (NH_3-N) concentration decreases to a minimum, to 23 mg/ℓ;
- organic nitrogen concentration increases to a maximum, to 28 mg/ℓ; and
- pH declines from 8.10 to 7.01.

In the second reactor (low pH_2 reactor):

- propionic and acetic acids decrease to minimum values of 22 and 30 mg/ℓ respectively;
- soluble COD reduces to about 240 mg/ℓ;
- free and saline ammonia (NH_3-N) concentration is reduced by only 4 mg/ℓ i.e. from 23 mg/ℓ to 19 mg/ℓ and thereafter remains constant;
- organic nitrogen concentration increases to a maximum from 13 to 41 mg/ℓ at the bottom of the reactor and then decreases to a stable value of 10 mg/ℓ; and
- pH increases to a stable value of 7.35.

The concentration profiles of the two-in-series reactor system show similar trends to those of the single reactor system except that in the two-in-series system a peak in the organic nitrogen is observed at the bottom of the low pH_2 reactor.

5. ALKALINITY

An aspect that will have important implications on the success of a UASB system is
**Fig 4.4:** Concentration and pH profiles observed in the two-in-series UASB reactor system with apple juice waste water (COD concentration ≈ 2600 mg/l, flow rate = 92 l/d).
the alkalinity requirement of the system. In a UASB system treating a carbohydrate-type substrate, phase separation into high and low H₂ partial pressures takes place. In the high pH₂ region there is a loss of alkalinity principally due to the generation of short chain fatty acids. In the single UASB reactor system studied here the influent alkalinity was 8387 mg/l as CaCO₃ (i.e. ALK/COD = 3,2 mgALK as CaCO₃/mgCOD influent). The total alkalinity declined to a minimum of 8181 mg/l at the point where the short chain fatty acids peaked, that is, there was a consumption of 206 mg/l as CaCO₃. However in the low pH₂ region virtually all the alkalinity lost was recovered to give an effluent alkalinity of 8317 mg/l as CaCO₃. The small net loss of alkalinity (70 mg/l as CaCO₃) was that due to organic nitrogen formation from the dissolved ammonia. Thus although there were significant changes in alkalinity in the bed, there was virtually no net consumption of alkalinity by the system between the influent and effluent. In this regard the UASB system has quite different alkalinity requirements from that of a completely mixed anaerobic system. In the latter system the alkalinity requirements are relatively small because there is no accumulation of SCFA, whereas in the former system the space transient generation of SCFA require appreciable alkalinity to counter pH changes in the high pH₂ zone.

A critical factor in maintaining stability in a UASB system will be the pH: In the high pH₂ zone, 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. The sole function off the alkalinity provision in the influent is to prevent undue pH decline in the high pH₂ zone.

Judging from the alkalinity requirements per influent COD, in this investigation, with strong wastes the alkalinity requirements will be high. To reduce the alkalinity requirement, consideration should be given to recycle the effluent to the influent. The effluent will contain alkalinity and by recycling the alkalinity/COD ratio of the influent will be increased. In full scale plants the alkalinity problem possibly would be ameliorated to some degree by intermixing effects due to the difficulty of ensuring
perfect plug flow conditions.

6. CONCLUSIONS
The following conclusions are drawn from the study:

(1) The single UASB system appears to operate as a plug flow system. For a carbohydrate-type waste three regions are identified within the sludge bed:

(i) A lower active zone: In this zone short chain fatty acids (acetic and propionic acids) concentrations rise to a maximum – the bed level at which the peaks occur defines the upper limit of this zone; free and saline ammonia (NH₃-N) concentration reduces to a minimum and soluble organic nitrogen concentration increases to a maximum; the soluble COD reduces to about half its influent value; alkalinity and pH decline to minimum values.

(ii) An upper active zone: In this zone the soluble COD concentration reduces to a minimum; the propionic and acetic acid concentrations decrease to near zero – the bed level at zero acids concentrations defines the upper limit of this zone; NH₃-N concentration remains near constant and soluble organic nitrogen decreases to a minimum; alkalinity and pH increases to stable values.

(iii) An upper inactive zone: This zone extends above the upper active zone to the top of the bed; no overt biokinetic reactions are observed except for pellet breakup, the debris being discharged as organic fines into the suspended blanket above the bed.

In the lower active zone the continuous increase in propionic acid concentration indicates that the rate of generation and the rate of utilization of hydrogen is such that a high pH₂ is maintained. Indeed the lower active zone can be said to terminate at the bed level where the pH₂ falls to such a low value that the acetogens can convert the propionic acid to acetic acid, hydrogen and carbon dioxide. This conversion, of the propionic to acetic acid, indeed is observed in the upper active zone providing supporting evidence that it is a low pH₂ zone. Throughout the two zones the methanogens convert the acetic acid and hydrogen plus carbon dioxide to
4.19

methane.

(2) In the single UASB system operating at a sludge age of 11 days the mass of COD removed was 226 000 mgCOD/d and the mass of VSS produced was 20 160 mgVSS/d, giving a specific yield of 0,09 mgVSS/mgCOD removed. This yield value is similar to that reported for a UASB system fed with sucrose as substrate (Hulshoff Pol et al., 1984). In the two-in-series UASB system, in the high pH2 reactor operating at a sludge age of 2,9 days, the mass of COD removed was 48 576 mgCOD/d and the mass of VSS produced was 20 202 mgVSS/d, giving a specific yield value of 0,42 mgVSS/mgCOD removed.

The VSS production in the high pH2 reactor was identical to that in the single UASB system under the same loading. This indicates, firstly, that all the VSS production took place in the high pH2 zone and, secondly that there appears to be little or no solubilization (i.e. no decay) of the VSS produced in the high pH2 zone. The VSS yield in the high pH2 zone (i.e. 0,42 mgVSS/mgCOD removed) is 14 times greater than normally reported for anaerobic fermentation processes (0,03 mgVSS/mgCOD removed).

(3) Pellet formation took place in the high pH2 zone with virtually no fines produced. Pellet break-up occurred in the low pH2 zone with substantial fines production. Pellet size in the high pH2 reactor was 2-3 mm in diameter whereas in the low pH2 reactor the pellet size decreased from an initial mean diameter of 1 to 2mm to less than 0,2 mm. Thus it would appear that the pelletized sludge bed volume, if allowed to build up, would stabilize at some maximum value and then the pellets would start breaking up and eventually discharge as fines in the effluent.

(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 short chain fatty acids principally in the high pH2 zone, the pH in this zone may drop significantly if the system is not buffered adequately. This could lead to failure of the system. Hence a sufficient buffering capacity of the reactor liquid contents is important to ensure that the pH does not decline at some point in the line of flow to less then pH 6,6.
CLOSURE
From the system behaviour a number of aspects were unusual and are summarized as follows:

(1) The net VSS yield per unit mass COD removed was exceptionally high in the high $pH_2$ zone of a UASB system.

(2) The growth of the pelletized sludge mass was confined to the high $pH_2$ zone.

(3) High removal of NH$_3$-N and a concomitant generation of organic nitrogen were observed in the high $pH_2$ zone, a situation not observed in normal anaerobic fermentation processes.
CHAPTER 5

HYPOTHESIZED MECHANISM OF PELLETIZATION

1. INTRODUCTION
Chapter Four concluded with a list of features not observed in normal anaerobic fermentation processes, briefly:

1. Pelletized sludge production was confined to the high pH zone of a UASB system;

2. VSS yield per COD removed in the high pH zone was about 14 times that normally observed;

3. There was a high NH$_3$-N removal in the high pH zone, with a concomitant generation of organic nitrogen.

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 rapid mass increase in organic nitrogen associated with rapid apparent growth, made death an unlikely cause, accordingly attention was focussed on the proposition that organic nitrogen generation was a product in the growth process. In this chapter an enquiry into this growth process is reported.

2. ORGANISM IDENTIFICATION
Zehnder and Wurhmann (1977) isolated a hydrogen utilizing methanoorganism, Methanobacterium strain AZ (M. Strain AZ), now classified as Methanobrevibacter arborophilus, from digested sewage sludge. Pure culture studies 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
5.2

exception of the sulphur containing amino acid, cysteine – an external cysteine source is necessary for growth;

- Provided all nutrients and cysteine are present the organism has a high specific growth rate;

- With a deficiency of cysteine during the growth phase, exceptionally high concentrations of amino acids are secreted to the surrounding medium;

- Growth is stimulated by yeast extract;

- The organism grows in rosette-type clusters.

The growth characteristics of the *M. Strain AZ* appears to provide a basis for an hypothesis on the formation of pelletized sludge in the UASB system:

When the *M. Strain AZ* is surrounded by excess substrate i.e. high H$_2$ partial pressure (high pH$_2$), 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 made in Chapter 4, on the lower active (high pH$_2$) zone, 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; and
5.3

(4) the effect of cysteine supplementation on volatile solids yield.

3. OBSERVATIONS

3.1 Ratios of COD/VSS and TKN/COD of pelletized sludge:

A number of measurements of the following parameters were made on the pelletized sludge: COD, VSS and TKN. Hence the COD/VSS and TKN/COD ratio of the sludge could be calculated. The COD/VSS values from a number of such measurements are shown plotted on normal probability paper, in Fig 5.1, giving a mean COD/VSS ratio of 1.23 with a standard deviation of the mean of ± 0.02. 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.

Micro-organisms utilizing 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 Wurmann (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. The theoretical COD/VSS ratio for a polypeptide type polymer consisting of these three amino acids, in the given molar ratios will be 1.21 mgCOD/mgVSS (and that for a polysaccharide type polymer will be unity (see Appendix A) and, for a 'standard' protoplasm composition the COD/VSS ratio is about 1.42 (McCarty, 1972). If the pelletized sludge consisted only of protoplasmic mass the COD/VSS ratio would be 1.42, but as the observed ratio is only 1.23 it must include one or both of the two types of polymer. Assuming the only polymer present is a polypeptide then in order to have a COD/VSS of 1.23 about 90 per cent of the pelletized sludge should be polypeptide and 10 per cent protoplasm. Assuming only a polysaccharide polymer is present then about 40 per cent of the sludge should be polysaccharide and 60 per cent protoplasm (see Appendix A). To determine which of the two types of polymer is generated, one needs to examine the TKN/COD ratio of the pelletized sludge.

The observed TKN/COD ratio obtained from a number of measurements is shown plotted on normal probability paper, Fig 5.2, giving a mean TKN/COD ratio of 0.090 with a standard deviation of the mean of ± 0.002. 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, would have a TKN/COD ratio 0.113 mgN/mgCOD (see
Fig 5.1: Statistical plot of COD/VSS ratio for pelletized sludge obtained from a number of measurements.
Fig 5.2: Statistical plot of TKN/COD ratio for pelletized sludge obtained from a number of measurements.
Appendix A); a polysaccharide would have a TKN/COD ratio of zero. For protoplasm the "standard composition" suggests a TKN/COD ratio of 0.086 mgN/mgCOD (McCarty, 1972). Applying these ratios to the mass proportions of polymer:biomass hypothesized in the pellets above (0.9:0.1 for the polypeptide:biomass and 0.4:0.6 for the polysaccharide:biomass) the calculated TKN/COD ratios for the pellet should be:

For the polypeptide:protoplasm, TKN/COD = 0.108 and, for the polysaccharide:protoplasm, TKN/COD = 0.034. Comparing these with the observed TKN/COD ratio of 0.09, it would appear that the most likely constitution of the pellets is a polypeptide:protoplasm combination in the proportion of approximately 0.9:0.1, that is protoplasm constitutes about 10 per cent of the pelletized sludge mass.

3.2 Free and saline ammonia (NH$_3$-N) disappearance and organic nitrogen generation:

Referring to the free and saline ammonia concentration profile (Fig 4.2, Chapter 4), in the high pH$_2$ zone the concentration decreased by 38 mgN/ℓ and the total soluble COD concentration decreased by 1427 mgCOD/ℓ. If this mass of free and saline ammonia were utilized 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 mgN/mgVSS. Accepting a biomass yield of 0.03 mgVSS/mgCOD removed (ten Brummeler et al., 1985), the COD associated with the utilization of 38 mgN/ℓ would be (38/0.122)/0.03 i.e. 10 383 mgCOD/ℓ. However the observed COD utilized was only 1427 mgCOD/ℓ, consequently the disappearance of the free and saline ammonia cannot be associated with protoplasmic mass generation only.

Referring to the organic nitrogen (orgN) concentration profile (Fig 4.2, Chapter 4), in the high pH$_2$ zone, there was a release of 49 (orgN)/ℓ to the surrounding medium. If one ascribes this generation of soluble orgN to death of the 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 observed behaviour however can be explained if it is accepted that the orgN is due to M. Strain AZ that secretes amino acids under high pH$_2$ when there is a deficiency in cysteine and an adequate supply of ammonia nitrogen.
3.3 **Volatile mass yield**

The volatile mass specific yield in the high $\mathrm{pH}_2$ reactor calculated in Chapter 4, was $0.42 \, \text{mgVSS/mgCOD}$ removed. This value is based on the measured total COD removed (i.e. COD influent – COD effluent) of 48 576 mg/d. However since part of the influent COD is converted into amino acids and reappears in the effluent COD, the total COD removed will be less. Using the data from Chapter 4 average volatile mass specific yield in the high $\mathrm{pH}_2$ zone can be calculated as follows:

Feed flow rate $= 92 \, \ell/d$

Mass influent COD (92·2703) $= 248 676 \, \text{mgCOD/d}$

Mass effluent COD (92·2175) $= 200 100 \, \text{mgCOD/d}$

Sludge concentration $= 28 860 \, \text{mgVSS/}\ell$

Sludge wastage per day $= 700 \, \text{ml/d}$

VSS generated (28,86.700) $= 20 202 \, \text{mgVSS/d}$

COD of free amino acids generated (see Appendix A) $= 7 087 \, \text{mgCOD/d}$

i.e. Total COD removed $= [248 676–(200 100–7 087)]$

$= 55 663 \, \text{mgCOD/d}$

Hence biomass yield $= \frac{\text{VSS generated}}{\text{Total COD removed}}$

$= \frac{20 202}{55 663}$

$= 0.36 \, \text{mgVSS/mgCOD removed}$.  

A specific yield 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. However the high yield value can be explained accepting the formation of polypeptide polymers, 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 in the molar proportions reported by Zehnder and Wuhrmann,
1977) 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 polypeptide [see (3.1) above], the yield will be \((0.9,0.36 + 0.1,0.03) = 0.33 \text{ mgVSS/mgCOD}\), that is, the observed yield is consistent with the calculated yield of 0.36 mgVSS/mgCOD based on the assumptions and deductions developed so far.

3.4 Effect of cysteine supplementation on volatile solids yield:

In terms of the 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 oxidized 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. From the above ATP requirement for protoplasm formation is about 12 times that for the same molar mass of polypeptide formation. Consequently a higher fraction of hydrogen uptake would need to be oxidized to methane to provide the energy required. Hence the sludge yield would decrease and the methane production increases.

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 5.3. 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 hours after cysteine addition was the same as that subsequently, indicating no transition effect. The methane production unfortunately could not be checked due to a breakdown of instrumentation at that time.

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:
Fig 5.3: Effect of cysteine addition on pelletized sludge production in the high \( \mathrm{pH}_2 \) reactor.
(i) 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.

(ii) 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.

4. CRITERIA FOR PELLET FORMATION

Accepting the hypothesis for pelletization, the ecological conditions under which pelletization 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;
- a near neutral pH.

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

- no pelletization in systems where the influent substrate does not yield hydrogen in the fermentation processes, e.g. acetate as sole substrate,
- no pelletization in systems where the influent substrate can be broken down only under low $\text{pH}_2$ conditions, e.g. propionate and lipids,
- no pelletization in systems where the substrate yields hydrogen but in order to obtain complete conversion, operation requires a low $\text{pH}_2$, e.g. carbohydrates and proteins in completely mixed reactors,
- no or limited pelletization in systems where the substrate can generate a high
\( \text{pH}_2 \) but the \( H_2 \) generated is preferentially utilized by other organisms such as sulphate reducers,

- pelletization in systems where the substrate yields hydrogen and the operation allows zones for high \( \text{pH}_2 \) build-up, e.g. carbohydrates and proteins in plug flow reactors.

5. DISCUSSION
There is considerable information in the literature that can serve as a testing material of the hypothesis on pellet formation and the consequential requirements necessary for its formation.

**Acetate as substrate source:**
With acetate as the sole substrate no hydrogen is released; without hydrogen the hydrogen-utilizing methanogenic organisms including the \( M. \) Strain \( AZ \), necessarily must be absent. No experiments appeared to have been reported where acetate is the sole carbon substrate source. However 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 \( \text{pH}_2 \); 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 poor 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.

**Oil processing wastes:**
In the fermentation of long chain fatty acids, hydrogen release can take place only under low \( \text{pH}_2 \). Hence with oily wastes a high ATP/ADP ratio is unlikely to develop, that is, one of the conditions for polypeptide polymer formation is absent. It is likely, therefore, that with vegetable oil as substrate, even if excess free and saline ammonia is present, polymer formation will not take place. Experimentally, pelletization has not been observed in a UASB system with olive oil processing wastes (Boari et al., 1984). A well settling dense sludge did form but was of uniform
smooth consistency.

**Completely mixed anaerobic systems:**
No pelletization has been reported in completely mixed reactor systems. In a 'well balanced' completely mixed system, the pH will be maintained near neutral but the pH\textsubscript{2} will remain low due to hydrogen utilization by the methanogenic organisms including \textit{M. Strain AZ}. With a low pH\textsubscript{2} 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 pH\textsubscript{2} 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 pH\textsubscript{2}, methane fermentation is reduced 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 pH\textsubscript{2} state, the necessary conditions for pellet formation will exist in completely mixed systems only over relatively short transition periods.

**Plug flow systems:**
Pelletization has been reported in UASB systems with carbohydrate substrates (Lettinga \textit{et al.}, Ross 1984, Wu \textit{et al.}, 1987; experience in this investigation supports this finding. In the UASB system which is essentially a plug flow system, there is a phase separation along the line of flow in the system, with the acidogenic phase being dominant at the bottom of the reactor. In the acidogenic phase of the fermentation of carbohydrates, hydrogen is readily produced under both low and high pH\textsubscript{2}. In the UASB system production of hydrogen takes place in the lower active zone; under high loading conditions if the H\textsubscript{2}-utilizing methanogens cannot utilize the hydrogen at the rate it is generated, a zone of high pH\textsubscript{2} will form. With excess free and saline ammonia present, polypeptide polymer formation should take place according to the proposed hypothesis.

Pelletization also has been reported in UASB systems with protein (gelatine) as substrate (Schulze \textit{et al.}, 1988). This is to be expected as some amino acids, during deamination, produce hydrogen and under plug flow conditions may allow a build up
of pH₂.

Only limited pelletization has been obtained with a UASB system treating a waste from a paper re-pulping plant (50 per cent carbohydrate, 50 per cent SCFA, with COD ~ 5000 mg/l) containing sulphate of about 300 mgSO₄²⁻/l (Russo, 1987). Since sulphate-reducing organisms have a higher affinity for hydrogen than the H₂-utilizing methanogens (Kristjansson et al., 1982; Kristjansson and Schönheit, 1983), the generated hydrogen will be utilized virtually immediately to reduce sulphate ions to sulphide at the base of the reactor, thereby tending to reduce the availability of hydrogen for pelletization; this behaviour is supported by the observation that as sulphate concentration decreased so the rate of pellet formation increased (Russo, 1987).

**Acidogens as pellet producers**

The biochemical/mechanistic model for pellet formation, developed in this investigation, is based on the hypothesis that an hydrogenotroph, *M. Strain AZ*, or another species with similar characteristics, is responsible. The pellet formation is reputed to arise from a particular characteristic of the organism — an inability to synthesize the amino acid cysteine: In the zone of high hydrogen partial pressure, the presence of this substrate (hydrogen) activates the synthesis processes but due to the organism's inability to synthesize cysteine a disequilibrium develops to the degree cysteine is in short supply. The organism accommodates this by discharging excess amino acids in soluble form or as polypeptides.

An objection that could be raised against this hypothesis is that in the high hydrogen partial pressure zone, where pelletization takes place, the activity of the acidogens is also at a maximum. Could it be that the acidogens are responsible for polypeptide/pellet formation? Two arguments can be advanced why acidogens are not likely to be responsible:

- It is commonly accepted that extracellular polymers are produced by organisms when some nutrient deficiency is present. In the case of the hydrogenotrophs, this deficiency is present in that cysteine needs to be supplied from the liquid — we have seen that trace supplementation of cysteine sharply reduces polymer and thus pellet production. In the case of acidogens, we could not find in the literature an acidogen that exhibits a deficiency in generating cysteine; hence with our present understanding an acidogen could not be implicated in the sharp
decline of pellet production on addition of cysteine in trace amounts.

- If acidogens were responsible for polypeptide/pellet production then a reduction in pH, to say pH 6, should not affect such production because the acidogens are insensitive to pH, to as low as pH 3.5. While investigating pH control in UASB systems (see Chapter 8, Section 3.1), when insufficient alkalinity was added in the influent in some of the tests, the pH in the high hydrogen partial pressure reactor declined to pH 6.1. Within 24h it was observed that pellet break up was taking place to a significant degree and that volatile suspended solids production declined i.e. pellet production declined. Hence low pH had a significant adverse effect on pellet and associate polypeptide production. In contrast, the behaviour at low pH is consistent with that expected from the hydrogenotroph, *M. Strain AZ*. Zehnder and Wurhmann (1977) found that in pure cultures, *M. Strain AZ* attained a maximum growth rate at pH 6.6, but this growth rate declined rapidly to virtually zero at pH 6. If *M. Strain AZ* was responsible for pellet production then the observed decline in pellet production and pellet break up is not unexpected.

6. CONCLUSIONS

(1) There is strong evidence that pelletization is due to the action of *Methanobacterium* strain *AZ*, now classified as *Methanobrevibacter arboriphilus*. This organism utilizes H\(_2\) as its sole energy source. It can produce all its amino acid requirements 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) Pelletization is unlikely in completely mixed systems because in such systems the H\(_2\) partial pressure always is likely to be low, if the objective is optimal methane fermentation.

(3) Stable pellet formation appears to be possible only in plug-flow or semi plug-flow systems because in these systems partial phase separation into high
and low hydrogen partial pressure can occur along the line of flow. However phase separation is a necessary but not sufficient requirement — the influent substrate is also a determining factor. Pelletization is unlikely with the following substrates: (i) acetate, because acetate does not produce $\text{H}_2$ during fermentation, (ii) propionate, because the conversion of propionate to acetate and $\text{H}_2$ takes place only under low pH, (iii) edible oily wastes because the oil is broken down to short chain fatty acids and $\text{H}_2$ only under low hydrogen partial pressure. Pelletization is possible with (i) a carbohydrate or protein substrate in a plug flow system because $\text{H}_2$ is released during the conversion of a carbohydrate or protein to short chain fatty acids; under high loading conditions if the $\text{H}_2$-utilizing methanogens cannot utilize the $\text{H}_2$ at the rate it is generated, a zone of high hydrogen partial pressure will form; if excess free and saline ammonia is present, the two prime conditions for growth of $M$. Strain $AZ$ are satisfied, (ii) a carbohydrate or protein substrate in a completely mixed reactor provided the reactor is operated such that SCFA in addition to acetic acid is generated. Higher SCFA is generated only if the hydrogen partial pressure is high thereby satisfying a key requirement for polypeptide generation. However such a system is unlikely to be operated as a practical one because the conversion of COD to methane will be low and hence the effluent COD will be high.
CHAPTER 6

GROWTH OF BIOPELLETS ON GLUCOSE

1. INTRODUCTION
In the literature all the work reported on UASB systems at mesophilic temperatures had made use of influents that were in effect mixtures of substrates such as short chain fatty acids (SCFA), carbohydrates and proteins. This was also the case in this study; in the previous chapters the substrate utilized was apple juice waste water or apple juice concentrate supplemented with excess free and saline ammonia and a trace metal solution. This waste water is not a clearly defined substrate, almost certainly containing various organic carbon species in addition to sugars and SCFA (acetate and propionate). With such an undefined substrate (1) the biochemical reactions are uncertain, in particular it is not possible to assess the hydrogen flux from end product formation (because the biochemical pathways are not clearly defined) and (2) the influent feed may contain polymers that could promote pellet formation (Ross, 1984). Resolution of these difficulties would be obtainable 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 associate product formation is available and certainly the presence of polymers in the influent could be eliminated. Accordingly it was decided to study the response of a UASB system using glucose instead of apple juice as substrate.

The following tasks were set for the investigation:

(1) Response of a UASB system to glucose as substrate feed.

(2) Enquiry into the appearance or non-appearance of butyrate in UASB systems.

(3) Behaviour of the hydrogen-utilizing organisms in the high pH zone of a UASB system.

(4) Effects of the free and saline ammonia concentration in the influent, in excess of, and just sufficient for, normal anaerobic growth.
2. **FERMENTATION OF GLUCOSE**

Fermentation of glucose under the conditions normally encountered in methane fermentation processes occurs in three distinct phases: (1) acidogenesis; (2) acetogenesis, and (3) methanogenesis. Product formation arising in these three phases depend to a large degree on the pH$_2$ surrounding the organisms. The three phases in the fermentation of glucose are set out briefly below.

(1) **Acidogenesis:** This is the initial phase in fermentation of soluble carbohydrates carried out by a group of organisms, the acidogens. When the pH$_2$ is $< 10^{-4}$ atmospheres (atm), acetate, butyrate, hydrogen and carbon dioxide are generated, and when the pH$_2$ is $> 10^{-4}$ atm acetate, propionate, butyrate, hydrogen and carbon dioxide are generated, Fig 6.1(a, b and c). Butyrate is a normal fermentation product from a carbohydrate substrate irrespective of the pH$_2$ (Thauer et al., 1977). The stoichiometric reactions describing these pathways are as follows:

For pH$_2 < 10^{-4}$ atm

production of acetate,

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (6.1)$$

and production of butyrate,

$$C_6H_{12}O_6 \rightarrow CH_3CH_2 CH_2COOH + 2CO_2 + 2H_2 \quad (6.2)$$

for pH$_2 > 10^{-4}$ atm

production of acetate and propionate,

$$C_6H_{12}O_6 \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2 \quad (6.3)$$

and production of butyrate,

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (6.2)$$

The acidogens have a generation time of about 2 hours with a specific yield on glucose of approximately 0.12 to 0.14 mgVSS/mg glucose fermented.
Glucose

2NAD⁺ $\rightarrow$ 2NADH

2P
2ADP
2ATP

EMP

2PYR

2AcCoA

2HAc

2CO₂
2NAD⁺
2NADH
2H₂

(a) Low H₂ partial pressure

(<10⁻⁴ atm)

(b) high and low H₂ partial pressures

(c) High H₂ partial pressure

(>10⁻⁴ atm)

Fig 6.1: Acidogenic phase of glucose fermentation under low and high H₂ partial pressures to form acetic acid, butyric acid, propionic acid, H₂ gas and CO₂.

Abbreviations: EMP - Embden Myerhof pathway; PYR - pyruvic acid; AcCoA - acetyl coenzyme A; NAD⁺ - nicotiamide adenine dinucleotide (oxidized form); NADH - nicotiamide adenine dinucleotide (reduced form); ButyrlCoA - butyrl coenzyme A; HAc - acetic acid; HBr - butyric acid; HPr - propionic acid.
Acetogenesis: Short chain fatty acids, butyrate and propionate, generated in the acidogenic phase, are oxidized by a specific group of organisms, the acetogens, to acetic acid and hydrogen. Conversion of butyrate and propionate can take place only at low $\tilde{pH}_2$, but at different respective partial pressures.

For acetate formation from butyric acid, the reaction is:

$$\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{H}_2$$  \hspace{1cm} (6.4)

This reaction thermodynamically is energy yielding to the organisms for $\tilde{pH}_2 < 10^{-2.7}$ atm (McInerney et al., 1979). The generation time for butyrate oxidizers is approximately 2.3 days (Gujer and Zehnder, 1983) with a specific yield of about 0.03 gVSS/gCOD butyrate fermented.

For acetate formation from propionic acid, the reaction is:

$$\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{H}_2$$  \hspace{1cm} (6.5)

This reaction thermodynamically is feasible for $\tilde{pH}_2 < 10^{-4.1}$ atm (McInerney et al., 1979). The generation time for propionate oxidizers is between 4.6 to 5.8 days (Koch et al., 1983, Boone and Bryant, 1980) with a specific yield of between 0.023 to 0.034 gVSS/gCOD propionate fermented (Dolfing, 1987).

Methanogenesis: Specialized groups of methanogenic organisms can utilize hydrogen and/or acetic acid to generate methane. Three groups of methanogens have been identified: (i) obligate acetoclastic methanogens that utilize acetate only as energy source; (ii) obligate hydrogenotrophic methanogens that utilize $\text{H}_2$ only (also called $\text{H}_2$ utilizers) as energy source (with $\text{CO}_2$ as the carbon source). The hydrogenotrophs can operate over a wide range of $\tilde{pH}_2$, and (iii) methanogens that utilize both acetic acid and hydrogen as energy source. Generally this latter group utilizes hydrogen preferentially. The reactions for hydrogen and acetate oxidations are:
For hydrogen,

\[ 4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \]  

(6.6)

and for acetate,

\[ CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \]  

(6.7)

3. SINGLE UASB REACTOR SYSTEM RESPONSE TO GLUCOSE AS SUBSTRATE

A UASB reactor, effective volume 9 l, was seeded with 3 l of pelletized sludge developed on an apple juice waste water (described in Chapter 2). The reactor was fed with glucose as sole organic carbon source with COD concentration approximately 2800 mgCOD/l. The feed was supplemented with trace elements and nutrients for organism growth (details of composition are given in Chapter 3), with excess NH_3-N, and buffered by addition of 1,2 mg alkalinity as CaCO_3 per mg influent COD. The effects of loading on the system were investigated for the range 9,3 to 26,7 kgCOD/m³ reactor/d; pertinent operating data for the system are given in Table 6.1.

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

For the range of loadings investigated excellent pelletization 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 Figs 6.2, 6.3, 6.4 and 6.5 for the various loadings (varying influent COD concentration and flow rates). 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 observed:

(i) **a lower active zone**: In this zone, the total soluble COD concentration
### Table 6.1: Operating data for single UASB reactor with glucose as influent.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent COD concentration, mgCOD/l</td>
<td>2792–5395</td>
</tr>
<tr>
<td>Volumetric flow rate, l/d</td>
<td>15–45</td>
</tr>
<tr>
<td>Nominal velocity of flow in reactor, cm/min</td>
<td>0,14–0,41</td>
</tr>
<tr>
<td>Hydraulic retention time, h</td>
<td>14,4–4,8</td>
</tr>
<tr>
<td>Organic loading, kgCODm³ reactor/day</td>
<td>9,3–26,7</td>
</tr>
<tr>
<td>Operating temperature, °C</td>
<td>30</td>
</tr>
<tr>
<td>mg alkalinity as CaCO₃/mg influent COD</td>
<td>1,2</td>
</tr>
<tr>
<td>Influent pH</td>
<td>8,12</td>
</tr>
<tr>
<td>Free and saline ammonia (NH₃-N), mgN/l</td>
<td>65,8–141,7</td>
</tr>
</tbody>
</table>
Fig 6.2: Concentration and pH profiles observed in the single UASB system on glucose substrate (Influent COD concentration = 5000 mg/l, flow rate = 15 l/d).
Fig 6.3: Concentration and pH profiles observed in the single UASB system on glucose substrate (Influent COD concentration = 5345 mg/l, flow rate = 45 l/d).
Fig 6.4: Concentration and pH profiles observed in the single UASB system on glucose substrate (Influent COD concentration = 2792 mg/l, flow rate = 30 l/d).
Concentration and pH profiles observed in the single UASB system on glucose substrate (Influent COD concentration = 2712 mg/l, flow rate = 45 l/d).
decreased to about a half the initial value, acetate and propionate increased to a maximum, 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 zone reflects a monotonic increase in propionate and as such defines a high pH$_2$ zone (Sam-Soon et al., 1987).

(ii) **an upper active zone**: In this zone, the total soluble COD concentration reduced further, to a minimum value; acetate and propionate decreased 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. This zone reflected a monotonic decrease in propionate and as such defined a low pH$_2$ zone.

(iii) **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 in the UASB system the fermentation of glucose follows a pattern similar to that observed in the fermentation of apple juice waste water. Pellet formation on glucose as substrate clearly indicates that pelletization is not due to extraneous organics present in the influent feed (as hypothesized by Ross, 1984) but is due to some other biochemical mechanism, such as that proposed in Chapter 5. 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 pH$_2$ zone of the reactor. Accordingly it was decided to investigate the high pH$_2$ zone of a UASB system with glucose as substrate.

4. **HIGH pH$_2$ UASB REACTOR RESPONSE TO GLUCOSE SUBSTRATE**

The enquiry into the high pH$_2$ phase of the UASB system with glucose as substrate set the following specific tasks: (1) Pellet production, (2) Non-production of butyrate, and (3) Hydrogenotrophic organism behaviour.
A UASB reactor, to operate under high $\hat{p}H_2$ conditions, was set up as follows: A reactor with an effective volume of 3 l was inoculated with 1 l of pelletized sludge obtained from the high $\hat{p}H_2$ zone of a single UASB reactor fed with the same glucose substrate described in the section above. The reactor supplying the inoculum had been operated at a loading of 8,3 kgCOD/m³ reactor/d with influent COD concentration $\approx$ 5000 mg/l, flow rate: 15 l/d. Measurements of the pelletized sludge inoculum gave a mean pellet density = 35 349 mgVSS/l, COD/VSS = 1,19 mgCOD/mgVSS and TKN/COD = 0,0820 mgN/mgCOD.

In operating the high $\hat{p}H_2$ reactor the COD concentration of the glucose substrate was changed to be approximately the same as that in the single UASB reactor system described previously — the influent COD concentration was maintained around 2600 mg/l. Increase in COD loading to the unit was achieved by an appropriate increase in the influent flow rate. To ensure that the system comprised virtually only the high $\hat{p}H_2$ zone, the volume of pelletized sludge was kept at a value such that no propionate oxidation occurred. Propionate is not oxidized if $\hat{p}H_2 > 10^{-4}$ atm; by measuring propionate concentration along the line of flow, the bed volume was maintained at a value such that propionate concentration either increased continuously up the bed or remained constant up to the top of the bed. This was accomplished by maintaining the bed volume at 1 litre, by drawing excess pellets daily. This procedure also automatically established a sludge age for the system as follows:

$$\text{sludge age} = \frac{\text{mass of pelletized bed}}{\text{mass of pellets wasted per day}}$$

Measurements of the following parameters were made daily: Influent COD, filtered effluent COD, free and saline ammonia, TKN, pH of the reactor, gas production (corrected to standard temperature and pressure), volume of sludge generated, VSS and 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 consists only of CO₂ and CH₄).

The steady state responses of the system, to loadings of 0,166, 0,203 and 0,238 kgCOD/d, are shown in Table 6.2. From the sludge mass in the high $\hat{p}H_2$ reactor and the mass wasted daily, the sludge ages for the three loadings ranged from 1,9 to
Table 6.2: Steady state response of the high H₂ partial pressure reactor to loadings.

<table>
<thead>
<tr>
<th>Flow rate 1/d g/d</th>
<th>InfI COD gCOD/d</th>
<th>Effl. COD as COD</th>
<th>COD Recovery %</th>
<th>Glucose util.  molGl/d</th>
<th>Effl. SCFA mg/l</th>
<th>HAc mg/l</th>
<th>HPr mg/l</th>
<th>HBr mg/l</th>
<th>NH₃ In mgN/l</th>
<th>NH₃ Out mgN/l</th>
<th>OrgN mgN/l</th>
<th>NH₃ Gas Prod.  mgN/d</th>
<th>CO₂  mg/l</th>
<th>CH₄  mg/l</th>
<th>COD VSS mgCOD/mgVSS</th>
<th>TKN ratio mgN/mgVSS</th>
<th>Sludge age days</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>166.44</td>
<td>128.52</td>
<td>25.54</td>
<td>11.85</td>
<td>99.7</td>
<td>0.867</td>
<td>722</td>
<td>389</td>
<td>333</td>
<td>86.8</td>
<td>40.3</td>
<td>17.7</td>
<td>8.5</td>
<td>0.51</td>
<td>0.49</td>
<td>1.42</td>
<td>0.1260</td>
</tr>
<tr>
<td>75</td>
<td>203.86</td>
<td>167.85</td>
<td>26.90</td>
<td>8.70</td>
<td>99.0</td>
<td>0.965</td>
<td>750</td>
<td>367</td>
<td>250</td>
<td>86.8</td>
<td>40.3</td>
<td>19.5</td>
<td>5.8</td>
<td>0.99</td>
<td>0.41</td>
<td>1.30</td>
<td>0.1296</td>
</tr>
<tr>
<td>90</td>
<td>237.78</td>
<td>186.84</td>
<td>30.47</td>
<td>10.68</td>
<td>99.2</td>
<td>1.140</td>
<td>509</td>
<td>350</td>
<td>278</td>
<td>86.0</td>
<td>40.3</td>
<td>23.0</td>
<td>12.3</td>
<td>0.47</td>
<td>0.53</td>
<td>1.23</td>
<td>0.1126</td>
</tr>
<tr>
<td>60</td>
<td>166.44</td>
<td>138.30</td>
<td>3.12</td>
<td>2.29</td>
<td>96.3</td>
<td>0.670</td>
<td>689</td>
<td>270</td>
<td>300</td>
<td>10.5</td>
<td>5.4</td>
<td>3.8</td>
<td>1.6</td>
<td>0.48</td>
<td>0.52</td>
<td>1.45</td>
<td>0.0780</td>
</tr>
</tbody>
</table>

* NH₃-N limited system.
2.1 days (see Table 6.2).

4.1 Pellet production

In the high pH reactor the VSS production rate was estimated by daily removing excess pellets generated and measuring the VSS content. The principal fermentation reactions taking place are: (1) acidogenesis, (2) methane formation from H\(_2\) and CO\(_2\) and (3) a degree of methane formation from acetate cleavage. Acетogenesis did not appear to take place due to the high pH (propionate showed no decrease in the profile). Accordingly the microbial content of the VSS produced comprised principally acidogens, hydrogenotrophs and acetoclastic methanogens.

With glucose as substrate in acidogenesis: (1) approximately 10 per cent (molar basis) of the glucose fermented reappears as acidogenic mass, (2) approximately 12 per cent (COD basis) of the glucose fermented is released as hydrogen gas (Cohen et al. 1979), the balance of the glucose is transformed to short chain fatty acids such as acetate, propionate and butyrate, 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 0.043·0.12 = 0.0052 i.e. 0.5 per cent of the glucose (COD) fermented reappears as hydrogenotrophic mass.

Some of the acetate is converted to methane by the acetoclastic methanogens. Even if all the acetate 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(acetate) removed. Later in the chapter, by appropriate mass balance, a method to estimate the mass of acetate utilized 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 (see Table 6.2), glucose fermented was 0.867 mol/d. The expected VSS generation is:

(i) For acidogenic organisms,

\[
\text{VSS generated} = 0.867 \cdot 1.067 \cdot 180 \cdot 0.1 = 16.65 \text{ gCOD/d}
\]
(ii) For hydrogenotrophic organisms,
\[ \text{VSS generated} = 0.867 \cdot 1.067 \cdot 180 \cdot 0.12 \cdot 0.043 \]
\[ = 0.86 \text{ gVSS/d} \]
\[ = 1.22 \text{ gCOD/d} \]

(iii) For acetoclastic organisms (from Table 5.2 and 6.3 below)
\[ \text{VSS generated} = 4.48 \cdot 0.03 \]
\[ = 0.134 \text{ gVSS/d} \]
\[ = 0.19 \text{ gCOD/d} \]

Total expected VSS production
\[ = 16.65 + 1.22 + 0.19 \]
\[ = 18.06 \text{ gCOD/d} \]

The observed VSS generated for this loading was 25.54 gCOD/d. For each of the loadings investigated similar discrepancies arose. A logical 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 is a peptide type.

4.2 Butyrate production
For each of the three loadings investigated, butyric acid was observed in the high pH2 system, in addition to acetic and propionic acids (see Table 6.2). The presence of butyric acid was a feature not observed previously in either the parent single reactor maintained on a glucose substrate, or, in either the single or two-in-series (high and low pH2) 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 pH2 glucose system were compared with that of the high pH2 reactor in the two-in-series system system treating apple juice waste. In the high pH2 glucose system the sludge age ranged between 1.9 and 2.1 days whereas in the high pH2 apple juice system the sludge age was about 3.0 days. Now the generation time for acetogenic butyrate oxidizing bacteria is about 2.3 days (Gujer and Zehnder, 1983). Therefore with a sludge age of about 2 days one may expect washout of these butyrate oxidizers, and hence appearance of butyric acid in the profiles. For sludge ages greater than 2.3 days, as in the high pH2 apple juice system, these organisms could become established and oxidize any butyric acid generated.
In UASB systems with both high and low pH zones present in the same reactor operating on glucose, the sludge age always would be well in excess of 2.3 days so that one would still expect butyrate generation and utilization to take place in this system and follow a similar pattern to propionate, i.e. generation in the lower region of the system and disappearance in the upper region. However butyrate had not been observed in the high pH zones; this possibly was 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 oxidizers are introduced to the region where butyrate is generated. Provided the pH in this region is below \(10^{-2.7}\) atm, butyrate oxidation should take place but not propionate oxidation (propionate oxidation occurs only at \(pH < 10^{-4.1}\) atm). Hence the disappearance of butyrate, but not propionate, would infer a pH of between \(10^{-4.1}\) and \(10^{-2.7}\) atm. In contrast if a sludge age is established (as in the high pH reactor) at a level lower than that required for butyrate oxidizers, 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 performed this hypothesis on the presence or absence of butyrate had not yet been formulated and consequently no experimental evaluation was undertaken.

4.3 Hydrogenotrophic organism behaviour in the high pH 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.

(i) Conversion of glucose to SCFA and acidogen mass

In the high pH UASB reactor, controlled to a sludge age of about 2 days, the only fermentation processes would be acidogenesis and methanogenesis. Acetogenesis would not be established because the generation time of the acetogens is longer than the sludge age of 2 d. This was supported from the experimental data in that butyric and propionic acids showed a monotonic increase to a maximum along the line of flow in the reactor.

Glucose will be fermented via the various acidogenic pathways set out in Fig 6.1(a, b and c); provided none of the propionate and butyrate is oxidized one could readily
make a glucose balance [using Eqs (6.1 to 6.3)] as follows:

Glucose fermented \([GT]\), in moles, is utilized in molar masses \([G1]\), \([G2]\), \([G3]\) and \([G4]\):

(a) under high \(\tilde{p}H_2\), from Eq (6.3),

For every one mole propionate appearing one mole glucose is fermented and one mole acetate also appears, i.e,

\[1 \cdot [HPr]_{G1} = 1 \cdot [HAc]_{G1} = 1 \cdot [G1]\]

(b) under low \(\tilde{p}H_2\), from Eq (6.1),

For every two moles acetate appearing one mole glucose is fermented, i.e,

\[\frac{1}{2} [HAc]_{G2} = [G2]\]

(c) under both high and low \(\tilde{p}H_2\), from Eq (6.2),

For every one mole butyrate appearing one mole glucose is fermented, i.e,

\[[HBr]_{G3} = [G3]\]

(d) The molar mass of glucose fermented reappearing as acidogenic mass is given approximately empirically by Zoetemeyer et al. (1982):

\[[G4] \approx 0.1 [GT]\]

Hence \([GT] = [G1] + [G2] + [G3] + [G4]\) (6.9a)

Substituting for \([G1]\), \([G2]\), \([G3]\) and \([G4]\) from above, i.e \([GT] = [HPr]_{G1} + \frac{1}{2} [HAc]_{G1} + \frac{1}{4} [HAc]_{G2} + [HBr]_{G3} + 0.1 \ [GT]\) (6.9b)

Now, the total acetate, \([HAc]_T\), is made up of \([HAc]_{G2}\) and \([HAc]_{G1}\) i.e,
\[[\text{HAc}]_T = [\text{HAc}]_{G2} + [\text{HAc}]_{G1}\]

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

Substituting for $[\text{HAc}]_{G2}$ in Eq (6.9b),

\[[\text{GT}] = [\text{HPr}]_{G1} + \frac{1}{4} \{[\text{HAc}]_T - [\text{HPr}]_{G1}\} + [\text{HBr}]_{G3} + 0.1 \cdot [\text{GT}] \quad (6.10)\]

Usually some of the HAc generated will be oxidized to methane, $[\text{HAc}]_{\text{ox}}$, thereby reducing the total HAc, $[\text{HAc}]_T$, to give the observed HAc, $[\text{HAc}]_{\text{obs}}$. To incorporate $[\text{HAc}]_{\text{obs}}$ into Eq (6.10) we note that

\[[\text{HAc}]_T = [\text{HAc}]_{\text{obs}} + [\text{HAc}]_{\text{ox}}\]

i.e. \[[\text{GT}] = [\text{HPr}]_{G1} + \frac{1}{4} \{[\text{HAc}]_{\text{obs}} + [\text{HAc}]_{\text{ox}} - [\text{HPr}]_{G1}\} + [\text{HBr}]_{G3} + 0.1 \cdot [\text{GT}] \quad (6.11)\]

All the terms in Eq (6.11) except for $[\text{HAc}]_{\text{ox}}$ can be measured directly or estimated. $[\text{GT}]$ can be measured directly, or alternatively, if no glucose measurements are available on the effluent, $[\text{GT}]$ can be determined as follows:

Measure the following on the filtered effluent:

- Total COD concentration.
- SCFA concentrations (i.e. acetate, propionate and butyrate), hence the COD\textsubscript{SCFA}.
- Organic nitrogen concentration (from the difference between TKN and NH\textsubscript{3}-N).

Thus the total effluent COD is comprised of:

\[\text{Total COD}_{\text{eff}} = \text{COD}_{\text{SCFA}} + \text{COD}_{\text{orgN}} + \text{COD}_{\text{glucose not fermented}} \quad (6.12)\]

From the hypothesis on pelletization, Sam-Soon \textit{et al.} (1987) proposed 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, \( (\text{COD}_{\text{orgN}}) \), can be estimated as set out in Appendix B. Knowing the effluent total COD, COD_{SCFA} and COD_{orgN} the COD of the glucose not fermented can be estimated from Eq (6.12).

The amount of glucose fermented is then calculated from the difference between the total glucose influent (as COD) and the COD_{glucose} not fermented.

The procedures above were applied to the observations obtained on the three loadings listed in Table 6.2. As an example to illustrate the procedures consider the flow rate 60 l/d with influent glucose COD of 2600 mg/l:

\[
\begin{align*}
\text{Total COD}_{\text{eff}} &= 2142 \, \text{mgCOD/l} \\
\text{COD}_{\text{orgN}} \text{(see Appendix B)} &= 164.5 \, \text{mgCOD/l}
\end{align*}
\]

From Table 6.2, SCFA are:

\[
\begin{align*}
\text{HAc} &= 722 \, \text{mg/l}; \quad \text{HPr} = 389 \, \text{mg/l} \quad \text{and} \quad \text{HBr} = 333 \, \text{mg/l} \\
\text{i.e.} \quad \text{COD}_{\text{SCFA}} &= 722 \cdot 1.067 + 389 \cdot 1.512 + 333 \cdot 1.816 \\
&= 1963 \, \text{mgCOD/l}
\end{align*}
\]

and from Eq (6.12),

\[
2142 = 1963 + 164.5 + \text{COD}_{\text{glucose not fermented}}
\]

i.e \( \text{COD}_{\text{glucose not fermented}} = 14.5 \, \text{mgCOD/l} \).

This amount can be taken as negligible, that is, all the glucose has been fermented. For the other loadings (see Table 6.3), substantial amounts of glucose in the feed were not fermented.

From Eq (6.11) \( \text{HAc}_{\text{oxidized}} \) for methane production is,

\[
(0.867) = (0.315) + \frac{1}{4}(0.722-0.315) + \frac{1}{4}[\text{HAc}]_{\text{oxidized}} + (0.227)
\]

i.e.

\[
[\text{HAc}]_{\text{oxidized}} = 0.07 \, \text{mols/d}
\]
Table 6.3: Calculated values of hydrogen flux, acetate cleavage, hydrogen oxidation and H₂ utilizers VSS yields.

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Glucose fermented</th>
<th>Glucose not fermented</th>
<th>Methane generated</th>
<th>HAc oxidized to CH₄</th>
<th>Hydrogen oxidized to CH₄</th>
<th>Hydrogen flux</th>
<th>Total VSS generated</th>
<th>VSS generated by H₂ utilizers</th>
<th>Hydrogen used for anaerobic acids</th>
<th>Yield H₂ utilizers</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>l/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
<td>gCOD/d</td>
</tr>
<tr>
<td>60</td>
<td>166.46</td>
<td>-</td>
<td>11.89</td>
<td>4.48</td>
<td>7.37</td>
<td>27.57</td>
<td>25.54</td>
<td>8.85</td>
<td>9.87</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>185.28</td>
<td>18.53</td>
<td>8.70</td>
<td>-</td>
<td>9.70</td>
<td>30.88</td>
<td>26.90</td>
<td>8.28</td>
<td>13.61</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>218.88</td>
<td>17.91</td>
<td>14.68</td>
<td>11.14</td>
<td>7.54</td>
<td>36.13</td>
<td>30.47</td>
<td>8.58</td>
<td>19.27</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>60*²</td>
<td>130.18</td>
<td>36.30</td>
<td>2.28</td>
<td>-</td>
<td>2.28</td>
<td>25.0</td>
<td>3.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*¹ see Appendix B for computation
*² NH₃-N limited system
- Nil
(ii) **Hydrogen flux**

Hydrogen flux through the system can be determined using the stoichiometric relationship between SCFA generated and associated hydrogen release, see the fermentation pathways in Fig 6.1(a, b and c), i.e.

\[
[H_2] = \left[\text{HPr}\right] + 2\left[HBr\right] + 2 \left\{[\text{HAc}]_{\text{obs}} - \left[\text{HPr}\right]_{G1} + [\text{HAc}]_{\text{oxidized}}\right\}
\]

From Eq (6.13) the hydrogen flux through the system

\[
[H_2] = (0.315) + 2 \cdot (0.227) + 2 \cdot (0.722-0.315) + 2 \cdot (0.070)
\]

\[
= 1.723 \text{ mol/d}
\]

\[
= 27.57 \text{ gCOD/d}
\]

The \([\text{HAc}]_{\text{oxidized}}\) and \([H_2]\) for the three loadings are listed in Table 6.3.

(iii) **VSS yield of hydrogenotrophs**

Knowing the hydrogen flux, acidogenic mass and measured total VSS generated per day, one can estimate the VSS mass generated by the hydrogenotrophic organisms 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. Knowing the VSS for the acidogens, it is possible to determine the VSS for the hydrogenotrophic organisms from the total VSS (pellets) measured, i.e.

\[
\text{VSS}_{\text{H}_2 \text{ utilizers}} = \text{Total VSS measured} - \text{VSS acidogens}
\]

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

\[
\begin{align*}
\text{VSS}_{\text{measured}} &= 25.54 \text{ gCOD/d} \\
\text{VSS}_{\text{acidogens}} &= 16.65 \text{ gCOD/d} \\
\text{i.e. VSS}_{\text{H}_2 \text{ utilizers}} &= 8.89 \text{ gCOD/d} \\
&= 8.89/1.35 \\
&= 6.59 \text{ gVSS/d}.
\end{align*}
\]
It is possible now to determine the specific yield associated for the $\text{H}_2$ utilizing methanogens from the VSS ascribed to the $\text{H}_2$ utilizers and the hydrogen flux determined above, i.e.

$$Y_{(\text{H}_2 \text{ utilizers})} = \frac{\text{VSS}}{(\text{Hydrogen flux})} \tag{6.15}$$

for the 60 l/d feed the yield is

$$Y_{(\text{H}_2 \text{ utilizers})} = \frac{6.59}{27.57} = 0.24 \text{ gVSS/gCOD(} \text{H}_2)$$

For the loading rate 0.203 kgCOD/d the repeat calculations showed that $[\text{HAc}]_{\text{ox}}$ was zero. Hence in this instance the calculated hydrogenotrophs yield would be unaffected by the acetoclastic organism mass. The specific yield value was $Y_{(\text{H}_2 \text{ utilizers})} = 0.21 \text{ mgVSS/mgCOD(} \text{H}_2)$ utilized. This value is very close to that calculated above when acetate oxidation did take place. The reason why it is acceptable to neglect the acetoclastic mass is that its specific yield is only 0.03 mgVSS/mgCOD (acetate)$_{\text{oxidized}}$ (ten Brummeler et al., 1985, Dolfing, 1987).

The hydrogenotrophs yield values calculated above are approximately 6 times that normally observed for these organisms (0.043 gVSS/g COD $\text{H}_2$ 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($\text{H}_2$)$_{\text{oxidized}}$.1 It remains to determine the type of polymer most likely formed; this is considered in (iv) below.

(iv) *Nitrogen for polypeptide formation*

Nitrogen was added to the high $\text{pH}_2$ reactor as NH$_3$-N in the influent. For the three loadings investigated, the NH$_3$-N removed in VSS synthesis, and the dissolved

---

1The gross specific yield values 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 (see Table 6.2) 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 (see Appendix B) and adding this to the pelletized VSS gives a gross specific yield for the $\text{H}_2$ utilizers of approximately 0.56 gVSS/gCOD($\text{H}_2$) generated, i.e. a value about 14 times higher than the specific yield of the hydrogenotrophs normally expected in anaerobic systems.
organic N generated are listed in Table 6.2. 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 utilizing 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 either released to the surrounding medium or incorporated in polypeptides in the pellet mass, or both.

Taking, for example, the data for the 60t/d feed shown in Table 6.2,

Nitrogen removed from the system per day, $\Delta M(\text{NH}_3\text{-N})$

$$\Delta M(\text{NH}_3\text{-N}) = M(\text{NH}_3\text{-N})_{\text{in}} - M(\text{NH}_3\text{-N})_{\text{out}}$$

$$= (86.8 \cdot 60 - 40.3 \cdot 60)$$

$$= 2790 \text{ mgN/d}$$

where $M(\text{NH}_3\text{-N})_{\text{in, out}} = \text{mass of free and saline ammonia per day in the influent, effluent respectively.}$

Nitrogen incorporated in acidogenic mass per day, $M(\text{NH}_3\text{-N})_{\text{acid}}$

$$M(\text{NH}_3\text{-N})_{\text{acid}} = \Delta M X_{\text{acid}} \cdot \left(\frac{\text{TKN}}{\text{COD}}\right)$$

$$= 16.65 \cdot 0.086$$

$$= 1432 \text{ mgN/d}$$

where $\Delta M X_{\text{acid}} = \text{mass of acidogens generated/d.}$

Nitrogen incorporated into hydrogenotrophic mass, $M(\text{NH}_3\text{-N})_{\text{hyd}}$

$$M(\text{NH}_3\text{-N})_{\text{hyd}} = \Delta M(\text{NH}_3\text{-N}) - M(\text{NH}_3\text{-N})_{\text{acid}}$$

$$= 2790 - 1432$$
Nitrogen removed by the hydrogenotrophs for cell synthesis can be determined from the hydrogen flux; the normally expected yield value should be about 0.043 mgVSS/mgCOD(H₂) removed (Shea et al., 1968) i.e. expected mass H₂ utilizers generated per day, \( \Delta MXv_{H_2} \), is

\[
\Delta MXv_{H_2} = Y_{H_2} \cdot (H_2 \text{ flux})
\]

\[
= 0.043 \cdot 27.57
\]

\[
= 1.19 \text{ gVSS/d.}
\]

where \( Y_{H_2} \) = specific yield of hydrogenotrophs, mgVSS/mgCOD(H₂).

Assuming a TKN/COD = 0.086 and a COD/VSS = 1.42 then the TKN/VSS = 0.122 mgN/mgVSS for organism cell mass, and the expected N removal by hydrogenotrophs for cell synthesis, \( M(NH_3-N)_{hyd.cell} \)

\[
M(NH_3-N)_{hyd.cell} = \Delta MXv_{H_2} \cdot (TKN/VSS)
\]

\[
= 1.19 \cdot 0.122
\]

\[
= 145 \text{ mgN/d.}
\]

This value (145 mgN/d) is about one tenth of the total nitrogen estimated to have been removed by the hydrogenotrophs (1358 mgN/d). The difference is hypothesized to be due to the formation of polypeptides and the discharge of amino acids to the surrounding liquid.

### 4.4 Effect of NH₃-N concentration

As polypeptide formation requires an adequate free ammonia supply in the influent it would seem that limitation on the free ammonia supply should bring about an associated reduction in polypeptide production and hence an associated reduction in VSS generation. The influence of NH₃-N concentration on VSS yield was investigated by operating a high pH UASB reactor (32) at a constant loading of 0.166 kg COD/d (COD concentration ≈ 2600 mg/l; flow rate 60 l/d). Two NH₃-N concentrations were investigated:

- An NH₃-N concentration of 86.8 mgN/l i.e. a N/COD ratio of 0.0334
mgN/mgCOD, well in excess of that normally required for anaerobic growth.

- An NH₃-N concentration of 10.5 mgN/l; i.e. a N/COD ratio of 0.004 mgN/mgCOD which should be just sufficient for normal anaerobic growth.

Reactor operation under the two NH₃-N influent concentrations were identical, and the methods of analysis similar to those described in the sections above dealing with the UASB response to glucose as substrate and non-appearance of butyrate.

When the reactor was operated with excess NH₃-N in the feed (86.8 mgN/l), the measured VSS yield was 0.52 mgVSS/mgCOD removed, with an NH₃-N removal of 46.5 mgN/l. When the NH₃-N concentration in the influent was decreased to 10.5 mgN/l, within two days the measured VSS yield decreased to 0.11 mgVSS/mgCOD removed (see Fig 6.6) and the NH₃-N removal was approximately 5.5 mgN/l. With this low NH₃-N influent the system was maintained for seven more days during which time the measured VSS yield remained constant at 0.11 mgVSS/mgCOD removed. On increasing the influent NH₃-N concentration back to 86.8 mgN/l, the original high VSS yield of 0.52 mgVSS/mgCOD removed was reattained within a day (see Fig 6.6), with an NH₃-N removal of 46.5 mgN/l.

In Table 6.4 are compared the responses of the various processes in the two systems, at excess and at limiting NH₃-N influent concentrations respectively. From Table 6.4 it would appear that when NH₃-N was present in excess concentration in the influent:

- An overall mass balance performed on the system gave a COD recovery of 99 per cent.

- Hydrogen, generated during fermentation of glucose, was utilized to generate hydrogenotrophic cell mass plus polypeptide plus amino acids in the liquid surrounding (± 68 per cent of the hydrogen mass generated), the balance was oxidized to methane (± 32 per cent).

- Methane was formed by both hydrogen oxidation and acetate cleavage.

- The VSS wasted from the system was in granular form. Moreover the effluent was turbid even after being left standing for a long period, apparently from
Fig 6.6: Effect of limiting NH$_3$-N addition on VSS yield in the high H$_2$ partial pressure reactor.
Table 6.4  Responses of the high pH<sub>2</sub> reactor at excess and at limiting NH<sub>3</sub>-N influent concentrations.

<table>
<thead>
<tr>
<th></th>
<th>NH&lt;sub&gt;3&lt;/sub&gt;-N excess system</th>
<th>NH&lt;sub&gt;3&lt;/sub&gt;-N limiting system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading</td>
<td>0,166</td>
<td>0,166</td>
</tr>
<tr>
<td>COD removed per day (gCOD/d)</td>
<td>37,92</td>
<td>14,7</td>
</tr>
<tr>
<td>COD removal (%)</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>NH&lt;sub&gt;3&lt;/sub&gt;-N (mgN/ℓ) In</td>
<td>86,8</td>
<td>10,5</td>
</tr>
<tr>
<td>Out</td>
<td>40,3</td>
<td>5,1</td>
</tr>
<tr>
<td>Dissolved orgN-effluent (mgN/ℓ)</td>
<td>17,7</td>
<td>3,8</td>
</tr>
<tr>
<td>VSS generated per day (gVSS/d)</td>
<td>18,02</td>
<td>2,15</td>
</tr>
<tr>
<td>VSS yield (mgVSS/mgCOD removed)</td>
<td>0,52</td>
<td>0,11</td>
</tr>
<tr>
<td>Nature of VSS generated</td>
<td>granules</td>
<td>finely dispersed solids</td>
</tr>
<tr>
<td>Methane generated per day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ℓ CH&lt;sub&gt;4&lt;/sub&gt;/d at stp)</td>
<td>4,2</td>
<td>0,877</td>
</tr>
<tr>
<td>Methane COD (gCOD(CH&lt;sub&gt;4&lt;/sub&gt;)/d</td>
<td>12</td>
<td>2,28</td>
</tr>
<tr>
<td>Glucose fermented per day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mol Gl/d)</td>
<td>0,867</td>
<td>0,678</td>
</tr>
<tr>
<td>SCFA (effluent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate (mgHAc/ℓ)</td>
<td>635</td>
<td>672</td>
</tr>
<tr>
<td>Propionate (mgHPr/ℓ)</td>
<td>315</td>
<td>278</td>
</tr>
<tr>
<td>Butyrate (mgHBr/ℓ)</td>
<td>270</td>
<td>297</td>
</tr>
<tr>
<td>Effluent quality</td>
<td>turbid</td>
<td>clear</td>
</tr>
</tbody>
</table>
presence of amino acids.

- The sludge yield was 0.52 mgVSS/mgCOD removed.

When NH₃-N concentration was present in limiting concentration in the influent:

- An overall mass balance gave only 86 per cent COD recovery.

- A glucose "balance" (see Eq 6.8) using an acidogen specific yield value of 0.13 gVSS/g glucose fermented, could not be attained.

- Methane production was much lower than that when excess NH₃-N was present.

- The VSS wasted from the system was in the form of finely dispersed solids with clear effluent in between.

- The sludge yield was 0.11 mgVSS/mgCOD removed.

The response of the system with limited NH₃-N in the feed can be explained as follows: From Eq (6.11), assuming that 10 percent of the glucose influent is converted to acidogenic organism mass and using the data from Table 6.2, then:

In the limited NH₃-N system, by applying Eq (6.12) it was found that a fraction of the glucose influent was not fermented, approximately 605 mgCOD/l, whereas in the excess NH₃-N system, at the same loading, virtually all the glucose influent was fermented.

Hence mass of glucose fermented was 0.678 mols/day, i.e., from Eq (6.11) \([\text{HAc}]_{\text{ox}}\) is:

\[
(0.678) = (0.225) + \frac{1}{4}(0.689-0.225) + (0.205) + (0.0678) + \frac{1}{4}[\text{HAc}]_{\text{ox}}
\]

i.e. \([\text{HAc}]_{\text{ox}} = -0.0896\) mol/d.

Since \([\text{HAc}]_{\text{ox}}\) cannot have a negative value it is likely that, for the system with the low NH₃-N influent concentration, the mass of glucose fermented which reappeared as acidogen mass was less than the 0.13 gVSS/(g glucose fermented) assumed when
NH$_3$-N was in excess. In the limited NH$_3$-N fed system, NH$_3$-N was present in the effluent so that it was unlikely that the NH$_3$-N concentration was limiting for biological growth (Influent NH$_3$-N = 10.5 mgN/l; effluent NH$_3$-N = 5.4 mgN/l).

Assuming that all NH$_3$-N removed (306 mgN/d) was incorporated into acidogen mass then the volatile suspended solids (VSS) associated with this mass of NH$_3$-N is given by:

$$\Delta M_{V_{\text{acid}}} = \frac{M(\text{NH}_3\text{-N})_{\text{acid}}}{(\text{TKN/COD})} = \frac{306}{0.086} = 3.56 \text{ gCOD/d} \approx 0.019 \text{ mole glucose/d}$$

i.e. 0.019 mole glucose/day was converted into acidogen mass.

This calculated VSS mass of 3.56 gCOD/d is very close to the measured VSS mass of 3.12 gCOD/d.

Using the calculated VSS yield for acidogens, Eq (6.11), and the data from Table 6.2 for the limited NH$_3$-N system, a glucose balance gives the following:

$$(0.678) = (0.225) + 4(0.689 - 0.225) + 1[HAc]_{\text{ox}} + (0.205) + (0.019)$$

$$[HAc]_{\text{ox}} \approx 0.$$  

From the glucose balance it would now appear that no acetate was oxidized to methane. Furthermore the hydrogen flux associated with the glucose fermentation can be calculated using Eq (6.13),

$$\text{Hydrogen flux} = [HPr] + 2[HBr] + 2 \left\{ [HAc]_{\text{obs}} - [HPr] \right\} + [HAc]_{\text{oxidized}}$$

$$= (0.225) + 2(0.205) + 2(0.464) + 2(0)$$

$$= 1.563 \text{ mol/d}$$

$$= 25.01 \text{ gCOD/d}.$$  

The methane generated is derived only from the oxidation of hydrogen, therefore the number of moles of hydrogen oxidized to methane is:
Hydrogen oxidation to methane = 2,28 gCOD/d
= 0,143 mol H2/d.

From the assumption that all the NH3-N removed was for cell synthesis of the acidogens, then no VSS mass was generated by the H2 utilizers. Hence the mass of hydrogen not accounted for is (25,01 - 2,28) = 22,73 gCOD/d. Hence it would appear, very likely, that hydrogen being a gaseous intermediate during glucose fermentation, was lost from the system. It was mentioned earlier that an overall COD mass balance gave only 86 per cent COD recovery. If the COD, hypothesized to be lost as hydrogen gas is taken into account, the overall mass balance is as follows:

COD flow into the system:

\[ \text{COD}_{\text{in}} = 2774 \cdot 60/1000 \]
\[ = 166,44 \text{ gCOD/d} \]

COD flow out of the system:

- Soluble COD (SCOD) = 2305 \cdot 60/1000 = 138,3 \text{ gCOD/d}
- COD incorporated into CH4 = 2,28 \text{ gCOD/d}
- COD incorporated into sludge = 3,12 \text{ gCOD/d}
- COD hydrogen = 22,73 \text{ gCOD/d (see above)}
- i.e. Total COD_{\text{out}} = 166,43 \text{ gCOD/d}

percentage COD recovery (including COD of the hydrogen gas)
\[ = 100 \cdot 166,43/166,44 \]
\[ = 99,99 \text{ per cent.} \]

This excellent overall COD mass balance would suggest that the mass of hydrogen not accounted for indeed appeared to have been lost as a gas. Furthermore from the mass of NH3-N removed from the system and the glucose balance it would appear that limiting the NH3-N concentration in the feed affected not only the acidogen rate of synthesis, but more importantly, the VSS mass generated by the H2 utilizers. The decrease in yield of the acidogen could possibly be explained by the findings of Teixiera de Mattos et al. (1984a, 1984b). They found that the growth of Klebsiella
*aerogenes* decreased and the rate of glucose consumption reduced when the glucose concentration increased to about 20 g/l. However when they sparged the culture with nitrogen gas, the residual glucose level decreased with a concomitant increase in cell yield. They concluded that metabolic gases (hydrogen and/or carbon dioxide), in particular carbon dioxide was the cause of the growth inhibition. A similar behaviour was observed by Crabbendam *et al.* (1985) with growth of *Clostridium butyricum* on glucose. In this study, under NH\textsubscript{3}-N limitation, the hydrogenotrophs became inoperative, hence an accumulation of gases such as CO\textsubscript{2} and H\textsubscript{2} could be expected. This, in terms of the findings of Teixiera de Mattos *et al.* and Crabbendam *et al.* would have affected the acidogens.

The analysis above would indicate that the hydrogenotrophs became inactive under 'Limiting' NH\textsubscript{3}-N conditions in that very little of the hydrogen generated is utilized. An hypothesis to explain the decrease in the species activity of the hydrogenotroph *M*. Strain AZ, is as follows:

Under conditions of excess H\textsubscript{2} (substrate) and excess NH\textsubscript{3}-N the intracellular ATP/ADP level within the organism will be high; if cysteine is available the ATP/ADP level is lowered by cell synthesis; if cysteine is limited, the ATP/ADP level is lowered by the generation of amino acids and polypeptides. In this fashion there is a continuous uptake of H\textsubscript{2} (substrate) by the species. However where NH\textsubscript{3}-N is limited the species is unable to decrease the ATP/ADP level, resulting in H\textsubscript{2} (substrate) uptake inhibition.

5. **CONCLUSIONS**

From the study of UASB systems with glucose as substrate feed the following conclusions can be summarized:

1. The responses of a single UASB system fed with glucose as substrate feed were similar to those obtained with apple juice waste water.

2. Excellent pellet formation was observed with glucose indicating that the presence of extraneous organics such as polymers is not a prerequisite for pellet formation.

3. In the single UASB system fed with glucose no butyrate was observed along the line of flow, a response similar to that observed with apple juice waste water.
4. Separating the single UASB system into a two-in-series UASB system with the first reactor operating as a high pH reactor and the second as a low pH reactor, butyrate was formed in the high pH reactor whereas no butyrate was observed with apple juice waste water in a similar system. This difference in behaviour was attributed to the difference in sludge ages of the high pH reactor in the two systems: In the glucose system the sludge age ranged between 1.9 to 2.1 days whereas in the apple juice system the sludge age was 3.0 days. Recognizing that the generation time of butyrate oxidizers is about 2.3 days (Gujer and Zehnder, 1983), in the high pH glucose system one would expect washout of these bacteria and hence appearance of butyrate.

5. In a single (high/low pH) UASB system where the sludge age is well in excess of 2.3 days one would expect butyrate generation and utilization to take place and follow a similar pattern to propionate i.e. generation in the lower active zone and disappearance in the upper active zone. The fact that this was not observed indicates that (i) the pH in the lower region is between $10^{-4.1}$ and $10^{-2.7}$ atm, i.e. the pH is such that butyrate oxidation occurs instantaneously, and (ii) substrate utilization rate by butyrate oxidizing organisms is greater than butyrate generation rate by acidogens.

6. The non-appearance of butyrate in the lower zone of the single UASB system indicates the presence of butyrate oxidizers in this region. This region also corresponds to the zone of pellet generation with relatively short solids retention time (less than about 2 days). Consequently the presence of butyrate oxidizers in this zone indicates a degree of solids back mixing. The flow regime therefore would appear to be closely plug flow with respect to the liquid phase but not necessarily so for the solid phase.

7. Using the reported acidogenic yield, from a glucose balance the fraction of acetate oxidized and hence the hydrogen flux could be estimated, and hence the gross specific yield (including organism mass + polymer) of the hydrogenotrophs. The gross specific yield ranged from 0.21 to 0.24 mgVSS/mgCOD(H₂) removed. These values are approximately 6 times larger than reported values; this high yield value is attributed to polymer generation. Observed excess mass concentration of NH₃-N removed indicates the polymer to be composed predominantly of peptides.
8. Decreasing the NH$_3$-N concentration, from a concentration in excess to a concentration just sufficient for anaerobic growth, resulted in a concomitant decrease in VSS specific yield from 0.52 to 0.11 mgVSS/mgCOD removed respectively. No VSS was generated by the hydrogenotrophs; this was shown to be so by the non uptake of hydrogen (energy source): their decreased activity is ascribed to the intracellular high ATP/ADP level within the hydrogenotrophs (due to high pH$_2$ substrate source), which the species cannot decrease, through generation of amino acids and polypeptides, when NH$_3$-N is limiting.
CHAPTER 7

HYPOTHESIS VERIFICATION

1. INTRODUCTION

According to the hypothesis on pelletization proposed in Chapter 5, two prime conditions must be satisfied for pellet formation: (1) a high H₂ partial pressure (high pH₂) and (2) an excess supply of free and saline ammonia (NH₃-N). The following situations were identified where one or both of these conditions would not be satisfied and accordingly pelletization will be limited or not take place at all:

(1) System operation such that low pH₂ is the norm, i.e. completely mixed anaerobic systems operated for high methane production and organic removal.

(2) Treatment of substrates in a UASB system that do not apparently yield hydrogen such as acetate.

(3) Limitation of the NH₃-N supply with substrates producing high pH₂ in UASB systems – limiting NH₃-N will limit the over production of amino acids, and hence limit polypeptide production.

(4) Treatment of substrates in UASB systems that can be broken down only under low pH₂, e.g. short and long chain fatty acids as sole substrate, or with lipids as sole substrate.

(5) Presence of electron acceptors such as sulphate ions (SO₄²⁻) in the influent feed. Under normal operating conditions even though the substrate (for example a carbohydrate) does produce H₂ sufficiently fast to generate a high pH₂, secondary reaction (sulphate reduction) abstracts hydrogen preferentially thereby reducing pH₂ and hence limiting pelletization.

With regard to (1) above, in Chapter 2 it was stated that there has been no report in the literature that completely mixed systems exhibit pelletization.

With regard to (2) where acetate has been used as sole carbon source in a UASB
system, no pelletization has been observed, see Chapter 2.

With regard to (3), in Chapter 6, it was shown that in a UASB system with glucose as substrate, when NH$_3$-N in the feed was limited to a concentration sufficient for cell synthesis, pellet production decreased markedly.

With regard to (4), in Chapter 2 literature reports of mixtures of short chain fatty acids such as acetate and propionate as substrate in UASB systems produced poor quality pellets; their formation was ascribed to the presence of a substantial concentration (as COD) of yeast extract. With olive oil processing wastes (lipid) no pelletization was observed; the sludge bed formed was uniform and smooth in consistency with good settleability.

With regard to (5), Russo (1987) observed that in a UASB system treating a paper re-pulping waste (50% carbohydrate, 50% short chain fatty acids, with COD $\approx$ 5000 mg/l) containing sulphate (SO$_4^{2-}$ $\approx$ 300 mg/l) pellet formation was limited and of poor quality. He observed complete removal of sulphate with production of hydrogen sulphide and an increase/reduction of the pellet size when the batch SO$_4^{2-}$ concentration was lower/higher.

Although the evidence in (4) and (5) appears to support the hypothesis on pelletization, the evidence is not unequivocal. The olive oil processing waste is complex and contains organics such as polyphenols, sugars, polyalcohols, proteinaceous and lipid compounds (Boari et al., 1984), – there is no certainty that a pure lipid can in fact be treated in a UASB system. Similarly, in the study of Russo (1987) the waste was complex and the mass production of pellets was not predictable quantitatively so that the effects of SO$_4^{2-}$ could be evaluated only qualitatively.

In this chapter, the objectives were to enquire into the response of UASB systems to:

(1) an influent with different concentrations of the electron acceptor SO$_4^{2-}$, with glucose as sole substrate, and

(2) an influent with the defined long chain fatty acid, oleic acid, as the sole substrate.
2. BIOCHEMICAL BACKGROUND

2.1 Sulphate reduction

Sulphate reducing microorganisms (sulfdogens) utilize similar intermediate anaerobic fermentation products as the methanogens. With glucose as substrate both groups have species that utilize hydrogen or acetate as energy sources.

Within the sulfdogens the principal species mediating sulphate reduction is believed to be Desulforibrio desulphuricans (Thauer, 1982). This organism utilizes hydrogen as energy source (electron donor) and sulphate ($\text{SO}_4^{2-}$) as terminal electron acceptor. With $H_2$ as electron donor reduction of $\text{SO}_4^{2-}$ can be expressed as:

$$\text{SO}_4^{2-} + 4H_2 \rightarrow H_2S + 2H_2O + 2OH^- \quad (7.1)$$

That is, for each mole of $\text{SO}_4^{2-}$ reduced, 4 moles of $H_2$ are consumed. (In methanogenesis, where $CO_2$ is reduced, 4 moles of $H_2$ also are consumed). Equation (7.1) also shows that reduction of 1 mole $\text{SO}_4^{2-}$ by 4 moles of $H_2$ produces two moles of alkalinity (100 mg alkalinity as $CaCO_3$) i.e. alkalinity is generated and hence pH of the medium would tend to increase.

Sulfdogens such as Desulfotomaculum acetoxidans and Desulfobacter postgatei can use acetate as the energy source and $\text{SO}_4^{2-}$ as the terminal electron acceptor. With acetate as electron donor, reduction of $\text{SO}_4^{2-}$ can be expressed as:

$$\text{CH}_3\text{COOH} + \text{SO}_4^{2-} \rightarrow H_2S + 2\text{HCO}_3^- \quad (7.2)$$

That is, for each mole of $\text{SO}_4^{2-}$ reduced, 1 mole of acetate is consumed. (Stoichiometrically this reaction is similar to acetate cleavage during methanogenesis). Equation (7.2) shows that reduction of 1 mole $\text{SO}_4^{2-}$ by oxidation of 1 mole acetate generates 2 moles of alkalinity (100 mg alkalinity as $CaCO_3$).

From studies on microbial reduction, Laanbroek et al. (1981) concluded that acetate-oxidizing sulphate reducing organisms generally are not present in low salinity environments such as anaerobic digesters. Isa et al. (1986) observed that sulphate reduction was not promoted with acetate as substrate. These would indicate that sulphate reduction usually should take place with $H_2$ as the energy source.
2.2 Lipid fermentation

Lipids (fats and greases) are first hydrolysed to organic monomers such as long chain fatty acids (LCFA) and glycerol. Neutral fats are hydrolysed as follows (Hanaki et al., 1981):

\[
\begin{align*}
\text{CH}_2\text{OCOR}_1 & \quad \text{R}_1\text{COOH} & \quad \text{CH}_2\text{OH} \\
\text{CHO}_2\text{COR}_2 + 3\text{H}_2\text{O} & \rightarrow \quad \text{R}_2\text{COOH} & \quad + \quad \text{CHOH} \\
\text{CH}_2\text{OCOR}_3 & \quad \text{R}_3\text{COOH} & \quad \text{CH}_2\text{OH}
\end{align*}
\]

(neutral fat \quad long chain fatty acids \quad glycerol)

where \(R_1, R_2, \) and \(R_3\) are alkyl groups.

The hydrolysis reaction above, to yield free LCFA, is reported to be rapid (Heukelekian and Mueller, 1958). The LCFA are degraded further by the obligate proton-reducing (\(H_2\) forming) acetogenic bacteria via \(\beta\)-oxidation to short chain fatty acids (SCFA), \(CO_2\) and \(H_2\); even-carbon numbered LCFA are degraded to acetate, \(H_2\) and \(CO_2\) and odd-carbon LCFA to acetate, propionate and \(H_2\) with proton serving as the electron acceptor (McInerney et al., 1981; Jeris and McCarty, 1965). These reactions are:

Even-numbered

\[
\begin{align*}
\text{CH}_3(\text{CH}_2)_{14}\text{COO}^- & + 14\text{H}_2\text{O} & \rightarrow 8\text{CH}_3\text{COO}^- + 14\text{H}_2 + 7\text{H}^+ \quad (\text{palmitate})
\end{align*}
\]

Odd-numbered

\[
\begin{align*}
\text{CH}_3(\text{CH}_2)_{8}\text{COO}^- & + 4\text{H}_2\text{O} & \rightarrow \text{CH}_3\text{CH}_2\text{COO}^- + 2\text{CH}_3\text{COO}^- + 4\text{H}_2 + 2\text{H}^+ \quad (\text{octanoate})
\end{align*}
\]

Thermodynamically these reactions are feasible only if the hydrogen partial pressure (\(pH_2\)) is kept very low — Novak and Carlson (1970) indicated that \(H_2\) generated during the degradation of LCFA inhibited the reaction; Heukelekian and Mueller (1958) reported that LCFA were not degraded during the acid-forming phase where methane was not produced.

2.3 Oleic acid fermentation pathway

Oleic acid, an unsaturated fatty acid with an 18 carbon chain is degraded primarily
by beta-oxidation to acetate, hydrogen and carbon dioxide (Weng and Jeris, 1976). This reaction is mediated by H₂-producing acetogenic bacteria. Weng and Jeris (1976) found that either acetic acid only or acetic plus propionic acids can be produced during anaerobic fermentation of oleic acid. They proposed two biochemical pathways for the fermentation of oleic acid to explain the two respective processes. These two pathways are set out in Fig 7.1 and 7.2 respectively.

In both pathways the reactions are thermodynamically unfavourable unless the partial pressure of hydrogen (pH₂) is maintained at an extremely low level.

3. EFFECT OF SULPHATE
A UASB reactor, effective volume 9ℓ, was seeded with 3ℓ of pelletized sludge obtained from a UASB reactor, at 30°C, fed with glucose. Glucose was used as the sole organic carbon source with COD concentration approximately 5000 mg/ℓ. The feed was supplemented with trace elements and nutrients for organism growth (details of composition are given in Chapter 3), with excess NH₃-N, and buffered by addition of 1,6 mg alkalinity as CaCO₃ per mg influent COD. The loading on the reactor was maintained at 8,3 kgCOD/m³ reactor/d (influent COD concentration = 5000 mg/ℓ, flow rate: 15ℓ/d). The feed was supplemented with SO₄²⁻ in the form of anhydrous sodium sulphate. The effects of different SO₄²⁻ concentrations were investigated for the range 100-5000 mgSO₄²⁻/ℓ.

The pelletized sludge initially was acclimatized to a sulphate concentration of 100 mgSO₄²⁻/ℓ. In less than three weeks 90 per cent of the SO₄²⁻ was reduced to sulphide and the overall COD, NH₃-N and SO₄²⁻ removals remained constant. It was accepted therefore that steady state had been established. (Subsequently on average it was found that after each incremental increase in SO₄²⁻ concentration, three weeks were needed before steady state was reattained).

Once a steady state had been attained, samples were taken along the line of flow and measurements made of: total soluble COD, short chain fatty acids, free and saline ammonia, total Kjeldahl nitrogen (hence organic nitrogen by difference), SO₄²⁻ and pH. Measurements were made according to the methods described in Chapter 3. Sulphate concentration was determined by the turbidimetric method as outlined in Standard Methods (1985). For measurement of total soluble COD, the sample first was stripped of the sulphide by bubbling nitrogen gas through the sample (the S²⁻ is oxidized to SO₄²⁻ during the COD test and hence will given an inflated COD value).
Possible pathway for oleic acid fermentation producing propionic and acetic acids (after Weng and Jeris, 1976).
Fig 7.2: Possible pathway for oleic acid fermentation producing acetic acid only (after Weng and Jeris, 1976).
4. RESULTS

4.1 Single UASB system

With glucose as substrate at a constant loading of 8.3 kgCOD/m³/d (influent concentration ≈ 5000 mgCOD/l, flow rate: 15l/d), six SO₄⁻ concentrations were tested i.e. (i) 100, (ii) 200, (iii) 400, (iv) 1000, (v) 3000 and (vi) 5000 mgSO₄²⁻/l.

Profiles of the parameters: soluble COD, propionate, acetate, NH₃-N, organic nitrogen (orgN), SO₄⁻ and pH, measured along the line of flow in the reactor, are shown in Figs 7.3 to 7.8 for the different SO₄⁻ concentrations. With each SO₄⁻ concentration the system attained stability and the COD removal remained above 90 per cent.

The concentration profiles (Figs 7.3 to 7.8) show trends of behaviour similar to those of a UASB system with glucose as substrate but with no SO₄⁻ present in the feed. From Figs 7.3 to 7.8 and Table 7.1, in the lower active zone (up to sampling port No.1), as SO₄⁻ concentration increased from 100 to 5000 mg/l in the influent feed so the magnitudes of some parameters were increasingly affected. At sampling port No.1:

- All the acetate and propionate profiles showed maxima but the maxima decreased with increased influent SO₄⁻.

- For SO₄⁻ concentrations below 400 mg/l, except for a relatively small constant residual (~10-20 mgSO₄²⁻/l) all of the SO₄⁻ was removed from solution, that is, SO₄⁻ removal was virtually equal to the influent SO₄⁻ (see Fig 7.9); for SO₄⁻ concentrations of 1000 up to 5000 mg/l, a relative constant mass of SO₄⁻ from 590 to 610 mgSO₄²⁻ was removed (Fig 7.9). With the fixed COD loading applied, in this range of SO₄⁻ loading, a maximum SO₄⁻ removal was achieved (~600 mgSO₄²⁻/l), i.e. a specific COD loading has associated with it a specific maximum capacity for SO₄⁻ removal.

- Ammonia removal (ΔNH₃-N) decreased linearly with regard to SO₄⁻ removal (ΔSO₄⁻), from 86.8 mgN/l to 64.9 mgN/l (see Fig 7.10).

- COD removal (ΔCOD) increased linearly with ΔSO₄⁻, from 2979 to 3596 mgCOD/l, for ΔSO₄⁻ from 90 to 600 mg/l (see Fig 7.11).

- Short chain fatty acids (SCFA) concentrations, both acetate and propionate,
Fig 7.3: Concentration and pH profiles observed in single UASB system with glucose substrate and sulphate (influent COD concentration = 5910 mg/l, flow rate = 15 l/d; influent SO\textsubscript{4}\textsuperscript{2-} concentration = 100 mgSO\textsubscript{4}\textsuperscript{2-}/l).
Concentration and pH profiles observed in single UASB system with glucose substrate and sulphate (influent COD concentration = 5202 mg/l, flow rate = 15 l/d; influent SO$_4^{2-}$ concentration = 192 mgSO$_4^{2-}$/l).
Concentration and pH profiles observed in single UASB system with glucose substrate and sulphate (influent COD concentration = 5356 mg/l, flow rate = 15 l/d; influent SO\(_4\) concentration = 400 mgSO\(_4\)/l).

Fig 7.5:
Concentration and pH profiles observed in single UASB system with glucose substrate and sulphate (influent COD concentration = 5531 mg/l, flow rate = 15 l/d; influent SO\textsuperscript{2-} concentration = 1000 mgSO\textsuperscript{2-}/l).
Concentration and pH profiles observed in single UASB system with glucose substrate and sulphate (influent COD concentration = 5457 mg/l, flow rate = 15 l/d; influent SO$_4^-$ concentration = 3000 mgSO$_4^-$/l).
Fig 7.8: Concentration and pH profiles observed in single UASB system with glucose substrate and sulphate (influent COD concentration = 5422 mg/l, flow rate = 15l/d, influent SO\textsuperscript{4}\textsuperscript{-} concentration = 5000 mgSO\textsuperscript{4}\textsuperscript{-}/l).
### Table 7.1: Response of the lower active zone of a single UASB reactor to SO$_4^{2-}$ concentrations.

<table>
<thead>
<tr>
<th>SO$_4^{2-}$ mg/l</th>
<th>ΔCOD mgCOD/l</th>
<th>ΔNH$_3$ mgN/l</th>
<th>ΔSO$_4^{2-}$ mg/l</th>
<th>HAc mg/l</th>
<th>HPt mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2272</td>
<td>89.2</td>
<td>0</td>
<td>1244</td>
<td>944</td>
</tr>
<tr>
<td>100</td>
<td>2979</td>
<td>86.8</td>
<td>92</td>
<td>867</td>
<td>833</td>
</tr>
<tr>
<td>200</td>
<td>3014</td>
<td>79.8</td>
<td>180</td>
<td>955</td>
<td>777</td>
</tr>
<tr>
<td>400</td>
<td>3353</td>
<td>73.7</td>
<td>379</td>
<td>578</td>
<td>444</td>
</tr>
<tr>
<td>1000</td>
<td>3455</td>
<td>66.5</td>
<td>590</td>
<td>231</td>
<td>371</td>
</tr>
<tr>
<td>3000</td>
<td>3572</td>
<td>65.6</td>
<td>620</td>
<td>244</td>
<td>356</td>
</tr>
<tr>
<td>5000</td>
<td>3596</td>
<td>64.9</td>
<td>615</td>
<td>256</td>
<td>312</td>
</tr>
</tbody>
</table>
Fig 7.9: \( \text{SO}_4^{2-} \) removal versus influent \( \text{SO}_4^{2-} \) concentration in the lower active zone of a UASB system with glucose as substrate.

Fig 7.10: \( \text{NH}_3-N \) removal versus influent \( \text{SO}_4^{2-} \) removal in the lower active zone of a UASB system with glucose as substrate.
Fig 7.11: COD removal versus influent $SO_4^{2-}$ removal in the lower active zone of a UASB system with glucose as substrate.

Fig 7.12: Short chain fatty acid concentrations versus $SO_4^{2-}$ removal in the lower active zone of a UASB system with glucose as substrate.
decreased linearly with $\Delta SO_4^{2-}$, acetate from 867 to 256 mgHAc/l and propionate from 833 to 312 mgHPr/l (see Fig 7.12).

- For the same mass of alkalinity in the feed, over the range of $SO_4^{2-}$ concentration, the pH was virtually unaffected by the $SO_4^{2-}$ concentration; the pH in the influent dropped from 8.33 to about 6.6 to 6.8 at port No.1 where the lowest pH in the profiles was observed, thereafter it increased sharply to a stable value between 7.3 and 7.4.

- Pellet size decreased with increasing influent sulphate concentration from 2-3 mm in diameter ($SO_4^{2-} \leq 400$ mg/l) to 1-2 mm in diameter ($SO_4^{2-} \geq 1000$ mg/l). Concomitantly the sludge bed volume decreased by about 700 ml, attaining a stable low volume at concentrations of 1000 mgSO$_4^{2-}$/l and above.

The data points to the following behaviour pattern: The observation that propionate was always present even at the highest $SO_4^{2-}$ influent concentrations (from 1000 to 5000 mg/l) would indicate that a high $\hat{p}H_2$ always was generated in the lower active zone of the UASB reactor, and accordingly pellet generation would take place. One may ask why is the $\hat{p}H_2$ not completely suppressed at high $SO_4^{2-}$? An explanation is possible from the investigations of Kristjansson et al. (1982) and Kristjansson and Schönheit (1983). Kristjansson et al. (1982) showed that at high $\hat{p}H_2$ (high H$_2$ substrate concentration) both methanogenesis and sulphate reduction (both using hydrogen as the energy source) can take place simultaneously. They observed that at high $\hat{p}H_2$ the ratio of the rates of production of H$_2$S to CH$_4$ (H$_2$S/CH$_4$) in mixed cell suspensions in D. vulgaris (a sulphate reducer utilizing H$_2$ as energy source) and M. Strain AZ was H$_2$S/CH$_4 = 1$; when $\hat{p}H_2$ was lowered the rate ratio H$_2$S/CH$_4$ increased to 5 demonstrating that at low $\hat{p}H_2$, the sulfidogen would tend to dominate over the hydrogenotrophic methanogens. In the experiment reported in this chapter, at the bottom of the reactor where $\hat{p}H_2$ is high the methanogens therefore can compete successfully for H$_2$ against the sulfidogens. With excess SO$_4^{2-}$, it would appear that the methanogens utilized a relatively constant fraction of the H$_2$ generated: In the three profiles at SO$_4^{2-}$ concentration of 1000, 3000 and 5000 mg/l, at port No.1, (i) the propionate concentration remained virtually constant, and (ii) NH$_3$-N uptake was approximately constant. Furthermore the sludge bed volume also remained constant. With regard to NH$_3$-N uptake, Fig 7.10 shows that NH$_3$-N uptake is linearly related to the SO$_4^{2-}$ removed; the higher the SO$_4^{2-}$ removal the lower the NH$_3$-N uptake. This behaviour is a direct consequence of the decreasing
availability of hydrogen to the hydrogenotroph *M. Strain AZ* due to the increasing removal of \( \text{H}_2 \) by the sulfidogens. (Because there is a limit to the mass of \( \text{SO}_4^- \) removal there is also a lower limit to \( \text{NH}_3-\text{N} \) removal).

4.2 High \( \text{pH}_2 \) UASB system

In order to obtain an estimate of the VSS yield with excess \( \text{SO}_4^- \) in the influent, two 3l UASB reactors were set up to operate in parallel as high \( \text{pH}_2 \) reactors. The first reactor was fed a trace of \( \text{SO}_4^- \) (1,06 mg\( \text{SO}_4^-/l \)) sufficient for normal anaerobic growth. The second reactor was fed an excess of \( \text{SO}_4^- \) (5000 mg\( \text{SO}_4^-/l \)). The first reactor was seeded with pelleted sludge obtained from the lower active zone of a single UASB system fed with glucose substrate (influent COD concentration \( \approx 5000 \) mg/l, flow rate: 15l/d, \( \text{NH}_3-\text{N}: 173,6 \) mgN/l); the reactor was seeded up to the 2nd port (equivalent to port No.2 in a single UASB system), a volume of 0,7l. The second reactor likewise was seeded with 0,7l of sludge obtained from the lower active zone of the single UASB system described in the previous section, also with glucose as substrate (influent COD concentration \( \approx 5000 \) mg/l, flow rate: 15l/d, \( \text{NH}_3-\text{N}: 173,6 \) mgN/l, \( \text{SO}_4^- \approx 5000 \) mg/l). Both systems that supplied the seed sludges had been operating in a stable state for six months.

In Table 7.2 are listed the responses of the two high \( \text{pH}_2 \) systems at excess and trace \( \text{SO}_4^- \) concentrations. Taking the system with trace \( \text{SO}_4^- \) as the reference, the system with excess \( \text{SO}_4^- \) exhibited the following deviant responses:

- Mass COD removed per day was greater by 11,88 gCOD/d; from 29,18 to 42,06 gCOD/d.
- \( \text{NH}_3-\text{N} \) removal decreased markedly from 86,8 to 58 mgN/l.
- Dissolved \( \text{orgN} \) in the effluent was significantly lower, from 33,6 to 4,1 mgN/l.
- SCFA concentrations were lower; acetate from 987 to 690 mg\( \text{HAc}/l \) and propionate from 815 to 495 mg\( \text{HPr}/l \).
- VSS wasted from the system was in the form of fines with some granules of smaller diameter.
- Net VSS yield declined from \( 8,3 \cdot 10^{-2} \) to \( 1,6 \cdot 10^{-2} \) mgVSS/mgCOD influent.
Table 7.2: Responses of the high pH reactor at excess and at limiting SO$_2^-$ influent concentrations.

<table>
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<tr>
<th></th>
<th>Excess SO$_2^-$ system</th>
<th>Limited SO$_2^-$ system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading (kgCOD/d)</td>
<td>0.076</td>
<td>0.077</td>
</tr>
<tr>
<td>COD in (mg/l)</td>
<td>5080</td>
<td>5100</td>
</tr>
<tr>
<td>COD out (mg/l)</td>
<td>2276</td>
<td>3185</td>
</tr>
<tr>
<td>COD removed per day (gCOD/d)</td>
<td>42.06</td>
<td>29.18</td>
</tr>
<tr>
<td>COD removal (%)</td>
<td>55</td>
<td>43</td>
</tr>
<tr>
<td>NH$_3$-N (mgN/l) In</td>
<td>173.6</td>
<td>173.6</td>
</tr>
<tr>
<td>NH$_3$-N (mgN/l) Out</td>
<td>115.6</td>
<td>86.8</td>
</tr>
<tr>
<td>Dissolved orgN—effluent (mgN/l)</td>
<td>4.1</td>
<td>33.6</td>
</tr>
<tr>
<td>Volume of sludge wasted (ml/d)</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>VSS generated (mgVSS/d)</td>
<td>1224</td>
<td>6361</td>
</tr>
<tr>
<td>Net yield (mgVSS/mg influent COD)</td>
<td>1.6·10$^{-2}$</td>
<td>8.3·10$^{-2}$</td>
</tr>
<tr>
<td>Nature of VSS generated</td>
<td>mainly fines with some granules of $\phi \approx 0.5-1.0$</td>
<td>mainly granules with $\phi$ between 1 and 3mm</td>
</tr>
<tr>
<td>Sludge VSS (gVSS/l)</td>
<td>30.40</td>
<td>35.34</td>
</tr>
<tr>
<td>Sludge TSS (gTSS/l)</td>
<td>33.17</td>
<td>37.95</td>
</tr>
<tr>
<td>SCFA (effluent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate (mgHAc/l)</td>
<td>690</td>
<td>987</td>
</tr>
<tr>
<td>Propionate (mgHPr/l)</td>
<td>495</td>
<td>815</td>
</tr>
<tr>
<td>Butyrate (mgHBr/l)</td>
<td>~</td>
<td>~</td>
</tr>
</tbody>
</table>

Note: $\Delta$ represents difference between influent and port 1 values.
With regard to the reported yield values the VSS yield parameter mgVSS/mgCOD removed is not the appropriate one for comparative purposes. It is deficient for the following reasons: The volume of the sludge bed in the high pH reactor was 0.7. This volume is in excess of the volume comprising the high pH zone. However at the time the experiment was undertaken it was not certain if the pH zone extended to between sample ports Nos.1 and 2; to ensure that the whole pH zone was present the sludge up to sampling port No.2 was used as the seed sludge for the high pH reactor. However at sampling port No.2 in the single UASB reactor, the results obtained clearly show that the propionate concentration already was reduced to a low value, indicating that pH was sufficiently low that propionate conversion to acetate, CO₂ and hydrogen was thermodynamically feasible. As a consequence some COD removal due to CH₄ generation had taken place, to some unknown degree. For comparative purposes the VSS yield rather should be expressed in terms of some constant COD parameter such as the influent COD. In Table 7.2 the specific VSS yields for the two high pH systems are expressed in terms of the influent COD, viz. 8.3·10⁻² and 1.6·10⁻² mgVSS/mg influent COD for the trace SO₄⁻ and excess SO₄⁻ systems respectively. That is, the VSS yield for the trace SO₄⁻ system was more than 5 times greater than the VSS yield of the excess SO₄⁻ system. [Note that the yield for the trace SO₄⁻ system was slightly less than that obtained in the high pH system (10.4·10⁻² mgVSS/mg influent COD) in Chapter 6. The reason for this is that in the trace SO₄⁻ system, the sludge bed included both high and low pH zones and hence some destruction of the VSS can be expected in the low pH zone of the bed. However in Chapter 6 the VSS yield determination was made on the sludge bed comprising only a high pH zone so that pellet destruction was at a minimum].

The decrease in orgN concentration (from 33.6 mgN/l for the SO₄⁻ trace system to 4.1 mgN/l for the SO₄⁻ excess system) is in conformity with the hypothesis on pelletization in that a decrease in the generation and release of amino acids by M. Strain AZ can be expected due to a reduction in hydrogen availability in the SO₄⁻ excess system.

From the discussions above the behavioural pattern of a UASB system with glucose as substrate, is crucially influenced by the pH. Sulphate reduction, because it abstracts hydrogen, is the main cause for modifying the pH. Recognizing this, the observed reduction in pellet formation and organic nitrogen generation can be

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In Chapter 6 this parameter was used, but it was for different purposes - the COD fraction utilized in the generation of the pelletized sludge mass.
explained in terms of the hypothesis on pelletization proposed in Chapter 5. The experimental results showed that (i) the presence of SO\textsuperscript{2}\textsuperscript{-} in the influent feed of a UASB system with glucose as substrate will reduced pellet formation and, (ii) this reduction is in conformity with the predictions of the hypothesis on pelletization.

5. SINGLE UASB SYSTEM WITH OLEIC ACID AS SUBSTRATE

5.1 Substrate selection

In the introduction it was noted that hydrolysis of a lipid to its monomers (LCFA) is rapid, the limiting rate in the fermentation of a lipid to methane is in the conversion of LCFA to SCFA. Hence in studying the response of a UASB system to a lipid substrate one needs only to use the monomer as the influent substrate.

Of the LCFA, oleic acid was selected as an appropriate substrate because:

(i) It is more soluble than other LCFA at room temperature,

(ii) Its degradation rate is reported to be faster than any of the saturated LCFA (Vishwanathan et al., 1962) and,

(iii) Its exact metabolic pathway has been described (Weng and Jeris, 1976).

5.2 Experimental

The behaviour of a UASB system with oleic acid as substrate was investigated at 30°C. A 9l UASB reactor was seeded with 3l of pelletized sludge obtained from a UASB reactor at 30°C which had been fed with a glucose substrate (COD concentration \(\approx 2500 \text{ mg/}l\); flow rate: 30l/d. To acclimatize the seeding sludge, a mixture consisting of glucose (COD: 2000 mg/\( l \)) and oleic acid (COD: 500 mg/\( l \)) with total COD 2500 mg/\( l \) was fed at a flow rate of 15l/d (i.e. organic loading: 4.17 kgCOD/m\( ^3 \)/d). The feed was supplemented with trace elements and essential nutrients (detailed compositions are given in Chapter 3); the nutrient NH\textsubscript{3}-N was supplemented in excess (68 mgN/\( l \)). For pH control, the influent was buffered by addition of 100g NaHCO\textsubscript{3} per 15l of feed. The feed was made up with warm tap water to ensure that the oleic acid dissolved completely. The feed bucket was kept at room temperature (20°C) and the bucket contents continuously stirred.

Over a period of about 5 months the oleic acid concentration in the feed was increased in steps of 500 mgCOD/\( l \), and simultaneously the glucose concentration
was decreased by 500 mgCOD/l, until the feed consisted only of oleic acid (2500 mgCOD/l). A step change in feed composition was effected only after the overall COD removal had shown a stable response for five consecutive days. As the oleic acid fraction increased in the feed, the overall COD removal decreased, and when the feed consisted of 100 per cent oleic acid (2500 mgCOD/l), the overall COD removal stabilized around 65 per cent.

Initially when the feed consisted of glucose (2000 mgCOD/l) and oleic acid (500 mgCOD/l), 100g NaHCO₃ per 15l of feed were required to maintain the pH around 7.2 in the liquid above the sludge bed. As the oleic acid fraction in the feed increased so the pH in the reactor tended upwards and the mass of NaHCO₃ added had to be decreased to maintain the pH around 7.2. By the time the feed consisted of 100 per cent oleic acid, the mass of NaHCO₃ added had been reduced to less than one quarter of the initial mass added. However even with this low alkalinity supplementation, the pH continued to increase to above 7.7. At this stage however it was decided that the NaHCO₃ mass addition should not be reduced further (less than 25g NaHCO₃ per 15l influent), to ensure that a CO₂ limitation (for methanogenesis from H₂) may not arise. Accordingly the minimum NaHCO₃ addition was fixed at 25g/15l influent and the pH controlled by addition of a strong acid, HCl. With 100 per cent oleic acid in the feed, addition of 100 ml 1% (v/v) HCl to 15l of feed was sufficient to keep the pH of the reactor at approximately 7.3.

5.3 Results
The UASB reactor fed with 100 per cent oleic acid as substrate, at a loading of 4.17 kgCOD/m³/d (influent concentration 2500 mgCOD/l, flow rate: 15l/d), operated in a stable state with removals from influent to effluent of: ΔCOD = 65 per cent and ΔNH₃-N = 5.2 mgN/l. Effluent organic nitrogen was 1.9 mgN/l, pH of the reactor 7.40 and effluent acetate: 13 mgHAc/l. Concentration profiles, of soluble COD, SCFA, NH₃-N, orgN and pH, measured along the line of flow in the reactor are shown in Fig 7.13 (a, b and c). The profiles exhibit:

- No distinct zones of behaviour (c.f. profiles with glucose as substrate, Fig 6.5)
- No propionate and very little acetate concentration, maximum 47 mgHAc/l.
- Very low NH₃-N removal, 5.2 mgN/l.
Concentration and pH profiles observed in single UASB system with oleic acid substrate (influent COD concentration = 2518 mg/l, flow rate = 15/d).

Fig 7.13.
• A relatively low percentage COD removal, 65 per cent.

• Virtually no pH change in the sludge bed.

With regard to the behaviour of the sludge bed, as the fraction of oleic acid in the feed increased so the pellets in the sludge bed progressively disintegrated. However pellet debris discharged to the liquid above the sludge bed was not observed. The pellets and the debris appeared to be encapsulated by the gelatinous mass. By the time the feed consisted of 100 per cent oleic acid, the sludge had changed to a uniform gelatinous mass, off white in colour. Microscopic examination of the mass, however, indicated that pellet debris was still present but did not show up in the visual appearance of the sludge. The bed volume of the sludge decreased over the course of the experiment (about 6 months) and with 100 per cent oleic acid feed stabilized at a volume of about 2.4l from an initial bed volume of 3l. The density of the sludge was low, ≈ 15 000 mgVSS/l compared to that observed in the pelletized bed (about 35 000 mgVSS/l). However the sludge mass was well defined with a compact appearance; did not appear to be disturbed under the shear action of escaping gas bubbles — the bubbles travelled up the bed leaving a well defined trail which slowly closed up. Neither did the sludge mass shed fines so that no sludge blanket formed above the bed.

5.4 Discussion

Weng and Jeris (1976) proposed two detailed metabolic pathways for the degradation of oleic acid whereby the SCFA generated could be either propionate and acetate or acetate only. They found that propionate was produced only in trace amount occasionally and concluded that the pathway producing acetate only is the predominant one. In this investigation with 100 per cent oleic acid in the feed, the acetate and propionate bed profiles (Fig 7.13a) show that only acetate was observed; this would indicate that the metabolic pathway producing acetate only was the predominant one. Even if propionate was generated this would not be indicative of a high \( \hat{p}H_2 \). The production of SCFA such as propionate and butyrate during the fermentation of odd-carbon number LCFA can only take place under low \( \hat{p}H_2 \) conditions, hence the appearance of propionate or butyrate will indicate that most likely the LCFA has been fermented. Accepting that fermentation of a lipid to methane can take place only if \( \hat{p}H_2 \) is low; one of the requirements for pelletization is not fulfilled and pellet formation cannot be expected to take place. In the absence of direct measurement of \( \hat{p}H_2 \) this behaviour is confirmed by: (i) the low uptake of
NH₃-N and, (2) the low production of organic nitrogen in the bed. In terms of the hypothesis on pelletization, the NH₃-N uptake and organic nitrogen production should have been high if pellet formation had taken place.

The interesting feature in the treatment of oleic acid in the UASB system is that a well defined sludge bed was formed, but of a gelatinous texture. This type of bed and its texture appears to be very similar to that obtained in a UASB system treating an olive oil processing waste water (Boari et al., 1984). Furthermore Boari et al. (1984) noted that the sludge bed condition was very poised; if disturbed by mechanical agitation the integrity of the bed was destroyed and the bed was lost in the effluent.

6. CONCLUSIONS
The following conclusions can be summarized:

1. In UASB systems with glucose substrate pellet formation was reduced markedly when sulphate (SO₄²⁻), an electron acceptor, was added to the influent feed. Under normal operating conditions even though the fermentation of glucose produces hydrogen sufficiently fast to generate a high pH₂, sulphate reducers utilize hydrogen preferentially thereby reducing pH₂ and hence limiting pelletization. This is supported by the following experimental observations:

(a) The reduction in the NH₃-N uptake as SO₄²⁻ increased. NH₃-N decreased linearly with SO₄²⁻ removal (ΔSO₄²⁻).

(b) COD removal increased linearly with (ΔSO₄²⁻).

(c) Short chain fatty acids concentrations, both acetate and propionate, decreased linearly with ΔSO₄²⁻. The reduction in propionate concentration indicated that the pH₂ was sufficiently low so that acetogenesis took place.

(d) Pellet size decreased with increasing influent SO₄²⁻ concentration from 2-3 mm in diameter (SO₄²⁻ ≤ 400 mg/l) to 1-2 mm in diameter (SO₄²⁻ ≥ 1000 mg/l). Concomitantly the sludge bed volume decreased by about 700 ml, attaining a stable low volume at concentrations of 1000 mgSO₄²⁻/l and above.
(e) In the high \( \tilde{\text{p}}\text{H}_2 \) reactors: the VSS yields were \( 8,3 \cdot 10^{-2} \) and \( 1,6 \cdot 10^{-2} \) mgVSS/mg influent COD for the trace and excess SO\( _4^- \) systems respectively; i.e. the VSS yield for the trace SO\( _4^- \) system was more than 5 times greater than that of the excess SO\( _4^- \) system. The NH\( _3^-\text{N} \) uptake decreased from 86,8 to 58 mgN/l with a concomitant reduction of organic nitrogen generated from 33,6 to 4,1 mgN/l for the trace SO\( _4^- \) and excess SO\( _4^- \) systems respectively. This behaviour is in conformity with the hypothesis on pelletization in that a decreased in the NH\( _3^-\text{N} \) uptake, with a concomitant reduction in the generation and release of amino acids by \( M. \) Strain AZ can be expected due to a reduction in hydrogen availability in the SO\( _4^- \) excess system.

2. In a UASB system with oleic acid as substrate, pelletization is not promoted. Fermentation of oleic acid to methane can take place only if \( \tilde{\text{p}}\text{H}_2 \) is low; accepting this, one of the requirements for pelletization is not fulfilled and pellet formation will not take place. This behaviour is confirmed from concentration profiles as follows:

(a) no distinct zones of behavior along the line of flow

(b) low NH\( _3^-\text{N} \) uptake (5,2 mgN/l)

(c) low generation of organic nitrogen (2,8 mgN/l)

(d) acetate was the only SCFA detected. This is in conformity with the metabolic pathway for the fermentation of oleic acid where acetate only is formed (Weng and Jeris, 1976).

The sludge bed formed with oleic acid substrate was well defined but of a gelatinous texture. This type of bed and its texture appears to be very similar to that obtained in a UASB system treating an olive oil processing waste water (Boari et al., 1984).
CHAPTER 8

EFFECTS OF A RECYCLE IN UASB SYSTEMS

1. INTRODUCTION

In the pH profile up the sludge bed of a UASB reactor treating apple juicing waste water, characteristically the pH commenced to decline, from the influent entry at the bottom, to a minimum at the point in the bed where the short chain fatty acids (SCFA) concentrations attained maxima. Thereafter the pH increased monotonically to some stable value in the upper part of the bed. In this study when the minimum pH declined to below 6.6, the overall COD removal efficiency decreased and the SCFA concentrations in the upper part of the bed increased, i.e. the system showed signs of incipient failure.

To limit the pH decline, alkalinity was added to the influent; about 1.6 mg alkalinity as CaCO$_3$/mg influent COD was necessary to maintain the minimum pH above 6.6.

In a normal functioning completely mixed anaerobic fermentation system the net alkalinity consumption is virtually zero. The reason for this is that the system operates at a low hydrogen partial pressure and at a relatively low average organic loading per unit mass of sludge; accordingly very little or no SCFA other than acetic acid are generated. With regard to the acetic acid generated, it is converted to methane at the rate generated, that is, from this source there is virtually no acidity generated and hence no alkalinity is required to counter the acidity effect. The only other source of acidity is the removal of free and saline ammonia (NH$_3$-N) for sludge production; this also is relatively minor in completely mixed anaerobic systems. In consequence usually no alkalinity addition to counter the acidity effects is necessary. However, the situation may be quite different in semi-plug or plug flow systems. In the UASB system treating carbohydrate wastes firstly there is a net loss of alkalinity due to a substantial removal of NH$_3$-N converted to organic nitrogen for pelletized bed formation. Secondly in the pelletized bed there is a partial phase separation of acidogenesis and methanogenesis giving rise to a build up of SCFA in the lower active zone of the bed. In this zone the SCFA generated remove a significant concentration of alkalinity giving rise to an associated drop in pH. In the upper active zone of the bed the SCFA generated is converted to methane and
alkalinity is recovered with an increase in pH — from influent to effluent there is a minimal net alkalinity loss in the system. Thus the function of alkalinity supplementation is principally to control the pH decline in the lower active zone — the alkalinity supplementation in the influent in effect eventually is wasted in the effluent.

Young and McCarty (1967) in their study of upflow anaerobic filters (operated as flow-through systems) observed a decline in pH at the bottom of the filter bed and subsequent recovery of the pH in the upper part. These filters operated as plug flow systems and, as in UASB systems, there was a phase separation of acidogenesis and methanogenesis. They observed a reduction in COD removal efficiency when the minimum pH declined to about 6.2. In these respects the filter and UASB systems appear to behave in a similar fashion.

In their study with protein-carbohydrate wastes, Young and McCarty (1967) found that at influent COD concentrations of 1500 and 3000 mg/l, alkalinity addition of 1500 mg/l as CaCO₃ was sufficient to maintain the pH above 6.6, i.e. 1 to 1 mg alkalinity as CaCO₃/mgCOD influent. Compared to the alkalinity addition found necessary in the UASB systems in this study (1.2 to 1.6 mg alkalinity as CaCO₃/mgCOD influent) their alkalinity addition was relatively small. Most likely this was due to the additional buffer capacity in the form of ammonia generated during fermentation of the proteinaceous component of the wastes, also more alkalinity was required for the UASB system due to the acidic nature of the apple waste water.

Capri (1973) when studying upflow anaerobic filters treating strong spent wine wastes (COD ~ 23 000 mg/l), countered the decline of pH by instituting a very high recycle ratio (recycle ratio ~ 35:1) without alkalinity supplementation. By this means the pH in the filter could be kept near 7 throughout the filter and allow treatment of the waste up to 4 kgCOD/m³/d. He noted that lower recycle ratios also might have been adequate. Cronje (1973) investigated the fermentation of glucose-starch wastes in upflow anaerobic filters with influent COD ~ 7000 mg/l; he observed a decline in pH in the lower region of the filter and found that the pH decline could be controlled by imposing a recycle. Relatively low recycle ratios of 0.9:1 were sufficient to control the minimum pH in the filter to 6.4 at the highest loading (~ 5 kgCOD/m³/d).¹

¹At loading > 4 kgCOD/m³/d, Cronje (1973) observed a massive increase in VSS
The success achieved in pH control by instituting a recycle on the filters raised the question whether recycling also would act as a pH control measure in UASB systems. However, whereas in the filter system the objective was only to maintain pH above 6.6, in the UASB system a minimum pH > 6.6 was required but without disturbing the high hydrogen partial pressure region. No information was available on the effect of the recycle on the intensity and extent of the high pH region.

This chapter records a study on the effect of recycling on the response of a UASB system with apple concentrate substrate at 30°C.

2. EXPERIMENTAL
In this investigation the same UASB reactor system was used as that described in Chapter 3. This system had been in operation for a number of months with diluted apple juice as substrate at a loading of 9.6 kgCOD/m³/d. For the purpose of this investigation the sludge bed volume was set at 3l because experience with this system had shown that with this sludge mass the biological reactions were complete well before the top of the sludge bed. The substrate was apple juice concentrate suitably diluted to the influent concentration to be tested. Nutrients and trace metal supplementations were as described in Chapter 3. The recycle stream was taken from the region above the bed at the sample port closer to the settler (see Fig 3.1). A multi channel variable speed peristaltic pump was used both for the influent stream to the reactor and the recycle stream. The recycle tube was connected to the influent feed tube approximately 85 cm from where the combined stream entered the bottom of the reactor.

3. EXPERIMENTAL INVESTIGATIONS
3.1 Preliminary experiments
The objective of this experimental series was to investigate what effects a recycle would have when imposed on a system that was operating efficiently without a recycle. The conditions under which the system was operated without a recycle are listed under Experiment 1, Table 8.1. Concentration profiles on the system are shown in Fig 8.1 (a, b and c). Note that the alkalinity supplementation 1.6 mgAlk as CaCO₃/mg influent COD, by addition of NaHCO₃, was sufficient to maintain a minimum pH of 6.71.
Concentration and pH profiles observed in single UASB system with apple concentrate substrate. (Influent COD concentration = 2872 mg/l, flow rate = 30 l/d, recycle ratio = 0).

Fig 8.1:
### Table 8.1: Process specifications and responses for a single UASB system with apple juice substrate (Temperature: 30°C).

<table>
<thead>
<tr>
<th>Exp No.</th>
<th>Flow rate (l/d)</th>
<th>Influent COD (mg/l)</th>
<th>Loading (kgCOD/m³/d)</th>
<th>Recycle ratio</th>
<th>Alkalinity addition per mgCOD influent as CaCO₃/mgCOD</th>
<th>Effective alkalinities mgalk as CaCO₃/mgCOD</th>
<th>Overall COD removal (%)</th>
<th>Minimum pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>2500</td>
<td>9.6</td>
<td>0:1</td>
<td>1.6</td>
<td>1.6</td>
<td>96</td>
<td>6.71</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>2500</td>
<td>9.6</td>
<td>1:1</td>
<td>1.6</td>
<td>3.2</td>
<td>97</td>
<td>7.01</td>
</tr>
<tr>
<td>3*</td>
<td>30</td>
<td>5000</td>
<td>16.7</td>
<td>0:1</td>
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<td>0.85</td>
<td>82</td>
<td>6.11</td>
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<tr>
<td>4**</td>
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<td>4250</td>
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<td>2.0</td>
<td>97</td>
<td>6.64</td>
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<tr>
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<td>14.6</td>
<td>1:1</td>
<td>0.71</td>
<td>1.26</td>
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<td>6.81</td>
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<td>8500</td>
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<td>2:1</td>
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<td>1.35</td>
<td>96</td>
<td>6.82</td>
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<tr>
<td>12</td>
<td>15</td>
<td>8500</td>
<td>14.6</td>
<td>3:1</td>
<td>0.46</td>
<td>1.84</td>
<td>92</td>
<td>6.81</td>
</tr>
</tbody>
</table>

* SCFA in effluent: HAc = 100 mg/l; HPr = 543 mg/l
** SCFA in effluent: HAc = 53 mg/l; HPr = 150 mg/l
*** Effective alkalinity = (Base inf Alk + eff Alk + recycle flow) / (flow + recycle flow)
A recycle of 1:1 was now imposed upon the system (Exp.2, Table 8.1). Profiles of the response are shown in Fig 8.2(a, b and c). The minimum pH increased from 6.71 to 7.01. The alkalinity concentration in the effluent remained approximately the same as the concentration in the base influent flow. Thus the alkalinity concentration of the combined flow (influent flow + recycle flow) was virtually equal to that in the base influent flow but the mass of alkalinity in the combined flow now was twice the mass in the base flow. Comparing the two sets of profiles (Figs 8.1 and 8.2) the SCFA concentration profiles were reduced to slightly less than half their former values. This was due to the diluting effect of the combined flow. However the SCFA fluxes (flow x concentration) at the maximum (sample port 1) for both systems remained approximately equal. In the lower active zone of the recycle system because the alkalinity/SCFA ratio had increased the minimum reactor pH also increased. In the upper active zone where the SCFA in both systems had been reduced virtually to zero, the pH of both systems was near identical because the alkalinity concentrations were the same.

From this experiment it was concluded that alkalinity supplementation per influent COD could be reduced by recycling from the effluent to the influent, but it was not clear by how much alkalinity supplementation could be reduced. Accordingly the influent COD concentration was increased from 2500 to 5000 mg/l, but the mass alkalinity addition was maintained as before i.e. the alkalinity/COD ratio was reduced from 1.6 to 0.85 mgAlk as CaCO₃/mg influent COD (see Exp 3, Table 8.1). Initially the system was operated with no recycle. Immediately the minimum pH declined to 6.11; over the next few days the COD removal declined from 97 to 82 per cent; the SCFA in the effluent increased from near zero to 100 mgHAc/l and 583 mgHPr/l; within 24h extensive pellet break up commenced, a layer of fine pellet debris collected at the top surface of the sludge bed and the VSS generated per day declined from 4.88 to 3.18 gVSS/d. It was uncertain whether imposition of a recycle would be sufficient for the system to recover; to assist recovery the influent COD was reduced to 4250 mg/l (see Exp 4, Table 8.1), that is, the alkalinity/COD ratio of the base flow increased to 1.0. Minimum pH increased to 6.23 which was still low, but the COD removal improved to 90 per cent and the effluent SCFA reduced to 11 mgHAc/l and zero HPr. A recycle of 1:1 was now instituted (Exp 5, Table 8.1), inducing an effective alkalinity/influent COD of 2.0 mgAlk as CaCO₃/mg influent COD. Minimum pH recovered from 6.23 to 6.64 within a day; the COD removal improved from 90 to 97 per cent and SCFA in the effluent reduced to 11 mgHAc/l and zero HPr. Profiles of the system after recovery are shown in Fig 8.3.
Concentration and pH profiles observed in single UASB system with apple concentrate substrate. (Influent COD concentration = 2848 mg/l, flow rate = 30 l/d, recycle ratio = 1:1).

Fig 8.2:
To check if the system was operating consistently after the changes made above, the influent COD was again increased from 4250 to 5000 mg/l, keeping the alkalinity supplementation at 0.85 mgAlk as CaCO₃/mgCOD influent and removing the recycle. Immediately in the single flow-through system the pH declined to 6.11 and COD decreased to 83 per cent as before.

From this preliminary study it was concluded that (1) a recycle reduced the alkalinity supplementation necessary to maintain the minimum pH above 6.6 and (2) the recycle appeared to have little or no adverse influence on the efficiency of the system provided the minimum pH required is satisfied.

### 3.2 High influent COD concentrations

In this series of experiments the objectives were to determine if high influent COD concentrations could be treated adequately in the UASB system and if so in what measure the alkalinity supplementation in the feed can be reduced by imposing a recycle. The influent COD was increased to 2 to 3 times the average COD normally encountered in apple juice waste water (viz. ≈ 2500 mgCOD/l).

Initially the loading on the system was set at 9.6 kgCOD/m³/d; influent COD concentration ≈ 5000 mg/l, flow rate: 15l/d, alkalinity supplementation ≈ 1.2 mgAlk as CaCO₃/mg influent COD and no recycle (Exp 6). The system response was stable; minimum pH 6.62 and overall COD removal 96 per cent, that is, an Alk/influent COD ratio of 1.2 was adequate to maintain the pH at 6.6. A 1:1 recycle was instituted (Exp 7) and at the same time the alkalinity supplementation in the feed reduced from 1.20 to 0.71 mgAlk as CaCO₃/mg influent COD. However, with the recycle in operation the effective Alk/influent COD was 1.42. Concentration profiles are shown in Fig 8.4(a, b and c); the minimum pH increased from 6.62 (no recycle) to 6.80 and overall COD removal was 98 per cent, i.e. an effective Alk/influent COD ratio of 1.42 was more than adequate to maintain minimum pH above 6.6. This points to the conclusion that an Alk/influent COD ratio of about 1.2 is the required minimum irrespective of whether this is obtained by supplementation only or supplementation plus recycle.

The recycle ratio was then increased to 2:1 (Exp 8), keeping the alkalinity supplementation in the feed at about 0.68 mgAlk as CaCO₃/mg influent COD; the
Concentration and pH profiles observed in single UASB system with apple concentrate substrate. (Influent COD concentration = 4223 mg/l, flow rate = 30 l/d, recycle ratio = 1:1).

Fig 8.3:
Concentration and pH profiles observed in single UASB system with apple concentrate substrate. (Influent COD concentration = 5481 mg/l, flow rate = 15 l/d, recycle ratio = 1:1).

Fig 8.4:
To investigate the effect of recycle at the maximum loading on the system, i.e. 14.6 kgCOD/m³/d (discussed in Chapter 3), the influent COD concentration was set at about 8500 mgCOD/L, flow rate: 15 L/d, alkalinity supplementation in the feed at 1.22 mgAlk as CaCO₃/mg influent COD and no recycle (Exp 9). The system operated in a stable fashion, minimum pH 6.74 and 92 per cent overall COD removal. Concentration profiles for the system are shown in Fig 8.6(a, b and c).

Imposing a 1:1 recycle (Exp 10) and decreasing the alkalinity supplementation to 0.71 mgAlk as CaCO₃/mg influent COD (effective Alk/COD ratio of 1.36 mgAlk as CaCO₃/mg influent COD), the minimum pH increased to 6.81 and overall COD removal to 96 per cent. Increasing the recycle ratio to 2:1 (Exp 11) and decreasing the alkalinity supplementation in the feed to 0.45 mgAlk as CaCO₃/mg influent COD (i.e. effective Alk/COD ratio was 1.35 with a 2:1 recycle), the minimum pH rose slightly to 6.82 but overall COD removal remained at 96 per cent. Concentration profiles for the system with a 2:1 recycle are shown in Fig 8.7(a, b and c). Further increasing the recycle ratio to 3:1 (Exp 12) but keeping the alkalinity supplementation of 0.46 mgAlk as CaCO₃/mg influent COD (i.e. effective Alk/COD ratio is 1.84), the minimum pH remained unchanged (i.e. pH 6.81) but the overall COD removal declined slightly to 92 per cent; concentration profiles for this system are shown in Fig 8.8(a, b and c).

3.3 Discussion

In all the experiments with recycle, good pelletization was observed in the system, this indicates that in a system that produces pelleted sludge, imposition of a recycle ratio up to 3:1 is unlikely to induce detrimental effects on pellet formation. Furthermore, provided the loading is below the maximum, imposition of a recycle appears to have no or only minimal adverse effects on the overall COD removal; indeed it would appear that there is a positive effect shown by a slight increase in the COD removal.

It is not possible to assign a substantive reason for the slight improvement in COD removal when introducing a recycle. Very likely the fine volatile suspended solids present in the recycle stream may contribute to improving the system performance; Pellet debris accumulates in the region above the sludge bed and the VSS
Concentration and pH profiles observed in single UASB system with apple concentrate substrate (Influent COD concentration = 5792 mg/l, flow rate = 15 l/d, recycle ratio = 2:1).

Fig 8.5:
Fig 8.6: Concentration and pH profiles observed in single UASB system with apple concentrate substrate (Influent COD concentration = 8397 mg/l, flow rate = 15 l/d, recycle ratio = 0).
Concentration and pH profiles observed in single UASB system with apple concentrate substrate (Influent COD concentration = 8320 mg/l, flow rate = 15 l/d, recycle ratio = 2:1).
Fig 8.8: Concentration and pH profiles observed in single UASB system with apple concentrate substrate (Influent COD concentration = 8438 mg/ℓ, flow rate = 15 ℓ/d, recycle ratio = 3:1).
concentration ranges between 6 and 13 gVSS/ℓ depending on the loadings. In a UASB system this VSS shows no biological activity (as shown by the near constant COD concentrations within this region, see Figs 8.1 to 8.8). However recent work in the laboratory at the University of Cape Town indicates that when this debris is fed with glucose at COD concentration above 2000 mg/ℓ, biological activity is observed and COD is removed from solution. Hence in the recycle experiments the debris material could have assisted in the COD reduction.

Below the maximum COD loading, the recycle certainly led to reduction in the alkalinity supplementation in the feed. It would seem that the minimum provision for flow-through UASB systems treating carbohydrate waste is approximately 1,2 to 1,6 mgAlk as CaCO₃/mg influent COD in order to maintain the minimum pH in the reactor above 6,6. With recycle, the reduction in alkalinity supplementation to the feed can be estimated by multiplying the base requirement by the fraction flow/(flow + recycle flow).

The experiments in which the maximum loading was imposed (14,6 kgCOD/m³/d), imposition of a recycle did reduce the alkalinity supplementation to the feed while maintaining the minimum pH at 6,6. However leakage of SCFAs through the bed became significant at 3:1 recycle. The SCFAs that leaked through were recycled to the influent in effect increasing the influent COD load; this possibly was the cause for the slight decline in the overall COD removal.

The response data very clearly show that imposing a recycle reduces the alkalinity supplementation to the feed. Although there is no substantive evidence it would seem that the recycle does not allow the maximum loading to be increased above that obtained for a flow-through system with adequate pH control. At the maximum loading (thus defined) leakage of SCFAs through the bed, and hence present in the recycle to the influent, may in fact have a cumulative negative effect, by increasing the effective load on the system, and could conceivably lead to a rapid breakdown of the system.

4. Design

It was stated above that cause(s) that determine(s) the maximum loading have not been established conclusively; for the present the maximum loading must be determined experimentally on say a flow through system with adequate pH control. This maximum loading can be accepted to apply also when a recycle is imposed —
the study tends to indicate that maximum loading does not appear to be affected in a significant way by adding a recycle to the system.

In this study, for convenience, the loading was expressed as kgCOD/m³ reactor volume/d. The loading preferably should be expressed per unit of sludge bed volume. In this investigation the sludge bed volume was fixed at 1/3 of the total reactor volume; it follows that the loading in fact was 3 times that expressed in terms of the total reactor volume i.e. maximum loading rate $3 \cdot 14,6 = 43,8$ kgCOD/m³ sludge bed volume/d. This implies a minimum sludge volume provision of $1/43,8 = 0,023$ m³ of sludge/kg influent COD.

The information above allows preliminary design estimates for sizing a UASB reactor to treat apple juicing waste waters (and very likely other similar carbonaceous wastes):

The loading should not exceed half the maximum loading obtained on the laboratory-scale units; i.e. loading $\leq 22$ kgCOD/m³ sludge volume/d ($\approx 7,5$ kg COD/m³ reactor volume/d, in this study) and the sludge provision not less than about 0,03 m³ of sludge/kg influent COD. Minimum alkalinity supplementation in the feed should be about 1,2 to 1,6 mgAlk as CaCO₃/mg influent COD. This alkalinity requirement can be reduced by imposing a recycle. With a recycle the reduced alkalinity can be calculated by multiplying the base alkalinity requirement by the factor flow/(flow + recycle flow). The recycle ratio should be selected such that the influent COD based on the combined flow, (flow + recycle flow), is within the range 2500 to 5000 mgCOD/l. This range is selected because the experimental response in the range appears (subjectively) to be the most stable.

5. CONCLUSIONS
In this chapter the alkalinity requirements for a UASB system with apple juice waste water as substrate were determined and an assessment made on the effect of a recycle on the alkalinity requirements. The following conclusions are derived from this study:

1. The alkalinity supplementation to the feed for a flow-through UASB system with apple juice waste water was found to be 1,2 to 1,6 mgAlk as CaCO₃/mg influent COD in order to maintain the minimum pH $\geq 6,6$. 
2. Provided the loading on the system is below the maximum, this alkalinity requirement can be reduced by imposing a recycle; this reduction can be calculated by multiplying the base alkalinity requirement by the factor \( \frac{\text{flow}}{(\text{flow} + \text{recycle flow})} \).

3. Near the maximum loading, the recycle reduces the alkalinity requirement, but SCFA leakage from the sludge bed was observed. Recycling high SCFA concentrations may have adverse effects on the performance of the system and conceivably can cause failure by inducing an effective higher influent COD loading.

4. Imposition of a recycle up to 3:1 does not appear to have adverse effects on pellet formation.

5. Experimental recycle ratios (up to 3:1) appear to improve slightly the overall COD removal efficiency compared to that of the flow-through system. This improvement probably arises from recycling the suspended material (pellet debris) above the bed to the influent point where it is likely that the high substrate concentration could induce biological activity in the debris.
CHAPTER 9

MATHEMATICAL MODELLING OF UASB SYSTEM BEHAVIOUR

1. INTRODUCTION

In Chapter 4 the behaviour of a UASB system treating an apple juice waste water was reported and various zones of behaviour identified. In Chapter 5, using the experimental observations made on the UASB system, a biochemical model was hypothesized that could explain the pelletization phenomenon and the types of substrates that would, or would not, promote pellet formation. In Chapter 6 further evidence was presented that showed hydrogen to be the metabolite central to the pelletization process.

In this chapter the objective is to develop a mathematical model that describes the stoichiometric and kinetic behaviour of the various processes operating in a UASB system that produces a pelletized sludge mass.

To set up a mathematical model it is necessary to:

1. identify the essential compounds utilized and formed,
2. identify the processes acting on these compounds,
3. conceptualize a mechanistic model that qualitatively describes the kinetic and stoichiometric behaviour of the processes and compounds, and
4. formulate mathematically the process rates, stoichiometry and transport relationships.

It has been shown that pelletization develops in a UASB system only when substrates are treated that give rise to a high hydrogen partial pressure (high $\hat{\text{pH}_2}$) in a zone in the system, for example carbohydrates and proteinaceous substrates. The development of the kinetic model shall be restricted to one substrate only, a soluble carbohydrate.
2. COMPOUNDS

There appear to be 11 essential compounds directly involved in a pelletized sludge UASB system treating a carbohydrate substrate. Some of these compounds were directly observable; with others, the means for measuring these compounds were not available in the laboratory. As a consequence their existence had to be inferred, either from the hypothesized biochemical behaviour, or from the requirement of mass balances.

Five compounds are directly observable, the concentrations of

1. soluble COD,
2. short chain fatty acids (SCFA),
3. soluble ammonia/ammonium,
4. organic nitrogen,
5. methane.

Six compounds are inferred, the concentrations of

1. hydrogen,
2. glucose,
3. amino acids,
4. polypeptide polymer,
5. unbiodegradable particulate COD,

3. PROCESSES

The processes that act on the compounds were identified by observing changes in the compounds under a variety of conditions, such as different influent COD concentrations, flow rates and NH₃-N concentrations. Twelve essential processes were identified:

Growth:  
1. Acidogens on glucose under high pH₂  
2. Acidogens on glucose under low pH₂  
3. H₂-utilizing methanogens on hydrogen  
4. Acetoclastic methanogens on acetic acid  
5. Acetogens on propionic acid
9.3

Death:
(6) Acidogens
(7) H₂-utilizing methanogens
(8) Acetoclastic methanogens
(9) Acetogens

Other processes:
(10) Ammonification of soluble organic nitrogen
(11) Pellet break up
(12) Adsorption/enmeshment of soluble organic nitrogen

4. CONCEPTUAL MODEL

4.1 Microbial population

In conceptualizing the behaviour of the UASB system it is necessary to identify the biological agents that mediate the processes and utilize or generate the compounds. Four microbial populations are involved in the fermentation of glucose (soluble carbohydrate):

(1) Acidogens, (2) Acetogens, (3) Acetoclastic methanogens, and (4) H₂ utilizing methanogens.

(1) Acidogens: The acidogens convert glucose to SCFA (acetic, propionic and butyric acids), carbon dioxide and hydrogen. The type of SCFA that is generated will depend on the hydrogen partial pressure, \( \tilde{p}_H_2 \), (Chapter 4). Under low \( \tilde{p}_H_2 \), acetic and butyric acids are generated together with hydrogen and carbon dioxide. Under high \( \tilde{p}_H_2 \), acetic, butyric and propionic acids are generated together with hydrogen and carbon dioxide. However butyric acid was not observed in any of the single UASB systems in the investigations with apple juice waste water and glucose as substrates (see Chapters 4 and 6) and hence this compound is not included in the model.

(2) Acetogens: The acetogens convert propionic acid to acetic acid, hydrogen and carbon dioxide. Propionic acid conversion becomes significant only when \( \tilde{p}_H_2 \) declines to less than \( 10^{-4} \), atm; at high \( \tilde{p}_H_2 \) propionic acid conversion becomes negligible or does not take place, that is, conversion of propionic acid to acetic acid is a function of \( \tilde{p}_H_2 \) or hydrogen concentration.

(3) Acetoclastic methanogens: Acetoclastic methanogens convert acetic acid to methane; this conversion is independent of \( \tilde{p}_H_2 \). (Dubourguier et al., 1985,
1988 concluded that the dominant acetoclastic methanogen in granular anaerobic sludges is *Methanothrix soehngenii*.

(4) **H₂-utilizing methanogens:** H₂-utilizing methanogens utilize hydrogen as sole energy source, and CO₂ as carbon source, to produce methane. It is assumed that *Methanobacterium* strain AZ (*M. Strain AZ*), (now known as *Methanobrevibacter arboriphilus*), is the main H₂-utilizing methanogen present in a UASB system treating a carbohydrate substrate. (Dubourguier et al., 1985, 1988 concluded that *Methanobrevibacter arboriphilus* is the dominant H₂-utilizing species present in granular anaerobic sludges).

4.2 **Pellet formation**

The function of *M. Strain AZ* species is central to describing the pelletization phenomena (see Chapter 5): This organism has this unusual characteristic that it cannot manufacture the amino acid cysteine and depends on external sources, such as cysteine liberated from death of other organisms, for its cysteine supply.

Under high pH₂ conditions (i.e. high H₂ substrate concentration), in the presence of excess of free and saline ammonia, a high production of all the amino acids (except cysteine) is stimulated. If the cysteine supply is limited, *M. Strain AZ* cannot utilize all the amino acids for cell synthesis. The excess amino acids are released to the surrounding medium, and/or are linked in polypeptide chains which are extruded from active sites. These polypeptide chains bind the *M. Strain AZ* species and other micro-organisms into pellets. Thus, the hydrogen oxidized by *M. Strain AZ* reappears in the compounds methane, organism mass, polypeptide polymer and/or free amino acids, the relative magnitude of the fractions produced depending on the cysteine availability. If, for example, the feed is supplemented with cysteine, theoretically this should result in an increase in the *M. Strain AZ* species and a reduction in both volatile polypeptide solids yield and free amino acids released to the surrounding medium, the net effect being a reduction in volatile solids. The last two predictions have been verified experimentally (Chapter 5).

Other limitations on polypeptide/free amino acids generation are as follows:

(1) NH₃-N concentration is limiting (Chapter 6). A reduction in VSS yield and soluble free amino acids is to be expected for reason that the H₂ is not fully utilized. Support for this conclusion is presented in Chapter 6 where it is
shown that with glucose as substrate, under limiting NH$_3$-N, from mass balance considerations, most of the generated hydrogen is lost in the gas phase.

(2) Preferential hydrogen abstraction. Even though the substrate has the potential to give rise to a high $\tilde{p}H_2$, if secondary reactions abstract $H_2$ preferentially (for e.g. sulphate reduction), this will reduce $\tilde{p}H_2$, and hence a reduced polypeptide generation (Chapter 7).

(3) The substrate itself does not yield hydrogen (e.g. acetate) or can be broken down only under low $\tilde{p}H_2$ (e.g. lipids). Because the $\tilde{p}H_2$ is low the rate of substrate diffusion ($H_2$) into the cell will be slow and excess amino acids production will be limited – polypeptide generation is not to be expected.

For modelling purposes it will be assumed that (1) the substrate can generate a high $\tilde{p}H_2$, (2) oxidizing agents are absent, (3) NH$_3$-N concentration is present in excess, but (4) cysteine concentration is limiting.

4.3 Substrate transfer:
The model assumes that the pellets behave as "gas pumps". Gases (methane and CO$_2$) produced inside the pellets are released via gas channels to the surrounding medium. It seems that as the gas escapes from the pellets, a pressure drop is generated in the channels causing liquid (substrate) to be drawn into the pellets. In such a situation very likely mass transfer resistance, due to diffusion limitations, becomes negligible and should not be a factor influencing the kinetics of the processes in the pelletized sludge bed. The assumption that the pellets behave as "gas pumps" is supported by observations of Robinson et al. (1984); they produced scanning electron micrographs of biofilms from anaerobic fixed-bed reactors, which clearly show the presence of an extensive network of channels and openings. They concluded that these channels may facilitate gas and nutrient exchange. Wiegant and de Man (1986) also demonstrated a similar network of channels and openings in granular methanogenic sludge.

4.4 Soluble organic nitrogen:
In the aqueous phase in the pelletized sludge bed, soluble organic nitrogen is present in substantial concentrations. This fraction is assumed to arise from the following sources: (1) death of organisms (acidogens, $H_2$-utilizing methanogens, acetoclastic
methanogens and acetogens), (2) pellet breakup and, (3) release of free amino acids by \textit{M. Strain AZ}. Sinks for the organic nitrogen are: (1) adsorption/enmeshment of nitrogenous material, probably free polypeptide chains (from pellet break up) and, (2) ammonification of soluble organic nitrogen.

With regard to the sources of soluble organic nitrogen in Chapter 5 it was estimated that in the production of pellet mass, 90 per cent was polymer and only 10 per cent was anaerobic micro-organism mass. In "normal" anaerobic methane fermentation systems the presence of soluble organic nitrogen is ascribed to the endogenous mass loss (or death) of the anaerobic micro-organisms. In a pelletized sludge system the concentration of soluble organic nitrogen in the bed is much higher than that to be expected from death of micro-organisms. The sources for this high soluble organic nitrogen are conceptualized to be due to: (1) generation of free amino acids by \textit{M. Strain AZ}, and (2) polypeptide polymer release due to break up of pellets (see Chapter 5). With the experimental set-up used in this study it was not possible to differentiate between these two processes. For the purpose of modelling it is assumed that the soluble organic nitrogen is linked to the polymer mass, the concentration derived from break up of the polypeptide chains.

The sinks for soluble organic nitrogen, ammonification and adsorption/enmeshment, are treated as separate processes. With regard to ammonification, in UASB concentration profiles (Chapters 4 and 6) it is observed that the ammonia concentration first decreases due to uptake for polymer formation and cell synthesis, thereafter it increases and then remains virtually constant; this increase is conceptualized to be due to ammonification of soluble organic nitrogen. With regard to adsorption/enmeshment of the soluble organic nitrogen, the existence of this process cannot be clearly identified from the experimental profiles; very likely it occurs. It is included in the model as a function of soluble organic nitrogen, but this is more for the sake of completeness than quantitative estimation.

5. **MODEL PRESENTATION**

In the mathematical model, the process rates and the stoichiometric relationships between the processes and compounds are formulated mathematically. The large number of complex interactions between compounds and processes necessitates that these be clearly presented. Following the proposals of the IAWPRC Task Group (1987) on "Mathematical Modelling of Waste Water Treatment", the processes and compounds are set out in a process-compound matrix (Table 9.1). This format
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<tr>
<td>8 Death acetoclastic methanogens</td>
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<td>9 Death acetogens</td>
<td>-1</td>
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<tr>
<td>10 Ammonification</td>
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<tr>
<td>11 Pallet break up</td>
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<tr>
<td>12 Adenoprism/Ensemble of soluble organ</td>
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Table 9.1: Process kinetics and stoichiometry for the UASB system with glucose substrate.
facilitates clear and unambiguous presentation of the processes and compounds and their interaction. The setting up of such a matrix, how to interpret it and how it is incorporated in the mathematical solution procedures are described briefly in Appendix C. For greater detail on solution procedures for the matrix, see Billing (1987).

6. MODEL DESCRIPTION

The matrix of processes and compounds is set out in Table 9.1. The compounds, *i* in number, are listed across the top of the matrix; the processes, *j* in number are listed down the left hand side of the matrix. The stoichiometric conversion factors from one compound to another are listed horizontally for each process below the compounds. The kinetic rate expression, $\rho$, for each process is listed on the right hand of the matrix as a column in the matrix, the rate expression being in the same row as the process.

Following the IAWPRC recommendation, VSS specific yields and other associated parameters are expressed in COD units. Nitrogenous compounds are expressed in terms of nitrogen.

To facilitate the discussion, the processes in the matrix are subdivided into: (1) Growth of organisms ($j = 1-5$), (2) death of organisms ($j = 6-9$), (3) ammonification ($j = 10$), and (4) physical effects ($j = 11-13$).

6.1 Growth of organisms ($j = 1-5$)

The microbial growth rate and substrate utilization rate expressions for the 4 groups of organisms present in the UASB system are based on the Monod's formulation. The growth of each group of organisms is discussed below.

(1) Acidogen growth ($j = 1-2$)

From the conceptual model two processes are needed to describe acidogen growth. The first process ($j = 1$) takes place under high pH$_2$ conditions and the second process ($j = 2$) under low pH$_2$ conditions. The two processes have a number of factors in common: For every 1 COD unit of active mass appearing, $(1+Y_A)/Y_A$ COD units of glucose ($i = 9$) are consumed. With acidogen growth nitrogen is incorporated in cell mass giving rise to a reduction in ammonia concentration ($i = 13$) and an increase in the nitrogen content of biomass ($i = 7$).
However acidogen growth under high and low $\tilde{p}H_2$ differs in the stoichiometric production of acetic and propionic acids and hydrogen:

**Under high $\tilde{p}H_2$:** For every $(1+Y_A)/Y_A$ units of glucose consumed ($i = 9$), $1/3 \cdot 1/Y_A$ units of acetic acid ($i = 10$), $7/12 \cdot 1/Y_A$ units of propionic acid ($i = 11$) and $1/12 \cdot 1/Y_A$ units of hydrogen ($i = 15$), are generated, all in COD units.

**Under low $\tilde{p}H_2$:** For every $(1+Y_A)/Y_A$ units of glucose consumed ($i = 9$), $2/3 \cdot 1/Y_A$ units of acetic acid ($i = 10$), 0 units propionic acid ($i = 11$) and $1/3 \cdot 1/Y_A$ units of hydrogen ($i = 15$) are generated, all in COD units.

Derivations of these stoichiometric ratios are given in Appendix C.

The specific process rate of utilization of glucose by the acidogens (in the process rate column) is modelled using Monod's equation with maximum specific growth rate $\mu_A$ and half saturation coefficient $K_A$ with regard to the glucose concentration.

The extent to which process $j = 1$ or 2 predominates, depends on the hydrogen concentration. A switching function is utilized to switch from one process to the other based on the hydrogen concentration. The Monod rate expression is multiplied by the switching function $S_H/(K_H+S_H)$, where $S_H$ is the hydrogen concentration and $K_H$ is the switching constant. Consider acidogen growth on glucose, when $S_H$ is high $S_H/(K_H+S_H)$ approaches unity and process $j = 1$ is the predominant reaction; when $S_H$ decreases below $K_H$ then $S_H/(K_H+S_H)$ decreases eventually to near zero, process $j = 2$ becomes the predominant reaction and process $j = 1$ becomes inoperative.

(2) **$H_2$-utilizing methanogen growth (j = 3)**

In $H_2$-utilizing methanogenic growth both active organism and polymer masses are being formed simultaneously. The substrate source for both organism and polymer is hydrogen. Stoichiometrically, for every one unit of
active mass formed $Y_p/Y_{MH}$ units of polymer (i = 5) and $(1 - Y_p - Y_{MH})/Y_{MH}$ units of CH$_4$ (i = 16) are generated and $1/Y_{MH}$ units of hydrogen (i = 15) are utilized, all in COD units (Appendix C). With H$_2$-utilizing methanogen growth, nitrogen is incorporated both in cell mass and in polymer mass giving rise to a reduction in ammonia concentration (i = 13) and an increase in the nitrogen content of biomass (i = 7) and polymer mass (i = 6).

The kinetic growth constants are $\hat{\mu}_{MH}$ (maximum growth rate) and $K_{MH}$ (half-saturation constant) with regard to the hydrogen concentration.

(3) **Acetoclastic methanogen growth (i = 4)**

In acetoclastic methanogen growth on acetic acid, for every one COD unit of active mass formed, $(1 - Y_{MA})/Y_{MA}$ COD units of methane (i = 16) are generated and $(1 + Y_{MA})/Y_{MA}$ COD units of acetic acid (i = 10) are utilized. For methanogen growth, nitrogen is incorporated in cell mass giving rise to a reduction in ammonia concentration (i = 13) and an increase in the nitrogen content of biomass (i = 7).

The growth rate is modelled using Monod kinetics with kinetic growth rate constants $\hat{\mu}_{MA}$ (maximum growth rate) and $K_{MA}$ (half-saturation constant) with regard to the acetic acid concentration.

(4) **Acetogen growth (i = 5)**

From the conceptual model, acetogen growth on propionic acid is a function of the hydrogen concentration surrounding the organisms. As $pH_2$ increases (or equivalently as hydrogen concentration increases), acetogen growth decreases until the reaction thermodynamically is not feasible; i.e. growth rate becomes zero (Chapter 4). Mathematically this is achieved by multiplying the Monod rate expression by a switching function of the form $[1 - S_H/(K_H + S_H)]$ which is similar to the one used for acidogen growth at low $pH_2$. When $S_H >> K_H$ such that $S_H/(K_H + S_H)$ tends to unity, the switching function tends to zero and process rate tends to zero. However when $S_H << K_H$ then the value of the switching function approaches unity
and the process rate will then be given by the Monod equation.

For every 1 unit of active mass appearing \(4/7 \cdot 1/Y_{AP}\) units of acetic acid \((i = 10)\) and \(3/7 \cdot 1/Y_{AP}\) units of hydrogen \((i = 15)\) are produced and \((1 + Y_{AP})/Y_{AP}\) units of propionic acid \((i = 11)\) are consumed, all in COD units. With acetogen growth, nitrogen is incorporated in cell mass resulting in a reduction in ammonia concentration \((i = 13)\) and an increase in the nitrogen content of biomass \((i = 7)\). The kinetic constants are \(\mu_{AP}\) (maximum specific growth rate) and \(K_{AP}\) (half-saturation coefficient) with respect to the propionic acid concentration.

**6.2 Death of organisms \((i = 6-9)\)**

The rate of death of organisms is modelled as a first order reaction with respect to active mass. For every COD unit of organism disappearing \((i = 1-4)\) one COD unit of dead volatile material is generated \((i = 8)\). It is not possible to subdivide this material into, say, inert and biodegradable fractions. The mass release to the surrounding medium is small relative to the active mass; for the purpose of modelling it is sufficient to ascribe the products of death to an inert soluble mass — i.e. a soluble "unbiodegradable" COD. Associated with death of organisms is the loss of NH\(_3\)-N in the active mass \((i = 7)\); this nitrogen reappears as soluble organic nitrogen \((i = 14)\). The specific kinetic decay or death constants for acidogens, H\(_2\) utilizing methanogens, acetoclastic methanogens and acetogens are \(b_A\), \(b_{MH}\), \(b_{MA}\) and \(b_{AP}\) respectively, units (/d).

**6.3 Ammonification of soluble organic nitrogen \((i = 10)\)**

In line with experimental observations in Chapters 4 and 5, to account for the increase in NH\(_3\)-N concentrations, conversion of soluble organic nitrogen to ammonia is incorporated in the model \((j = 10)\). The rate of ammonification is modelled as a first order reaction with respect to the sum of all active masses with a specific rate constant \(K_{ND}\). For every 1 N unit of ammonia generated \((i = 11)\), one N unit of soluble organic nitrogen is consumed \((i = 12)\).
6.4 Physical processes ($j = 11-12$)

From the conceptual model, two physical processes have been identified as taking place in the pelletized sludge bed: (1) pellet breakup ($j = 11$) and, (2) adsorption/enmeshment of soluble organic nitrogen.

(1) Pellet breakup ($j = 11$)

The rate of pellet breakup is modelled as a first order reaction with respect to the growth rates of the 4 groups of organisms — Gas production is related to growth and break up of the pellets is assumed to be due to disruption by the gas. During organism growth gaseous products always are released. However, hydrogen gas released during acidogenic growth on glucose and acetogenic growth on propionic acid, is virtually removed instantaneously by the H$_2$ utilizing methanogens so that the gases released to the surrounding medium are methane and carbon dioxide. Since it is estimated that approximately 70 per cent of the methane generated comes from acetic acid fermentation (Jeris and McCarty, 1965; Smith and Mah, 1966) the stoichiometric coefficient for process rate $p_4$ is taken as 0.7 and those for process rates $p_1$, $p_2$ and $p_3$ as 0.1 with breakup kinetic constant $K_{BP}$.

Experimentally, it was not possible to obtain quantitative measures of this process. It is assumed that the broken up material is released as soluble inert polymer mass to the surrounding liquid and eventually discharged into the effluent.

From the matrix, for every one unit of polymer mass (as COD) disappearing ($i = 5$) one unit of soluble inert polymer mass is generated ($i = 12$). Associated with the disappearance of polymer mass is the loss of nitrogen incorporated in the polymer ($i = 6$); this nitrogen reappears as soluble organic nitrogen ($i = 14$).

(2) Adsorption/enmeshment of soluble organic nitrogen ($j = 12$)

The rate of adsorption/enmeshment of soluble organic nitrogen is modelled as a first order with respect to soluble organic nitrogen and the kinetic rate constant is $K_{EP}$.
From the matrix, for every one nitrogen unit of soluble organic nitrogen enmeshed \( (i = 14) \), one unit of nitrogen is added to the nitrogen content of polymer \( (i = 6) \). Associated with the enmeshment of organic nitrogen is the removal of soluble inert polymer mass (as COD) from solution \( (i = 12) \); this mass (COD) reappears as enmeshed polymer mass \( (i = 3) \).

7. **MATRIX SOLUTION**

The matrix presentation of the processes, compounds and rates defines the behaviour at a single point in the system. To obtain the response of the system, the system configuration (single or multiple reactors), hydraulic mixing regime in the reactor (plug flow or completely mixed), solids regime (suspended or fixed), recycle flows between reactors, or within a batch reactor, and mass transport of compounds in and out of each reactor need to be incorporated.

Within the requirements stated above for the UASB system the configuration is a single reactor, the hydraulic mixing regime (i.e. mixing regime of the aqueous phase) is of the plug flow type (from Chapter 4), the pelletized solids are assumed to be fixed and there is an option for a recycle from the effluent to the influent point of the reactor. Of these the distribution of the solids within the reactor needs to be described in greater detail:

7.1 **Pelletized bed VSS concentration:**

In the UASB reactor it was observed that pellet size varied from the bottom to the top of the pelletized bed, with maximum size at the bottom of the bed. One may expect, therefore, that the VSS concentration would vary up the sludge bed. In this study it was not possible to obtain a profile of the VSS concentration; only the average concentration could be obtained by draining the entire pelletized bed and sampling the bed after mixing to obtain a uniform concentration. In attempting to obtain a profile of VSS concentration on the laboratory scale reactors, because the diameter of the reactor was small, sampling disturbed the \textit{in situ} concentration at the sampling point. However profiles have been reported in the literature, obtained on laboratory-scale studies. Hamoda and Van den Berg (1984) measured the distribution of VSS up a UASB reactor and found that the VSS concentration profile exhibited three zones: (1) a lower zone, at the bottom of the bed, in which the VSS concentration was a maximum and constant, (2) a transitional zone in the upper part of the bed in which the VSS concentration decreased linearly with height to a
minimum at the top of the bed, and (3) a suspended blanket zone above the bed, in which the VSS concentration was low and constant. From experimental observations in this study, the sludge blanket above the sludge bed exhibited no biological activity and therefore was not considered in the mathematical model.

Hamoda and Van den Berg (1984) observed that within the bed concentration zones, the solid concentration profile was a function of organic loading — the VSS concentrations increased, and each zone extended further up the reactor, with increase in the organic loading. In this study a similar dependency was noted; the average VSS concentration increased with the organic loadings — average VSS ranged from about 28 gVSS/ℓ at a loading of 7.5 kgCOD/m³/d to about 37 gVSS/ℓ at a loading of 28 kgCOD/m³/d.

For modelling purposes a linear dependency of the average bed VSS concentration with loading was accepted. The VSS profile for any particular average VSS (with associated organic loading) was constructed as follows:

The depth of the pelletized bed was taken as that observed in the experimental study. For all loadings the VSS concentration at the top of the bed was fixed between 15 and 35 gVSS/ℓ; the zone of constant concentration in the bottom zone of the bed was assumed to extend from the bottom of the reactor up to ¼ of the bed depth; thereafter the concentration decreased linearly to the minimum at the top of the bed. The VSS concentration at the bottom of the bed was found by trial and error such that the average VSS concentration for the profile was equal to that estimated from the loading.

With the information supplied above, a solution procedure for the system is structured as follows:

The plug flow reactor is divided into a set of elements, the products of the preceding element become the input to the succeeding element. For each element the VSS concentration is assumed to be the average of the concentrations of the preceding and succeeding elements which are obtained from the hypothesized concentration profile. The influent compound concentrations and flows are used to obtain the initial concentrations and flows at the reactor base. Concentration profiles in the reactor of the various compounds in the matrix are obtained by integrating forward (using a predictor corrector method) from the initial concentrations, through the
reactor. For the integration, the rate equations are obtained from the matrix and the VSS concentrations from the hypothesized VSS profile, (as described above). Further details of solution techniques for the matrix are given in Appendix C.

8. **MODEL PARAMETERS**

Model parameters were obtained partly from experiments and partly from literature, others were obtained by 'curve fitting' by using a range of system operating conditions.

8.1 **Polymer and organism mass fractions**

In Chapter 5 the ratio polymer mass:organism mass was determined to be approximately 0.9:0.1. The organism mass consisted of different organism mass fractions; the subdivision into the different organism types was assumed to be the same as that reported by Dolfing (1987) for a granular methanogenic sludge grown on a waste water from a sugar factory, i.e. Acidogens : H₂-utilizing methanogens : Acetoclastic methanogens : Acetogens = 0.9 : 0.08 : 0.01 : 0.01. These ratios were assumed to apply also in the model.

8.2 **Kinetic and stoichiometric constants for organisms**

A search of the literature for kinetic and stoichiometric constants for the four groups of microorganisms (viz. acidogens, acetogens, acetoclastic methanogens and H₂-utilizing methanogens) indicates an order of magnitude variation for each; these are shown in Table 9.2. For example, the maximum specific growth rate ($\mu_{AP}$) and half saturation constant ($K_{AP}$) for the propionate utilizers range from 0.3 to 1.4 (/d) and 48-330 mgCOD/l respectively. However, there is a consistency in the reported values for anaerobic organism specific decay rate and specific yield values for each of the four groups of organisms. The kinetic and stoichiometric values used to verify the model are given in Table 9.3.

The gross specific yield for H₂-utilizers of 0.56 mgVSS/mgCOD(H₂) calculated in Chapter 6, includes polymer/free amino acids generated and organism masses. Assuming a specific yield of 0.043 mgVSS/mgCOD(H₂) for H₂-utilizers then the specific yield for polymer/free amino acids is 0.52 mgVSS/mgCOD(H₂). The nitrogen content of the polymer was calculated in Chapter 5 and has a value of 0.113 mgN/mgCOD.

The COD/VSS and TKN/COD ratios for organisms are determined from the
Table 9.2  Reported kinetic and stoichiometric constants.

<table>
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<tr>
<th></th>
<th>$\mu_{\text{max}}$</th>
<th>$K_s$ (mgCOD/l)</th>
<th>b</th>
<th>Y</th>
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<tr>
<td></td>
<td>(/d)</td>
<td></td>
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<tr>
<td><strong>ACIDOGENS</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hill and Barth (1977)</td>
<td>0.4</td>
<td>150</td>
<td>0.04</td>
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<td>Denae et al. (1986)</td>
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<td><strong>ACETOGENS</strong></td>
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<td></td>
</tr>
<tr>
<td>Lawrence and McCarty (1969)</td>
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<td>48</td>
<td>0.01</td>
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<td>Heyes and Hall (1983)</td>
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<tr>
<td>Gujer and Zehnder (1983)</td>
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<td><strong>ACETOCLASTIC METHANOGENS</strong></td>
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</tr>
<tr>
<td>Hill and Barth (1977)</td>
<td>0.4</td>
<td>25</td>
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<td>237</td>
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<tr>
<td>Ten Brummeler et al. (1985)</td>
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<td>0.038</td>
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<td>Smith and Mah (1978)</td>
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<td>320</td>
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<td>165</td>
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<td>Kaspar and Wurthmann (1978)</td>
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Table 9.3: Kinetic parameters and stoichiometric constants in the matrix.

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<th>Constant Description</th>
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<td>Maximum specific growth rate</td>
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<td>/d</td>
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<tr>
<td>( K_A )</td>
<td>half saturation constant</td>
<td>150</td>
<td>mgCOD/t</td>
</tr>
<tr>
<td>( b_A )</td>
<td>Decay (death) rate</td>
<td>0.041</td>
<td>/d</td>
</tr>
<tr>
<td>( Y_A )</td>
<td>Yield</td>
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<td>mgCOD volatile mass/mgCOD</td>
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<tr>
<td><strong>ACETOGENS</strong></td>
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<td></td>
</tr>
<tr>
<td>( \hat{\mu}_{AP} )</td>
<td>Maximum specific growth rate</td>
<td>1.1-1.2</td>
<td>/d</td>
</tr>
<tr>
<td>( K_{AP} )</td>
<td>half saturation constant</td>
<td>250-330</td>
<td>mgCOD/t</td>
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<tr>
<td>( b_{AP} )</td>
<td>Decay (death) rate</td>
<td>0.015</td>
<td>/d</td>
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<tr>
<td>( Y_{AP} )</td>
<td>Yield</td>
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<td>mgCOD volatile mass/mgCOD</td>
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<td>Decay (death) rate</td>
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<td>/d</td>
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<tr>
<td>( Y_{MH} )</td>
<td>Yield</td>
<td>0.041</td>
<td>mgCOD volatile mass/mgCOD</td>
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<td><strong>ACETOCLASTIC METHANOGENS</strong></td>
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<td>( \hat{\mu}_{MA} )</td>
<td>Maximum specific growth rate</td>
<td>0.35-0.40</td>
<td>/d</td>
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<td>( K_{MA} )</td>
<td>half saturation constant</td>
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<td>mgCOD/t</td>
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<td>( b_{MA} )</td>
<td>Decay (death) rate</td>
<td>0.037</td>
<td>/d</td>
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<tr>
<td>( Y_{MA} )</td>
<td>Yield</td>
<td>0.041</td>
<td>mgCOD volatile mass/mgCOD</td>
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<td><strong>OTHER CONSTANTS</strong></td>
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<td>( X_{BN} )</td>
<td>Nitrogen content of organism</td>
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<td>mgN/mgCOD</td>
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<td>( X_{PN} )</td>
<td>Nitrogen content of polymer</td>
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<td>mgN/mgCOD</td>
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<td>Polymer yield</td>
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<td>Half saturation switching function</td>
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<td>mgCOD/t</td>
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<td>Pellet breakup rate</td>
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<td>/d</td>
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<td>Enmeshment rate</td>
<td>10-15</td>
<td>/d</td>
</tr>
<tr>
<td>( K_{ND} )</td>
<td>Ammonification rate</td>
<td>0.010-0.018</td>
<td>/d</td>
</tr>
</tbody>
</table>
stoichiometric formula $C_8H_{10}O_2N$ for biosolids (McCarty, 1972), giving COD/VSS = 1.42 mgCOD/mgVSS and N/COD = 0.086 mgN/mgCOD respectively.

9. MODEL VERIFICATION

The predictive capability of the UASB model was tested against the experimental responses observed on laboratory scale flow-through systems and systems with a recycle from the unsettled effluent to the influent. The experimental systems were operated over a range of loadings.

For all the simulations the same set of kinetic and stoichiometric constants, as listed in Table 9.3, were used.

9.1 Flow-through systems

Responses were simulated for the flow-through experiments listed in Table 9.4.

Figs 9.1 to 9.4 show the response profiles in the sludge bed, predicted and observed, for the following parameters: Total COD, SCFA (acetic and propionic acids), free and saline ammonia and organic nitrogen.

Comparison of the experimental observed and simulated responses indicate a most satisfactory correlation. The following two comments need to be made:

(1) The same set of constants were used in all cases except in one instance, (Exp.3) at the loading of 14 kgCOD/m$^3$ reactor volume/d. In this particular case the half saturation constant of the acetogens ($K_{AP}$) had to be increased from 250 to 330 mgCOD/l for the model to give a good prediction. However the $K_{AP}$ value, of 330 mgCOD/l, is within the range of reported half saturation constant values for acetogens (Heyes and Hall, 1983).

(2) In Table 9.3 the value of the half saturation constant ($K_H$), in the switching function for the processes ($\rho_1$, $\rho_2$ and $\rho_3$), was taken as 10 mgCOD/l. Usually for switching functions the half saturation constant values are selected to be small so that switching from one process to another takes place at a very low concentration. In this study the magnitude of the selected value of $K_H$ on process behaviour could not be evaluated — the rate of utilization of glucose was very rapid so that glucose concentration reduced
### Table 9.4: Data for flow-through UASB system.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Influent COD mg/l</th>
<th>Flow rate l/d</th>
<th>Depth of sludge bed mm</th>
<th>Mean sludge bed density gVSS/l</th>
<th>Loadings kgCOD/m³/d reactor sludge bed volume</th>
<th>Sludge bed volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5079</td>
<td>15</td>
<td>400</td>
<td>29</td>
<td>8.5</td>
<td>25.5</td>
</tr>
<tr>
<td>2</td>
<td>2672</td>
<td>30</td>
<td>400</td>
<td>29</td>
<td>9.6</td>
<td>28.8</td>
</tr>
<tr>
<td>3</td>
<td>8397</td>
<td>15</td>
<td>406</td>
<td>20</td>
<td>14.0</td>
<td>42.9</td>
</tr>
<tr>
<td>4</td>
<td>5345</td>
<td>45</td>
<td>400</td>
<td>35</td>
<td>26.7</td>
<td>80.1</td>
</tr>
</tbody>
</table>

Diameter of reactor = 100 mm  
Effective reactor volume = 9l

### Table 9.5: Data for UASB system with recycle.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Influent COD mg/l</th>
<th>Flow rate l/d</th>
<th>Recycle ratio</th>
<th>Depth of sludge bed mm</th>
<th>Mean sludge bed density gVSS/l</th>
<th>Loadings kgCOD/m³/d reactor sludge bed volume</th>
<th>Sludge bed volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5481</td>
<td>15</td>
<td>1:1</td>
<td>400</td>
<td>29</td>
<td>9.1</td>
<td>27.3</td>
</tr>
<tr>
<td>2</td>
<td>2648</td>
<td>30</td>
<td>1:1</td>
<td>400</td>
<td>31.9</td>
<td>9.5</td>
<td>28.5</td>
</tr>
</tbody>
</table>

Diameter of reactor = 100 mm  
Effective reactor volume = 9l
Fig 9.1: Experimentally observed and simulated concentration profiles in the pelletized sludge bed of a single UASB system. (Influent COD concentration = 5075 mg/l, flow rate = 15 l/d).
Fig 9.2: Experimentally observed and simulated concentration profiles in the pelletized sludge bed of a single UASB system. (Influent COD concentration = 2872 mg/l, flow rate = 30 l/d).
Fig 9.3: Experimentally observed and simulated concentration profiles in the pelletized sludge bed of a single UASB system. (Influent COD concentration = 8337 mg/l, flow rate = 15 l/d).
Fig 9.4: Experimentally observed and simulated concentration profiles in the pelletized sludge bed of a single UASB system. (Influent COD concentration = 5345 mg/l, flow rate = 45 l/d).
to zero before the first sampling port at the bottom of the reactor. Moreover reducing the value of \( K_H \) to 5 did not appear to have an observable influence on the simulated response.

9.2 System with recycle

Two experiments on UASB systems with recycle were simulated; see Table 9.5 below.

The observed and simulated responses are shown in Figs 9.5 and 9.6. Clearly the simulated responses show deviations from the experimentally observed responses. In general the experimental data indicate that the process rates of the different compounds were more rapid than the simulated rates. The following factors probably contributed to these deviations:

In the region above the sludge bed a certain degree of settlement of the debris, discharged from the bed, took place. Consequently the concentration of solid material in the recycle stream was higher than in the discharged effluent. Although the model assumes that the solid debris discharged is inert, very likely this is not true and recycling of this material could have contributed in increasing the process rates in the sludge bed. Recent investigations in the laboratory at the University of Cape Town on the biological activity of the suspended bed material has indicated that biological activity occurs when substrate is added to this material. In future investigations, to test the model when a recycle is imposed on the system, it will be necessary either to induce sufficient turbulence in the suspended bed so that there is no increase in solid concentration (due to settlement) or the effluent particulate material must be separated and the clarified liquid recycled.

9.3 Discussion

A significant feature in this model was that it required virtually no calibration. The stoichiometric conversion constants were obtained from established biochemical pathways, and yield values from studies reported in the literature on pure and enhanced cultures; likewise for the maximum specific growth rates and half saturation constants (in the Monod formulation). Other input data such as the mass fractions of the different microorganisms in the pellets and the distribution of VSS concentration up the reactor also were obtained from reported data in the literature. Indeed the only constants that were obtained by curve fitting were:
Fig 9.5: Experimentally observed and simulated concentration profiles in the pelletized sludge bed of a single UASB system with recycle. (Influent COD concentration = 5481 mg/l, flow rate = 15 l/d, recycle ratio = 1:1).
Fig 9.6: Experimentally observed and simulated concentration profiles in the pelletized sludge bed of a single UASB system with recycle. (Influent COD concentration = 2848 mg/l, flow rate = 30 l/d, recycle ratio = 1:1).
(1) The specific rate for pellet break up ($K_{BP}$),

(2) The specific rate for adsorption/enmeshment of soluble organic nitrogen ($K_{EP}$), and

(3) The specific rate for ammonification of soluble organic nitrogen ($K_{ND}$).

The model therefore is much broader based, on fundamental aspects or independently obtained data, than it would have been if the constants were derived by curve fitting.

An omission in the model is that it does not simulate pH and alkalinity changes in the sludge bed. It assumes in fact that the alkalinity provision in the influent is sufficient to prevent pH decline below 6.6 at any point in the bed. Because of the rapid changes in SCFA concentrations in the lower part of the bed it is likely that regions might develop where the pH decline is such that inhibition of methanogenesis takes place (both acetoclastic and H$_2$-utilizing methanogens are widely reported to be inhibited by low pH). This situation can arise at loadings close to the maximum loading.

Improvements on the model therefore should include the parameters pH and alkalinity and the inhibitory effect of pH on the methanogens. Without these extensions to the model, a theoretical basis for estimating the maximum loadings the system can accommodate is unlikely. Any such extension to the model would necessarily require experimental information. In the present study the orientation was towards determining "normal" behaviour of the UASB system, the situation at or near failure was not pursued and consequently the factors giving rise to failure were not investigated in detail.

Assuming the maximum loading conditions are not exceeded and that the pH does not decline below 6.6 in the reactor profile, by simulating the behaviour under various reactor loading conditions the following information can be abstracted:

(1) For the same mass of sludge the cross-sectional area of the reactor does not appear to have an influence on process kinetics. If the cross-sectional area is doubled, the depth of the sludge will be reduced to half. Theoretically the
effluent products will remain the same in both situations. The profiles will retain the same slope with respect to relative depths of the sludge bed.

(2) Theoretically the imposition of a recycle reduces the concentration of products in the reactor in the ratio \( \text{flow}/(\text{flow} + \text{recycle flow}) \). (Experimentally the reduction was slightly greater). This we have seen in Chapter 8 leads to an associated reduction in the alkalinity supplementation and is of particular importance from the point of view in the treatment of high strength wastes of say \( > 10000 \, \text{mgCOD/l} \). By reducing the waste strength (by imposing a recycle) of an influent of \( 20000 \, \text{mgCOD/l} \) to say \( 2500-5000 \, \text{mgCOD/l} \), the alkalinity requirement is reduced to approximately 0,125 to 0,25 of that needed in a flow-through system; SCFA concentrations inside the sludge bed likewise are reduced into a range where the system appears to operate efficiently.

10. CONCLUSIONS
A mathematical model describing the product formation in the pelletized sludge bed in a UASB system with glucose substrate, has been presented. The model accounts for the product formation by an interaction between four groups of organisms, viz: (1) Acidogens, (2) Acetogens, (3) Acetoclastic methanogens and (4) H\(_2\)-utilizing methanogens; along the line of flow up the pelletized bed. Product formation and utilization is modelled using Monod kinetics. The crucial effects of hydrogen concentration (or hydrogen partial pressure) on acetogen growth and the distribution of SCFA generated by the acidogens are incorporated by the use of a switching function. The model neglects solid transport and biofilm diffusion.

The model adequately predicts the concentrations of total COD, SCFA (acetic and propionic acids) in the pelletized sludge bed under different loadings (i.e. varying conditions of influent flow rates and COD concentrations) below the maximum loading.
CHAPTER 10

CONCLUSIONS

This investigation was initiated with a simple objective — a feasibility study into the anaerobic treatment of an agricultural waste, apple juicing waste water, by means of an upflow anaerobic sludge bed (UASB) reactor. As the investigation progressed problem areas were opened up which on enquiry provided solutions but in turn generated new problems; so that at its termination the investigation had ranged over a number of fields of enquiry including, amongst others, the feasibility study, basic processes active in the UASB system, causes of pelletization, factors limiting the behaviour of the system and modelling of the system response. With such a number of aspects it is perhaps best to deal with each separately, the problems that were addressed and the solutions achieved.

Feasibility study:
The feasibility study comprised an investigation into treating apple juicing waste water at 25 and 30°C, to ascertain acceptable organic loadings at these to temperatures and to evaluate the interactive effects of influent COD concentration, flow rate, and hydraulic retention time in the reactor. The study gave rise to the following conclusions:

1. Apple juicing waste water is amenable to treatment by the UASB system at 25 and 30°C.

2. The COD of the waste water, based on filtration, is 99 per cent soluble with short chain fatty acids (acetic and propionic acids) comprising approximately 5 per cent of the COD influent. The remainder is made up principally of sugars.

3. The waste water is deficient in nitrogen and phosphorus. These nutrients must be supplemented through chemical addition in order to achieve complete anaerobic treatment.

4. The waste water is acidic with little buffering capacity. The pH is in the range 4,5 to 5,5. Alkalinity addition, to increase the buffer capacity, must be added to the waste prior to treatment. Addition of 1,6 mg alkalinity as CaCO₃ per
mgCOD influent was sufficient to maintain the pH in the UASB reactor above the sludge bed close to 7.

5. The maximum loadings attainable are dependent on temperature. With the sludge volume controlled to 1/3 of the reactor volume, under the same condition of loading increments (average 0.21 kgCOD/m³ reactor volume every day) the maximum loadings at 30°C and 25°C were 15 and 10 kgCOD/m³ reactor volume/d respectively (45 and 30 kgCOD/m³ sludge bed volume/d respectively), i.e. the maximum loading at 30°C was 1.5 times higher than at 25°C.

6. The maximum loading attainable appears to depend on the rate of increase of the load. At 30°C when the loading was increased by apparently too large increments or too short time increments the system failed at loadings well below the maximum possible.

7. At 30°C, with incremental load increase sufficiently low (average 0.125 to 0.167 kgCOD/m³ reactor volume every day), maximum loading of 28 kgCOD/m³ reactor volume/d was attained. COD removal appeared to be virtually independent of both concentration and hydraulic retention time. At the maximum loading of 28 kgCOD/m³ reactor volume/d, at influent COD of 2500 to 5000 mg/l, the COD removal was above 90 per cent. At COD loadings below the maximum the COD removal was apparently independent of loading. For example, at influent COD of 1250 mg/l, the COD removal was 90 per cent up to loading of 22 kgCOD/m³ reactor volume/d.

8. Pelletization of the sludge occurred with the apple juicing waste water and was observed in the reactors at both 25 and 30°C. The size of the pellets ranged from 5 mm at the bottom of the bed to 0.5 mm at the top; as the loading increased the pellet sizes also increased.

Product formation:
The feasibility study showed that pelletization is readily obtainable when treating apple juicing waste water. However the study provided information only on effluent quality; no information was given as to how the effluent quality was produced in the sludge bed. Visual observations of pellet movement in the sludge bed indicated that relative movements of the sludge pellets were minimal so that the flow regime in the bed was very near plug flow. It was concluded that a detailed study on product
formation along the line of flow in the sludge bed should provide information on the processes active in the bed. Such a study was undertaken and gave rise to the following findings:

(1) The single UASB system appears to operate as a plug flow system. For the carbohydrate-type waste three regions were identified within the sludge bed:

   (i) **A lower active zone**: In this zone short chain fatty acids (acetic and propionic acids) concentrations rose to a maximum – the bed level at which the peaks occurred defined the upper limit of this zone; free and saline ammonia (NH$_3$-N) concentration reduced to a minimum and soluble organic nitrogen concentration increased to a maximum; the soluble COD reduced to about half its influent value; alkalinity and pH declined to minimum values.

   (ii) **An upper active zone**: In this zone the soluble COD concentration reduced to a minimum; the propionic and acetic acid concentrations decreased to near zero – the bed level at zero acids concentrations defined the upper limit of this zone; NH$_3$-N concentration remained near constant and soluble organic nitrogen decreased to a minimum; alkalinity and pH increased to stable values.

   (iii) **An upper inactive zone**: This zone extended above the upper active zone to the top of the bed; no overt biokinetic reactions were observed except for pellet breakup, the debris being discharged as organic fines into the suspended blanket above the bed.

(2) In the lower active zone the continuous increase in propionic acid concentration indicated that the rate of generation and the rate of utilization of hydrogen is such that a high $pH_2$ is maintained. The lower active zone terminated at the bed level here the $pH_2$ declined to such a low value that the acetogens could convert the propionic acid to acetic acid, hydrogen and carbon dioxide. This conversion, of the propionic to acetic acid, was observed in the upper active zone indicating that it was a low $pH_2$ zone. Throughout the two zones the methanogens converted the acetic acid, and hydrogen plus carbon dioxide, to methane.
(3) A single UASB system operating at a sludge age of 11 days gave a net specific VSS yield of 0.09/mgCOD removed. This yield value was similar to that reported for a UASB system fed with sucrose substrate. The single UASB system was then operated as a two-in-series reactor system, a high \( \text{pH}_2 \) reactor containing the sludge from the lower active zone operating at a sludge age of 2.9 days, and a low \( \text{pH}_2 \) reactor with the balance of the sludge. The high \( \text{pH}_2 \) reactor gave a specific yield of 0.42 mgVSS/mgCOD removed; a value 14 times greater than that normally observed for completely mixed anaerobic systems; in the low \( \text{pH}_2 \) reactor there was a net reduction in the pelletized sludge bed mass due to pellet breakup.

(4) The loss in alkalinity between influent and effluent was small. However due to the generation of short chain fatty acids principally in the high \( \text{pH}_2 \) zone, the pH in this zone declined significantly and could lead to failure if the pH declined to below 6.6. Sufficient buffering capacity had to be provided to ensure that the pH did not decline, at any point in the line of flow, to less than pH 6.6; 3.2 mg alkalinity as CaCO\(_3\)/mg influent COD was adequate to control the minimum pH. Clearly because of the phase separation in the bed alkalinity requirements for the UASB systems treating a carbohydrate are substantially greater than for completely mixed anaerobic systems.

(5) High removal of \( \text{NH}_3\)-N and a concomitant generation of organic nitrogen were observed in the high \( \text{pH}_2 \) zone, a situation not observed in normal anaerobic fermentation processes.

*Biochemical model*

From the study on product formation, features not observed in normal anaerobic processes were: pelletized sludge production was confined to the high \( \text{pH}_2 \) zone; in this zone the specific yield of the pelletized sludge was about 14 times and the nitrogen removal 8 to 10 times greater than in completely mixed anaerobic systems, also an appreciable concentration of soluble organic nitrogen was generated. It was hypothesized that the magnitude of the soluble organic nitrogen generation made endogenous/death processes unlikely sources, that most likely the organic nitrogen was a product of the growth processes.

A search of the literature produced information on one anaerobic organism that does release organic nitrogen during growth — a methanogen, *Methanobacterium* strain AZ.
(M. Strain AZ), now classified as Methanobrevibacter arboriphilus. This species utilizes hydrogen as sole energy source and can produce its amino acid requirements very effectively with the exception of the sulphur containing amino acid, cysteine—an external cysteine source is necessary for growth. In a hydrogen rich environment, with an adequate supply of NH$_3$-N and a cysteine limitation, the species secretes high concentrations of amino acids (orgN) to the surrounding medium. These characteristics provided a basis for an hypothesis on pellet formation:

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 was found in the experimental observations on the lower active (high $\phi$H$_2$) zone, by examining:

- the COD/VSS and TKN/COD ratio of the pellet;
- the rate of disappearance of free and saline ammonia, coupled to the rate of generation of organic nitrogen;
- the yield of volatile solids; and
- the effect of cysteine supplementation on volatile solids yield.

Using the mass ratio of the three major amino acids released in pure culture studies, calculations indicated that the observed TKN/COD and COD/VSS ratios of the pellets (0.09 mgN/mgCOD and 1.23 mgCOD/mgVSS respectively) could be satisfied only if about 90 per cent of the pellets consisted of polypeptide polymer.
Hypothesis verification

In a UASB system treating a carbohydrate waste water, a high hydrogen partial pressure (high $\mathrm{pH}_2$) zone develops within the lower active zone, in the breakdown of carbohydrates to SCFA by acidogenesis. The high $\mathrm{pH}_2$ (> $10^{-4.1}$ atm) zone extends up to the level where the propionic acid concentration reaches a peak. It was in this high $\mathrm{pH}_2$ zone that pellet growth was observed, associated with a high generation of soluble organic nitrogen, a situation that could be explained by the activity of $M$. Strain $AZ$. This possibility was supported by the response of the high $\mathrm{pH}_2$ zone when the feed was supplemented with cysteine - immediately (within 24h) there was a reduction in the specific pellet yield, of about 50 per cent.

In the literature, presence of a polymer matrix had been observed in the pellets, however, its origin(s) was/were not identified; one investigator ascribed its presence to polymer present in the influent, incorporated in the pellets by an agglutination process. In order to ascertain the origin of the pellet polymer, a non-polymer defined carbohydrate substrate – glucose, was selected; excellent pellet formation was observed in the UASB system.

With glucose as substrate, a detailed enquiry into the biochemical reactions taking place in the lower active zone, was initiated (because the biochemical fermentation pathways of glucose are well established). A UASB system consisting of only the lower active (high $\mathrm{pH}_2$) zone was set up. From mass balance considerations the gross specific yield (organism + polymer) of the hydrogenotrophs was determined to be between 0.21 and 0.24 mgVSS/mgCOD ($\mathrm{H}_2$). Limiting the $\mathrm{NH}_3$-N in the influent to that required for cell synthesis resulted in a decrease in the overall gross specific pellet yield from 0.52 to 0.11 mgVSS/mgCOD removed. No VSS was generated by the hydrogenotrophs; this was shown to be so by the non uptake of hydrogen (energy source) and mass balances; their decreased activity was ascribed to the intracellular high ATP/ADP level within the hydrogenotrophs (due to high substrate concentration) which the species cannot decrease, through the generation of amino acids and polypeptides, when $\mathrm{NH}_3$-N is limiting.

Criteria for pellet formation and their verification

From the hypothesis and the study above, conditions necessary for pellet formation could be set out:

- An environment with a high $\mathrm{pH}_2$. 
• 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.

From the criteria formulated above the following situations were identified under which one could expect pelletization or not:

(1) Pelletization 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.

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

(3) No pelletization 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.

(4) No or limited pelletization where the substrate can generate a high $pH_2$ but the $H_2$ generated is preferentially utilized by other organisms such as sulphate-reducers.

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

With regard to (1) this study and others demonstrated that pelletization takes place in a UASB system with carbohydrate substrates.

With regard to (2) the literature reports no pelletization in UASB systems with acetate only as substrate.

With regard to (3) there is no report on pelletization in completely mixed reactors.

With regard to (4) a study with a UASB system was undertaken with glucose as
substrate and sulphate (SO$_4^{2-}$ ions) were added to the influent feed. Pellet formation was reduced markedly. Under normal operating conditions even though the fermentation of glucose produces hydrogen sufficiently fast to generate a high $pH_2$, sulphate reducers utilize hydrogen preferentially thereby reducing $pH_2$ and hence limiting pelletization. This was supported by the following observations:

- NH$_3$-N uptake decreased as SO$_4^{2-}$ concentration increased; NH$_3$-N uptake decreased linearly with SO$_4^{2-}$ removal.

- COD removal increased linearly with SO$_4^{2-}$ removal.

- Short chain fatty acids concentrations, peak acetate and peak propionate decreased linearly with SO$_4^{2-}$ removal. The reduction in peak propionate concentration indicated that the $pH_2$ had been reduced so that acetogenesis was initiated at a lower level in the bed.

- Pellet size decreased with increasing influent SO$_4^{2-}$ concentration from 2-3 mm to 1-2 mm in diameter. Concomitantly the sludge volume decreased by about 700 ml, attaining a stable low value at concentrations of 1000 mgSO$_4^{2-}$/l and above.

The data show that at high SO$_4^{2-}$ concentrations, under high $pH_2$, the utilization of H$_2$ by M. Strain AZ tends to a constant fraction of the H$_2$ produced.

- In the high $pH_2$ reactor, the VSS yields were $8,3 \cdot 10^{-2}$ and $1,6 \cdot 10^{-2}$ mgVSS/mg influent COD at trace and excess SO$_4^{2-}$ concentrations respectively; i.e. the VSS yield for at trace SO$_4^{2-}$ concentration was more than 5 times greater than that at excess SO$_4^{2-}$ concentration. At the same time the NH$_3$-N uptake decreased from 86,8 to 58 mgN/l with a concomitant reduction of organic nitrogen from 33,6 to 4,1 mgN/l at trace and excess SO$_4^{2-}$ concentrations respectively - a further indication that polypeptide formation was reduced because of preferential abstraction of H$_2$ by the sulfidogens.

With regard to (5) a UASB system was studied with oleic acid as substrate. Pelletization did not take place. This behaviour was confirmed by the lack of
distinct zones of behaviour in the sludge bed, low NH$_3$-N uptake, low generation of orgN and production only of acetate. With oleic acid as substrate the sludge bed was well defined but of a gelatinous nature.

**pH control**

One aspect that became very clear in the study on UASB system behaviour was its high alkalinity requirements with the apple juicing waste water and glucose as substrates. To ensure that the minimum pH, in the pH profile in the sludge bed, did not decline below 6.6, alkalinity supplementation of about 1.6 mg alkalinity as CaCO$_3$/mg influent COD was adequate. Whereas there is an alkalinity loss in the lower active region, this alkalinity is recovered in the upper zones so that only a small alkalinity loss from influent to effluent is observed. Thus, in effect the alkalinity added in the influent is wasted in the effluent.

The alkalinity requirement for a UASB system is substantial; it may become an important factor in operational costs. The alkalinity in the effluent can be recovered by instituting a recycle from the effluent to the influent and hence alkalinity supplementation to the influent can be reduced. However no detailed information was available on the effect of a recycle on the pelletization and performance of a UASB system. A study was initiated to determine the effects of recycle on alkalinity requirements, maximum loadings and process performance. The following findings were obtained:

- The minimum alkalinity supplementation for a carbohydrate waste in a flow-through system is about 1.2 mg alkalinity as CaCO$_3$/mg influent COD.

- Provided the loading on the system is below the maximum this alkalinity requirement can be reduced by imposing a recycle; this reduction can be calculated by multiplying the base alkalinity requirement by the factor flow/(flow + recycle flow).

- Near the maximum loading the recycle reduced the alkalinity requirements but SCFA leakage from the sludge bed was observed. Recycling high SCFA concentrations in effect induces a higher influent COD loading, increasing the loading beyond the maximum and may lead to precipitous failure of the system.

- At loadings below the maximum imposition of a recycle up to 3:1 did not appear
to have adverse effects on pellet formation.

- Tentatively the maximum loading a system without recycle can sustain should be determined with influent COD between 2000 and 5000 mg/l. If the waste water has a higher influent COD, a recycle should be imposed such that the concentration of the combined flow (base + recycle) is within the above range.

- It is suggested that the actual loading applied should be lower than the peak load determined experimentally, by a factor of 0.5. For example with apple juicing waste water at 30°C the peak loading was about 45 kgCOD/m³ sludge bed volume/d (i.e. 15 kgCOD/m³ reactor volume/d), giving an operating loading of ≈ 22 kgCOD/m³ sludge bed volume/d. Tentatively a minimum sludge volume provision in the reactor should be about 0.03 m³/kg COD influent. Pellet production always will be sufficient to provide this volume of sludge.

**Kinetic model**
A mathematical model was developed that described the stoichiometry and kinetic behaviour of the various processes operating in a UASB system producing a pelletized sludge mass. With glucose as substrate 11 compounds, and 12 processes acting on these compounds, were identified. A mechanistic model was conceptualized that qualitatively described the kinetic and stoichiometric behaviour of the processes and compounds. Finally the process rates, stoichiometry and transport relationships were formulated mathematically. Stoichiometric and kinetic constants for the biologically mediated processes (acidogenesis, acetogenesis, and methanogenesis from hydrogen and acetate) were obtained either from the literature or this study. The constants for only 3 processes had to be found by curve fitting.

Simulation of the system behaviour at COD loadings below the maximum gave good correlation for COD, SCFA, orgN and NH₃-N profiles for the flow-through system. For the system with a recycle the experimental response indicated better performance than the simulated response; this appears to be due to pellet debris in the recycle, which apparently is biologically active – this aspect was not included in the model.

**Future research**
The following areas of enquiry need to be investigated:
• The kinetic model needs to take into account the changes in pH and alkalinity along the line of flow. Incorporation of these parameters may contribute to establishing a theoretical basis for determining the maximum loading a system can sustain. In this regard knowledge of the behaviour of the system at or near failure is still inadequate.

• Investigations are required into the treatment of other waste waters such as proteinaceous and wine distillery waste waters that conceptually have the potential to produce pelletized sludges. Proteinaceous waste waters will generate a high ammonium concentrations which might be inhibitory to the anaerobic organisms, and will affect pH behaviour by internal generation of alkalinity due to release of NH₃ (which forms NH₄⁺ in solution). Wine distillery waste waters have a complex composition of carbohydrates, proteins and other organic materials, and has high COD strengths, 20 000 to 40 000 mgCOD/l. In the past treatment of this waste water in UASB-like system has not been successful in South Africa, however with the knowledge acquired in this study there are good prospects that successful treatment can be achieved.

• The responses in the pelletized sludge bed indicate that the reactions usually are complete within, at the most, 80 cm from the base of the bed. This raises the question as to whether the existing UASB full-scale designs are appropriate.

• Theoretical and experimental enquiries are needed into UASB systems treating vegetable oils and long chain fatty acids. These substrates do not promote pellet formation but do give rise to a sludge bed. Very little is known on the conditions required to give optimal performance.
LIST OF REFERENCES


APPENDIX A

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 Warhmann, 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 are: alanine:1.08, valine:1.64 and glutamic:0.98.

(d) the TKN/COD ratio of the amino acids are: alanine:0.146, valine:0.073 and glutamic:0.097

(i) COD/VSS ratio of polypeptide = \(0.47 \cdot 1.08 + 0.30 \cdot 1.64 + 0.98 \cdot 0.23\)
   \[= 1.21\]

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

\[1g \text{ sludge} = Xg \text{ organisms} + (1−X)g \text{ polypeptide}\]

Substituting the COD/VSS values above into Eq (a),
\[1.23 = X \cdot 1.42 + (1−X) \cdot 1.21\]
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:

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

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:

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

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

\[
\text{TKN/COD of sludge} = 0,90 \cdot 0,113 + 0,10 \cdot 0,086 \\
= 0,11 \text{ mgN/mgCOD}
\]

COD of amino acids released in the high H₂ partial pressure reactor

Average mmols organic nitrogen released = 8,2/14

= 0,59

Component : mmols x COD mass equivalent

<table>
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<th>Component</th>
<th>mmols x COD mass equivalent</th>
<th>(\text{mgCOD/\ell})</th>
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</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>(0,56 \cdot 0,59) \cdot 96</td>
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<tr>
<td>Valine</td>
<td>(0,28 \cdot 0,59) \cdot 192</td>
<td>31,72 \text{ mgCOD/\ell}</td>
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<td>Glutamic</td>
<td>(0,16 \cdot 0,59) \cdot 144</td>
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<td></td>
<td>77,03 \text{ mgCOD/\ell}</td>
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</table>

\`: Total COD in the form of free amino acids = 77,03 \cdot 92 \\
= 7087 \text{ mgCOD/\ell}`
APPENDIX B

CALCULATION OF THE COD ASSOCIATED WITH THE DISSOLVED ORGANIC NITROGEN MEASURED IN THE LOWER ACTIVE ZONE (HIGH pH ZONE) OF A UASB SYSTEM

Sam-Soon et al. (1987) hypothesized 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 Wurhmann, 1977) are: alanine, valine and glutamic acid in the mass ratios: alanine:valine:glutamic = 0.47:0.30:0.23.

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

For the 60l/d feed;

Average mmols organic nitrogen released/ℓ = 17.7/14 = 1.26 mmol

Component : mmol x COD mass equivalent

<table>
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<tr>
<th>Component</th>
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<th>mgCOD/ℓ</th>
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</thead>
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</tr>
<tr>
<td>Valine</td>
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<tr>
<td>Glutamic</td>
<td>(0.16 x 1.26) x 144</td>
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</table>

\[164.5\] mgCOD/ℓ

\[\because\] COD in the form of free amino acids : 164.5 mgCOD/ℓ.
APPENDIX C

DETAILS ON THE MATRIX

1. STOICHIOMETRIC RATIOS

The stoichiometric relationships in the production of various compounds during anaerobic fermentation of glucose are derived below.

1.1 Glucose to short chain fatty acids

As mentioned in Chapter 9, as no butyrate was observed in the single UASB system under varying loadings, butyrate generation is not considered.

(i) Under high pH2:

From Chapter 2, under high pH2, 1 mole glucose (24 electron equivalents, e^-), produces 1 mole propionic acid (14 e^-), 1 mole acetic acid (8 e^-), 1 mole hydrogen (2 e^-) and 1 mole carbon dioxide Eq (2.11), Chapter 2. In terms of COD units, 1 COD unit of glucose gives:

\[ 1 = \frac{8}{24} \text{HAc} + \frac{14}{24} \text{HPr} + \frac{2}{24} \text{H}_2 \]  

(C.1)

The energy derived from catabolism of glucose is used to incorporate \( Y_A \) COD units glucose into organism mass i.e.

\( Y_A = (Y_A) \text{organisms} \)  

(C.2)

Adding Eqs 1 and 2 gives:

\[ 1 + Y_A = (Y_A) \text{organisms} + \frac{8}{24} \text{HAc} + \frac{14}{24} \text{HPr} + \frac{2}{24} \text{H}_2 \]  

(C.3)

Since the rate equations in the matrix (Chapter 9) are expressed in terms of rate of organism growth so that the stoichiometric equation is expressed as unity with respect to organism mass. Therefore, dividing Eq (C.3) by \( Y_A \):
\[ \frac{1+Y_A}{Y_A} = (1) \text{organisms} + \frac{8}{24} \cdot \frac{1}{Y_A} \text{HAc} + \frac{14}{24} \cdot \frac{1}{Y_A} \text{HPr} + \frac{2}{24} \cdot \frac{1}{Y_A} \text{H}_2 \]  

(C.4)

Eq (C.4) can be interpreted as follows: For every one COD unit of organism mass formed \( \frac{1+Y_A}{Y_A} \) COD units of glucose are utilized, and \( \frac{8}{24} \cdot \frac{1}{Y_A} \) COD units of acetic acid, \( \frac{14}{24} \cdot \frac{1}{Y_A} \) of propionic acid and \( \frac{2}{24} \cdot \frac{1}{Y_A} \) of \( \text{H}_2 \) are produced.

Similar derivations are made for other stoichiometric equations below.

(ii) Under low \( \bar{pH}_2 \):
Under low \( \bar{pH}_2 \), 1 mole of glucose produces 2 moles acetic acid and 4 moles of hydrogen Eq (2.5), Chapter 2. The energy derived from catabolism is used to incorporate glucose into organism mass, i.e.

\[ 1 + Y_A = (Y_A) \text{organisms} + \frac{16}{24} \text{HAc} + \frac{8}{24} \text{H}_2 \]  

(C.5)

dividing by \( Y_A \)

\[ \frac{1+Y_A}{Y_A} = (1) \text{organisms} + \frac{16}{24} \cdot \frac{1}{Y_A} \text{HAc} + \frac{8}{24} \cdot \frac{1}{Y_A} \text{H}_2 \]  

(C.6)

Eq (C.6) gives the stoichiometric relationship between glucose and products formed under low \( \bar{pH}_2 \).

1.2 Conversion of propionic acid to acetic acid
1 mole of propionic acid is converted to 1 mole acetic acid, 3 moles hydrogen and 1 mole carbon dioxide Eq (2.12), Chapter 2. The energy derived from catabolism of propionic acid is used to incorporate \( Y_{AP} \) units propionic acid into organism mass. The equation is

\[ 1 + Y_{AP} = (Y_{AP}) \text{organisms} + \frac{8}{14} \text{HAc} + \frac{6}{14} \text{H}_2 \]  

(C.7)
\[
\frac{1 + Y_{\text{AP}}}{Y_{\text{AP}}} = (1) \text{ organisms} + \frac{8}{14} \cdot \frac{1}{Y_{\text{AP}}} \text{HAc} + \frac{6}{14} \cdot \frac{1}{Y_{\text{AP}}} \text{H}_2 \quad (C.8)
\]

i.e. for one unit of acetogenic mass formed, \(\frac{1 + Y_{\text{AP}}}{Y_{\text{AP}}}\) units of propionic acid are utilized, and \(\frac{8}{14} \cdot \frac{1}{Y_{\text{AP}}}\) units acetic acid and \(\frac{6}{14} \cdot \frac{1}{Y_{\text{AP}}}\) units of hydrogen are produced, all units as COD.

### 1.3 Conversion of acetate to methane

Conversion of acetate to methane by the acetoclastic methanogens is given by Eq (2.14), Chapter 2, i.e. 1 mole acetate generates 1 mole methane

i.e. \(1 = (Y_{\text{MA}}) \text{ organisms} + (1 - Y_{\text{MA}}) \text{CH}_4 \quad (C.9)\)

dividing by \(Y_{\text{MA}}\)

\[
\frac{1}{Y_{\text{MA}}} = (1) \text{ organisms} + \left[ \frac{(1 - Y_{\text{MA}})}{Y_{\text{MA}}} \right] \text{CH}_4 \quad (C.10)
\]

i.e. for one unit of acetoclastic methanogen mass formed, \(\frac{1}{Y_{\text{MA}}}\) units acetate are utilized, and \((1-Y_{\text{MA}})\) units methane produced, all units as COD.

### 1.4 Conversion of hydrogen to methane

The conversion of hydrogen to methane by the hydrogenotrophs is given by Eq (2.15), Chapter 2. From Chapter 5, it is hypothesized that the energy derived from oxidation of hydrogen to methane is used to generate (i) organism mass and (ii) polymer mass, i.e.

\(1 = (Y_{\text{MH}}) \text{ organisms} + (Y_{\text{P}}) \text{ polymer} + (1 - Y_{\text{P}} - Y_{\text{MH}}) \text{CH}_4 \quad (C.11)\)

dividing by \(Y_{\text{MH}}\)
\[
\frac{1}{\text{Y}_{\text{MH}}} = (1) \text{ organisms} + \left( \frac{\text{Y}_{\text{P}}}{\text{Y}_{\text{MH}}} \right) \text{ polymer} + \left[ \frac{(1-\text{Y}_{\text{P}} - \text{Y}_{\text{MH}})}{\text{Y}_{\text{MH}}} \right] \text{CH}_4
\]

(C.12)

From Eq (C.12) one unit mass of hydrogenotrophic mass is generated from \( \frac{1}{\text{Y}_{\text{MH}}} \) units of hydrogen, with the formation of \( \frac{\text{Y}_{\text{P}}}{\text{Y}_{\text{MH}}} \) units of polymer mass and \( \frac{(1-\text{Y}_{\text{P}} - \text{Y}_{\text{MH}})}{\text{Y}_{\text{MH}}} \) units of methane, all units as COD.

2. MATRIX METHOD FOR MODEL PRESENTATION

To fully understand the mathematical model presented in Chapter 9, Table 9.1, it is useful to gain an insight into the representation and workings of the matrix as described briefly below.

**Representation**

The matrix is represented by a number of columns and rows; one column for each compound and one row for each process. The symbols for the compounds are listed at the head of the appropriate column and the compounds are defined at the bottom of the corresponding column. The index "i" is assigned to identify a compound in the totality of compounds.

The processes are itemized one below the other down the left-hand side of the matrix. The index "j" is assigned to identify the process. The process rates are formulated mathematically and listed down the right-hand side of the matrix, in line with the respective process row. These process rates are given the symbol "\( p_j \)", where j identifies the process.

Along each process row the stoichiometric coefficient for conversion from one compound to another is inserted so that each column lists the processes that influence that compound. The stoichiometric coefficients are given the symbol "\( v_{ij} \)" where i denotes the index of the compound and j the index of the process. The stoichiometric coefficients \( v_{ij} \) are greatly simplified by working in consistent units; in this case concentrations are expressed in COD or nitrogen (N) units. Sign convention in the matrix for the stoichiometric coefficients is "negative for consumption" and "positive for production".
This matrix forms a succinct summary of the complex interactions between compounds and processes. The matrix in effect constitutes a fingerprint uniquely characterizing the phenomenon. It allows alterations in processes, compounds, stoichiometry and kinetics to be readily incorporated.

The matrix representation method has two main benefits:

1. It allows the effect of a particular process on the compounds to be easily determined, as follows: The reader moves along a particular row, i.e. process, and multiplies the stoichiometric coefficient \( v_{ij} \) by the process rate \( \rho_j \). This gives the reaction rate \( r_{ij} \) for the particular compound being affected by the single process, i.e.

\[
 r = v_{ij} \rho_j
\]  
(C.13)

In representing the matrix, by adding up the reaction rates for a particular process, a mass balance must be obtained.

2. It allows rapid and easy recognition of the fate of each compound, as follows: The reader moves down the column representing the compound of interest, and multiplies the stoichiometric coefficient \( v_{ij} \) by the process rate \( \rho_j \). The summation of these multiplications gives the overall reaction rate \( r_i \) for the compound, i.e.

\[
 r_i = \sum_j v_{ij} \rho_j
\]  
(C.14)

**Switching function**

Under certain conditions the process rate equations are not operative, e.g. propionic acid degradation is not operative under high \( \rho H_2 \). Mathematically, switching the process rate "on" and "off" can be achieved by multiplying the appropriate rate by a "switching" factor, which is zero when the process rate is inoperative, or unity when the process rate is operative. The general expression used for the switching function is:

\[
 \frac{C}{K + C}
\]  
(C.15)
where \( C \) = concentration of compound effecting the switch
\( K \) = constant.

This is a Monod-type expression. By selecting very small values for \( K \), the function is close to unity when \( C \) is present. The function decreases to zero only at very low concentrations of \( C \). A Monod-type expression is utilized as it provides continuity between the "off" and the "on" situation which helps to eliminate problems of numerical instability in computer calculations.

**Matrix solution**

Solution of the matrix can be in time (e.g. plug flow reactor, batch reactor), space (e.g. steady state multiple reactor system), or time and space (e.g. multiple reactor system with time varying flow). Solution procedures are described briefly below. For more detailed procedures the reader is referred to Billing, 1987.

1. **Solution in time**: This solution requires that the initial concentration be known whereafter changes in concentration are determined by integrating forward in time. Integration forward follows the basic Euler equation or equivalent:

\[
C(t + \Delta t) = C(t) + \left( \frac{dC}{dt} \right)_t \Delta t
\]

where 
\( C \) = compound concentration
\( t \) = time
\( \Delta t \) = step size in integration
\( \left( \frac{dC}{dt} \right)_t \) = reaction rate

The reaction rate is obtained from the summation down the particular compound's column of the multiplication terms \( v_{ij} \rho_j \) as described previously. Solution in time can be transposed to a solution in space by taking due cognizance of the flow rate, e.g. in a flow-through plug flow reactor where the flow rate and time step interval in the integration are used to fix the displacement along the length of the reactor.

2. **Solution in space**: Solution of the matrix in space requires that the transport terms be included. Inclusion of the transport terms and the rate
The mass of input and output are the transport terms and depend on the physical characteristics of the system being modelled. The mass of production for a particular compound is obtained from the matrix. Taking an example, in symbols, for completely mixed reactor:

\[
V \frac{dC_{\text{out}}}{dt} = Q_{\text{in}} C_{\text{in}} - Q_{\text{out}} C_{\text{out}} - r_i V
\]  

(C.17)

where

- \(V\) = Volume
- \(Q_{\text{in}}\) = Flow rate in
- \(Q_{\text{out}}\) = Flow rate out
- \(C_{\text{in}}\) = Concentration of compound in flow
- \(C_{\text{out}}\) = Concentration of compound in outflow (i.e. reactor concentration for completely mixed reactor)

\[
\frac{dC_{\text{out}}}{dt} = \text{rate of change of reactor concentration of compound } C
\]

\[
r_i = \sum_{j} \nu_{ij} \rho_j\] obtained from the matrix (see Eq C.14).

Dividing by \(V\) and recognizing that at steady state \(\frac{dC_{\text{out}}}{dt} = 0\)

\[
\frac{Q_{\text{in}}}{V} C_{\text{in}} - \frac{Q_{\text{out}}}{V} C_{\text{out}} - r_i = 0
\]

Mass balance equations are derived for each compound in every reactor (including the settler). This yields a set of simultaneous non-linear equations for each reactor which then may be solved to give values for all the compounds. As the equations are non-linear repetitive techniques must be employed in the solution.

(3) **Solution in time and space:** This solution is confined to the situation of single or multiple reactors under repetitive diurnal flows. Again mass
balances are set up but, unlike the steady state system, \( \frac{dC_{\text{out}}}{dt} \) no longer equal zero. Initial concentration values are selected and the mass balance equations are integrated forward until the solution is reached; this is achieved when the concentration of all compounds in each reactor at the start and at the end of the diurnal cycle are equal.
APPENDIX D

EXPERIMENTAL RESULTS: UASB CONCENTRATION PROFILES
Table D1: Experimental results with Glucose substrate, (flow rate: 151/d)

<table>
<thead>
<tr>
<th>Smpl Port</th>
<th>COD mg/l</th>
<th>TKN mg/l</th>
<th>NH3-N mg/l</th>
<th>OrgN mg/l</th>
<th>pH</th>
<th>HAc mg/l</th>
<th>HPr mg/l</th>
<th>ALK CaCo3 mg/l</th>
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Table D2: Experimental results with Glucose substrate, (flow rate: 301/d)

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<th>HAc mg/l</th>
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(flow rate : 451/d)

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Table D4: Experimental results with Glucose substrate, 
(flow rate : 451/d)

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### Table D5: Experimental results with Glucose and SO4,
(flow rate: 151/d)

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### Table D6: Experimental results with Glucose and SO4,
(flow rate: 151/d)

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Table D8: Experimental results with Glucose and SO4, (flow rate: 151/d)

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Table D9: Experimental results with Glucose and SO₄,  
(flow rate: 151/d)

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Table D10: Experimental results with Glucose and SO₄,  
(flow rate: 151/d)

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Table D11: Experimental results with Apple conc. substrate, (flow rate: 30l/d, recycle ratio: 0)

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Table D12: Experimental results with Apple conc. substrate, (flow rate: 30l/d, recycle ratio: 1:1)

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Table D13: Experimental results with Apple conc. substrate,  
(flow rate: 301/d, recycle ratio: 1:1)

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Table D14: Experimental results with Apple conc. substrate,  
(flow rate: 151/d, recycle ratio: 1:1)

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Table D16: Experimental results with Apple conc. substrate, (flow rate: 151/d, recycle ratio: 2:1)

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(flow rate: 151/d, recycle ratio: 3:1)

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Table D18: Experimental results with Apple conc. substrate, 
(flow rate: 151/d, recycle ratio: 0)

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## Table D19: Experimental results with Oleic acid substrate, (flow rate: 151/d)

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