# Effect of nitrogen limitation on pelletisation in upflow anaerobic sludge bed (UASB) systems

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## Abstract

In a UASB system treating the carbohydrate substrate glucose, in order to obtain unimpeded pellet formation, the influent TKN/COD must be greater than that required for cell synthesis and amino acid/polypeptide formation, estimated at about 0,02 mgN/mg influent COD. As the influent TKN/COD decreases below this level; (1) the growth of the pellet forming hydrogenotroph *M.* Strain *AZ* is increasingly inhibited, (2) associated pellet formation decreases and (3) an increasingly high excess hydrogen partial pressure develops, giving rise to hydrogen loss from the system as a gas. Hydrogen escaping can account for a loss of up to 14 per cent of the influent COD. If the influent TKN/COD falls below that required for cell synthesis, estimated at about 0,0086 mgN/mg influent COD, acidogen synthesis is inhibited and their specific yield is reduced.

## Introduction

In the biochemical model of Sam-Soon *et al.* (1987; 1990) on pelletisation in upflow anaerobic sludge bed (UASB) systems; the prerequisites for pellet formation are listed as follows:

(1) an environment with a high hydrogen partial pressure;

(2) a nitrogen source, in the free and saline ammonia ($NH_3$-N) form, well in excess of metabolic requirements of the anaerobic organisms;

(3) a limited source of the amino acid cysteine; and

(4) a near neutral pH.

Indirectly Sam-Soon *et al.* (1987; 1990) had shown that in a UASB system treating a carbohydrate waste, prerequisites (1) and (3) are satisfied in the lower active (high hydrogen partial pressure) zone of the sludge bed; prerequisites (2) and (4) are satisfied by $NH_3$-N and alkalinity supplementation respectively, where required.

An implication of the nitrogen prerequisite is that if the $NH_3$-N in the influent is reduced below some upper level, pelletisation also should be reduced, until at some lower level conceivably no pellets are likely to be produced and the UASB system may not operate as expected. From both practical and theoretical points of view it is important, therefore, to enquire into the effects of nitrogen limitation on the behaviour of a pellet forming UASB system; this is the objective of this paper.

## Experimental

A UASB reactor was set up to operate in the high hydrogen partial pressure (high $\overline{p}H_2$) phase at 30°C. The set-up and substrate/nutrient feed were identical to those described by Sam-Soon *et al.* (1990): A 3 ℓ reactor filled with pelletised sludge up to the 1 ℓ volume level and maintained at this level by wasting sludge generated; influent substrate glucose of about 2 774 mgCOD/ℓ at a flow rate (Q) 60 ℓ/d, i.e. a load of 0,166 kgCOD/d or 0,867 mol glucose/d, giving a loading rate of 166 kgCOD/m³ sludge volume.d. With this loading rate Sam-Soon *et al.* (1990) had found that the 1 ℓ sludge bed volume would be completely in the high $pH_2$ zone.

Two influent $NH_3$-N concentrations were investigated:

- An $NH_3$-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 $NH_3$-N concentration of 10,5 mgN/ℓ, i.e. a N/COD ratio of 0,0038 mgN/mgCOD. This ratio was selected based on N requirements for completely mixed anaerobic systems.

Under the two $NH_3$-N influent concentrations, reactor operation was identical to that described by Sam-Soon *et al.* (1990). The results observed are listed in Table 1.

When operated with **excess** $NH_3$-N in the feed (86,8 mgN/ℓ) the measured VSS yield was 0,47 mgVSS/mgCOD removed, $NH_3$-N removal 46,5 mgN/ℓ and soluble COD removal 632 mg/ℓ (37,9 gCOD/d). The effluent was turbid and remained so after filtration; no pellet debris was discharged from the bed; effluent soluble organic nitrogen was 17,7 mgN/ℓ and $NH_3$-N 40,3 mgN/ℓ. The high organic nitrogen level indicated substantial soluble organic nitrogen production. This effluent quality was virtually identical to that found by Sam-Soon *et al.* (1990) at the same loading.

When operated with **limited** $NH_3$-N in the feed (10,5 mgN/ℓ), the measured VSS yield decreased within two days from 0,47 to 0,08 mgVSS/mgCOD removed (Fig. 1), $NH_3$-N removal was approximately 5,4 mgN/ℓ and soluble COD removal 469 mg/ℓ (28,1 gCOD/d). The pellets showed signs of breaking up with debris discharging from the bed. The filtered effluent was clear, the soluble organic nitrogen concentration at a low level of 3,8 mgN/ℓ and $NH_3$-N concentration at 5,1 mgN/ℓ. The effluent organic nitrogen level (3,8 mgN/ℓ), although low in absolute terms, was still relatively high compared with the $NH_3$-N removed (5,5 mgN/ℓ). Very likely the organic nitrogen was not generated as extruded amino acids or extracellular polymer by the organisms in their growth phase, but was a result of pellet break-up.

The low $NH_3$-N influent was maintained for a total of 9 d during which time the VSS yield remained constant at approximately 0,08 mgVSS/mgCOD removed. On increasing the influent $NH_3$-N concentration back to 86,8 mgN/ℓ, the response also reverted back, within a day, to the response previously observed (Fig. 1), i.e. VSS yield of 0,47 mgVSS/mgCOD removed, $NH_3$-N removal of 46,5 mgN/ℓ, etc.

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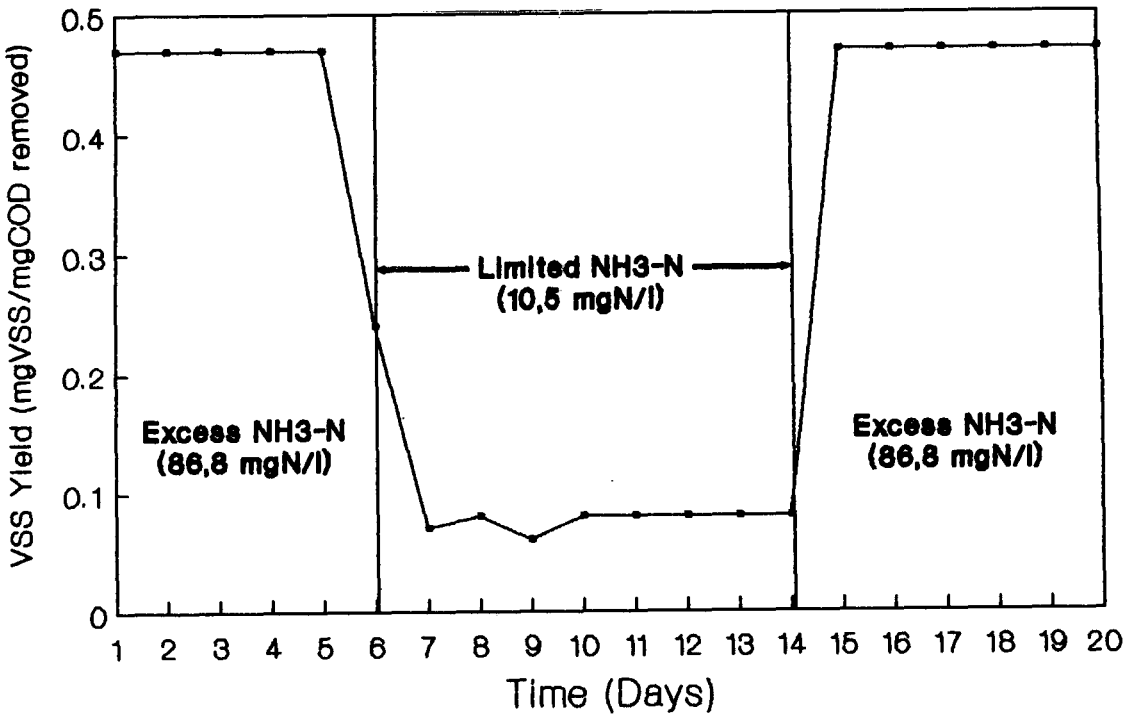


*Figure 1*
*Effect of limiting influent $NH_3$-N addition on VSS yield in the high hydrogen partial pressure reactor with glucose as substrate (COD = 2 774 mgCOD/ℓ; flow rate = 60 ℓ/d)*

## Analysis

The data were analysed following the identical procedures set out by Sam-Soon *et al.* (1990) viz. overall COD balance, glucose balance, hydrogen generation, hydrogenotrophic cell plus polypeptide mass generation, methane generated from hydrogen and acetate cleavage, VSS generated/d and gross specific sludge yield. Detailed analysis of the system with excess $NH_3$-N gave results identical to that by Sam-Soon *et al.* (1990); accordingly the analysis is not repeated here, the results obtained are listed in Table 2. An analysis of the system with **limited** $NH_3$-N (also listed in Table 2) is outlined below.

Following the method of analysis given by Sam-Soon *et al.* (1990) in their **Appendix 1,** the concentration of glucose not processed could be calculated using the data from Table 1 and applying Sam-Soon *et al's* Eq. (1.1), i.e.:

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

$\text{Total COD}_{eff}$ = 2 305 mg/ℓ
where $\text{Total COD}_{eff}$ = filtered effluent COD

$\text{COD}_{SCFA}$ = HAc x 1,067 + HPr x 1,1512 + HBr x 1,1714
= 689 x 1,067 + 278 x 1,512 + 300 x 1,714 = 1 670 mg/ℓ

where HAc, HPr and HBr are concentrations of acetic, propionic and butyric acids respectively, and the constants are for conversion to COD.

$\text{COD}_{orgN}$ was calculated following the procedure set out in **Appendix 2** of the paper by Sam-Soon *et al.* (1990) giving:

OrgN = 3,8 mgN/ℓ
hence $\text{COD}_{orgN}$ = 30 mg/ℓ

Substituting the values above in Eq. (1.1) and solving for $\text{COD}_{\text{glucose not processed}}$:

$\text{COD}_{\text{glucose not processed}}$ = 605 mg/ℓ (36,3 g/d)

i.e.
$\text{COD}_{\text{glucose processed}}$ = Influent $\text{COD}_{\text{glucose}}$ - $\text{COD}_{\text{glucose not processed}}$
= 2 774 - 605
= 2 169 mg/ℓ (130,14 g/d)

In molar masses per day:

mass glucose not processed = 605 x 60/(1,067 x 180 x 1000)
= 0,189 mol glucose/d.

And glucose processed per day = 0,867 - 0,189 = 0,678 mol glucose/d

The moles of acetate oxidised to methane ($[HAc]_{ox}$) now can be calculated from a glucose balance following the procedure of Sam-Soon *et al.* (1990), using their Eq. (11):

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

where
$[HPr]_{G1}$ = mol propionic acid in effluent/d
$[HAc]_{obs}$ = mol acetic acid in effluent/d
$[HBr]_{G3}$ = mol butyric acid in effluent/d
$[GT]$ = mol glucose processed/d
$Y_{acid}$ = specific yield of acidogens in mol glucose/mol glucose, usually taken equivalent to 0,1 (Sam-Soon *et al.*, 1990)

Converting the SCFA (from Table 2) to molar quantities and substituting in Eq. (11),

**TABLE 1**
**RESPONSE OF THE HIGH HYDROGEN PARTIAL PRESSURE UASB REACTOR AT EXCESS AND AT LIMITING $NH_3$-N INFLUENT CONCENTRATIONS TREATING GLUCOSE**

| | $NH_3$-N excess system | $NH_3$-N limited system |
|---|---|---|
| Flow (ℓ/d) | 60 | 60 |
| Influent COD (mgCOD/ℓ) glucose | 2 774*2 | 2 774*2 |
| Mass loading (gCOD/d) glucose | 166,4 (0,867 mol glucose) | 166,4 (0,867 mol glucose) |
| Filtered effluent COD (gCOD/d) | 128,5 | 138,3 |
| COD removed per day (gCOD/d) | 37,92 | 28,14 |
| COD removal (%) | 23 | 17 |
| $NH_3$-N (mgN/ℓ) In | 86,8 | 10,5 |
| Out | 40,3 | 5,1 |
| Dissolved orgN in effluent (mgN/ℓ) | 17,7 | 3,8 |
| VSS generated per day (gVSS/d) | 18,02 | 2,15 |
| VSS yield (mgVSS/ mg COD removed) | 0,47 | 0,08 |
| Nature of VSS generated | pellets | finely dispersed solids |
| Methane generated per day (ℓ $CH_4$/d at STP) | 4,2 | 0,88 |
| Methane COD [gCOD($CH_4$)/d] | 12 | 2,28 |
| Glucose processed per day (mol Gl/d) | 0,867 | 0,689 |
| Overall COD mass balance (%) | 99 | 86*1 |
| SCFA (effluent)*3 | | |
| Acetate (mgHAc/ℓ) | 635 | 689 |
| Propionate (mgHPr/ℓ) | 315 | 278 |
| Butyrate (mgHBr/ℓ) | 270 | 297 |
| Filtered effluent quality | turbid | clear |

*1 When $H_2$ gas loss is not included; 99 per cent when included.
*2 To convert COD (gram) to glucose (mol) divide by (180 x 1,067); 1 g glucose ≡ 1,067 gCOD.
*3 To convert HAc; HPr; HBr (mg) to COD (mg) multiply by 1,067; 1,512; 1,724 respectively. To convert HAc; HPr; HBr (mg) to (mmol) divide by 60;74;88 respectively.

**TABLE 2**
**CALCULATED VALUES OF HYDROGEN FLUX, ACETATE CLEAVAGE, HYDROGEN OXIDATION AND VSS YIELDS OF $H_2$-UTILISERS**

| Flow rate ℓ/d | Glucose processed gCOD/d | Glucose not processed gCOD/d | Methane generated (measured) gCOD/d | HAc oxidised to $CH_4$ gCOD/d | Hydrogen oxidised to $CH_4$ gCOD/d | Hydrogen flux gCOD/d | Total VSS generated gCOD/d | VSS generated by $H_2$-utilisers gCOD/d | Hydrogen used for*1 amino acids gCOD/d | Yield $H_2$-utilisers gVSS/ gCOD |
|---|---|---|---|---|---|---|---|---|---|---|
| 60 | 166,46 | zero | 11,85 | 4,48 | 7,37 | 27,57 | 25,54 | 8,89 | 9,87 | 0,24 |
| 60*2 | 130,18 | 36,30 | 2,28 | zero | 2,28 | 25,0 | 3,12 | zero | zero | zero |

*1 See **Appendix 2** Sam-Soon *et al.* (1990) for calculation procedures
*2 $NH_3$-N limited system

$$0,678 = 0,225 + {}^{1}/_{2}\left\{0,689 + [HAc]_{ox} - 0,225\right\} + \frac{0,205}{0,678} + 0,1 \text{ x}$$

Solving for $[HAc]_{ox}$:

$$[HAc]_{ox} = -0,0896 \text{ mol/d}$$

$[HAc]_{ox}$ cannot have a negative value because acetate cannot be generated from methane, i.e. the minimum value $[HA]_{ox}$ can take is zero. A zero value can be obtained by reducing the yield of the acidogens. Accepting $[HAc]_{ox}$ to be zero, the yield of the acidogens ($Y_{acid}$) can be calculated from Eq. (11), i.e.

$$0,678 = 0,225 + {}^{1}/_{2}\{0,689 + 0 - 0,225\} + 0,205 + Y_{acid} \text{ x } 0,678$$

i.e.

$Y_{acid} = 0,024$ mol glucose/mol glucose processed

$Y_{acid} = 0,024$ is substantially less than the $Y_{acid} = 0,1$ usually accepted for acidogens, Sam-Soon *et al.* (1990).

Assuming that the acetogenic, acetoclastic and hydrogenotrophic organism masses were minor compared to acidogenic mass (Sam-Soon *et al.*, 1990), then using $Y_{acid} = 0,1$ and $Y_{acid} = 0,024$ gives theoretical VSS generated/d of 12,9 gCOD/d and 3,11 gCOD/d respectively. One may now check which of these two values is to be preferred. The measured VSS generated/d, from Table 1, is 2,15x1,42 = 3,05 gCOD/d, which would support the lower yield value. A further check can be obtained from the mass of N incorporated in sludge/d. Again assuming that the acetogenic, acetoclastic and hydrogenotrophic organism masses are minor compared to the acidogenic mass, then the mass of N incorporated in acidogenic mass ($\Delta N_{acid}$) is given by the mass of $NH_3$-N in the influent ($NH_3$-$N_{in}$) less the mass of $NH_3$-N and soluble organic N in the effluent ($NH_3$-$N_{out}$ and $OrgN_{out}$ respectively), i.e.:

$$\begin{aligned}\Delta N_{acid} &= (NH_3\text{-}N_{in} - NH_3\text{-}N_{out} - OrgN_{out})Q \\ &= (10,5 - 5,1 - 3,8) \\ &= 96 \text{ mgN/d}\end{aligned}$$

Assuming the TKN/COD ratio of the sludge is 0,086, then:

$$\begin{aligned}\text{acidogenic mass generated} &= 96/(0,086 \text{ x } 1000) \\ &= 1,117 \text{ gCOD/d}\end{aligned}$$

This value of 1,117 gCOD/d is substantially less than the measured VSS of 3,05 gCOD/d.

It is likely that the effluent organic N could have been derived from pellet break up, and assuming this, the N utilised ($\Delta N_{acid}$) would be 5,4 x 60 = 324 mgN/d, in which event:

$$\begin{aligned}\text{acidogen mass generated} &= 324/0,086 \text{ x } 1000 \\ &= 3,77 \text{ gCOD/d}\end{aligned}$$

This value is not greatly at variance with the observed VSS generated (3,05 gCOD/d).

The analysis above, irrespective of the assumptions made, indicates that the reduced $Y_{acid} = 0,024$ is nearer reality than the value of 0,1 usually accepted. One possible reason for the low $Y_{acid}$ value is that the N supplied in the influent was completely inadequate. Assuming $Y_{acid} = 0,1$ mgCOD/mgCOD, then synthesis N requirement for the acidogens would be:

$$\frac{TKN}{COD} \text{ x } Y_{acid} \text{ x } COD_{\text{glucose to be processed}}$$

= 0,086 x 0,1 x 2774 = 23,9 mgN/ℓ influent flow.

However, the $NH_3$-N supplied was only 10,5 mgN/ℓ. As organism death of the acidogens is unlikely to be of any consequence in the acidogenic phase, it would now appear that the minimum estimated N requirement for cell synthesis was totally inadequate. In a normal completely mixed anaerobic system the net N requirement will be greatly reduced because organism death would be significant thereby providing a feedback of N to the system. This is an aspect that merits further experimental study. In this study it will be accepted that $Y_{acid} = 0,024$ mol glucose/mol glucose processed; consequently it follows, from Eq. (11) above, that $[HAc_{ox}] = 0$, that is, no methane is derived from acetate oxidation. Hence, the measured methane generated (2,28 gCOD/d, Table 1) is derived only from the oxidation of hydrogen, i.e.:

$$\begin{aligned}\text{Hydrogen oxidation to methane} &= 2,28 \text{ gCOD/d} \\ &= 0,143 \text{ mol } H_2\text{/d}\end{aligned}$$

Accepting $[HAc]_{ox} = 0$, the hydrogen flux associated with the glucose processing can be calculated using Eq. (12) of Sam-Soon *et al.* (1990):

$$\begin{aligned}\text{Hydrogen flux} &= [HPr] + 2[HBr] + 2\left\{[HAc]_{obs} - [HPr]_{G1} + [HAc]_{ox}\right\} \quad (12) \\ &= (0,225) + 2(0,205) + 2\{0,689 - 0,225 + 0,0\} \\ &= 1,563 \text{ mol/d} \\ &= 25,01 \text{ gCOD/d}\end{aligned}$$

The hydrogen generated is processed by the hydrogenotrophs, to form VSS (cell mass + polymer) and methane. Comparing the calculated acidogen mass generated (3,11 gCOD/d) with the measured VSS (3,05 gCOD/d), a negligible mass of VSS was generated by the hydrogenotrophs. Comparing the hydrogen oxidised to methane (2,28 gCOD/d) with the hydrogen generated (25,01 gCOD/d), a minor fraction of the generated hydrogen was oxidised to methane. Thus, the hydrogen was being processed only marginally. The mass of hydrogen not processed is (25,01 -2,28) = 22,73 gCOD/d. What had happened to this hydrogen? Very likely, because hydrogen is a gaseous intermediate during glucose fermentation, the hydrogen **was lost as gas from the system.** If the COD, hypothesised to be lost as hydrogen gas is taken into account, the overall COD mass balance is as follows:

**COD flow into the system**

$$\begin{aligned}COD_{in} &= 2\,774 \text{ x } 60/1000 \\ &= 166,44 \text{ gCOD/d}\end{aligned}$$

**COD flow out of the system**

| | |
|---|---|
| Soluble COD = 2 305 x 60/1000 | = 138,3 gCOD/d |
| COD incorporated into $CH_4$ | = 2,28 gCOD/d |
| COD incorporated into sludge | = 3,05 gCOD/d |
| COD loss in hydrogen gas lost | = 22,73 gCOD/d |
| i.e. Total $COD_{out}$ | = 166,36 gCOD/d |
| Percentage COD recovery | = 166,36/166,44 x 100 |
| | = 99,97 per cent. |

Note that of the mass of COD removed [(166,44 - 138,3) = 28,14 gCOD/d], a mass of 22,73 gCOD/d was lost as hydrogen gas, i.e. 81 per cent of the COD removed in the high $pH_2$ zone was lost as

$H_2$ gas, or, expressed in terms of the influent COD, 22,73/166,3 = 13,7 *per cent of the influent* COD was lost as hydrogen gas.

In Table 2 the calculated responses of the various processes in the high $\overline{p}H_2$ zone are listed, at excess and limiting $NH_3$-N influent concentrations. Comparing the two lists the following are of interest:

**At excess $NH_3$-N concentration**

- Soluble COD removal was only 23 per cent.
- An overall mass balance gave a COD recovery of > 99 per cent.
- From measurements of the effluent SCFA and total soluble effluent COD, and estimates of the COD in the effluent soluble organic N, all of the influent glucose was processed to SCFA and hydrogen.
- Using an acidogen specific yield of 0,1 mol glucose/mol glucose processes (≈ 0,075 gVSS/g glucose) in the glucose balance equation, the methane generated from acetate oxidation/cleavage ($[HAc]_{ox}$) was calculated to be 4,48 gCOD/d.
- Accepting $[HAc]_{ox}$ to be 4,48 gCOD/d, the hydrogen generated during processing of glucose was calculated. Of the hydrogen generated (27,57 gCOD/d) (Table 2) 8,89 gCOD/d was utilised for generation of hydrogenotrophic cells and 9,87 gCOD/d for polypeptide and excess amino acids. That is, 68 per cent of the hydrogen generated was used for these purposes and the balance, 32 per cent (8,82 gCOD/d), was oxidised to methane.
- Accepting methane production from acetate cleavage ($[HAc]_{ox}$) to be 4,48 gCOD/d, this was subtracted from the measured methane production (11,85 gCOD/d) to give methane generated from hydrogen oxidation (7,37 gCOD/d). This value (7,37 gCOD/d) conforms reasonably to the value obtained from the hydrogen balance (8,82 gCOD/d).
- The VSS wasted from the reactor was dominantly in pellet form; the filtered effluent was turbid even after being left standing for a long period, apparently due to the presence of amino acids.
- The gross sludge yield (organism mass + polypeptide) was 0,47 mgVSS/mgCOD removed.
- The $NH_3$-N removal was 46,5 mgN/ℓ. This value would form an estimate of the minimum N requirements to obtain unimpeded pellet formation. That is, minimum influent TKN/COD ratio for a glucose substrate should be about 46,5/2774 = 0,017 mgN/mg influent COD, say 0,02.

This behaviour was identical to that described by Sam-Soon *et al.* (1990).

**At limiting $NH_3$-N concentration**

- Soluble COD removal was only 17 per cent.
- An overall COD mass balance gave only 86 per cent COD recovery. However, when the mass of hydrogen calculated as lost from the system (22,73 gCOD/d) was included in the balance, an excellent overall COD mass balance of > 99 per cent was obtained.
- From measurements of the effluent SCFA and total soluble effluent COD, and estimates of the COD in the effluent soluble organic N, only 78,2 per cent of the influent glucose was processed to SCFA and hydrogen.
- Using the usual acidogen specific yield value of 0,1 mol glucose/mol glucose processed in the glucose balance equation, a negative value for methane generated from acetate oxidation/cleavage ($[HAc]_{ox}$) was obtained. Accepting $[HAc]_{ox} = 0$ gave an acidogen specific yield value of 0,024 mol glucose/mol glucose processed. This low specific yield value was confirmed by measured sludge generation. The reason for the low yield is hypothesised to be due to a completely inadequate influent TKN/COD ratio.
- Accepting $[HAc]_{ox}$ to be zero, the hydrogen generated during processing of glucose was calculated. Of the hydrogen generated (25,01 gCOD/d), a negligible mass was used for generation of hydrogenotroph VSS (cell mass + polypeptide) and only 9,1 per cent was oxidised to methane (2,28 gCOD/d). The balance of 90,9 per cent (22,73 gCOD/d) was lost from the system as hydrogen gas.
- Accepting methane generation from acetate cleavage to be zero, the observed methane production (2,28 gCOD/d) is due to hydrogen oxidation only. This is substantially less than that when excess $NH_3$-N was present (7,37 gCOD/d from hydrogen oxidation; 4,48 gCOD/d from acetate cleavage).
- The VSS wasted from the system was in the form of finely dispersed solids with clear effluent in between.
- The gross sludge yield was approximately 0,08 mgVSS/mgCOD removed.
- The influent $NH_3$-N supplied was 10,5 mgN/ℓ (i.e. 0,0038 mgN/mg influent COD). This is very much less than the calculated minimum $NH_3$-N necessary to supply acidogen cell synthesis N requirements in a UASB system, 23,9 mgN/ℓ (i.e. 0,0086 mgN/mg influent COD).

Clearly limiting the $NH_3$-N in the influent had a significant effect on the system response.

## Discussion

With limited $NH_3$-N in the feed, the hydrogen gas loss in the high $\overline{p}H_2$ zone implies that the hydrogenotroph *Methanobacterium* Strain *AZ*, was inhibited; this can be explained in terms of biochemical feed back control as follows: Under conditions of excess hydrogen substrate (high $\overline{p}H_2$) **and** excess $NH_3$-N, the intracellular ATP/ADP level will be high. If cysteine availability is not limited 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 both instances there is a continuous uptake of hydrogen substrate by the species. However, with excess hydrogen substrate and limited $NH_3$-N the species is unable to decrease its ATP/ADP level by either cell synthesis or polypeptide synthesis, resulting in a hydrogen substrate uptake inhibition a $pH_2$ build up with eventual $H_2$ release as a gas. In "normal" completely mixed anaerobic systems the $\overline{p}H_2$ remains low and nitrogen (and cysteine) are always in adequate supply for all types of hydrogenotrophs, hence no disequilibrium develops and there is only inconsequential or no hydrogen gas release.

Limiting the $NH_3$-N concentration in the feed appears to affect the acidogens also. The specific yield for the acidogens was

unusually low (0,024 mol glucose/mol glucose processed) which would have reduced the mass of acidogens, which in turn would have reduced the rate for processing the glucose. The consequence of this was that with $NH_3$-N limitation glucose became significant in the effluent (see system with $NH_3$-N limitation, Table 2).

## Conclusions

From the study of UASB systems with glucose as substrate, the reduction of the N concentration in the influent to very low values has the following two effects:

- If the N requirements for acidogenic growth are not satisfied, acidogen synthesis is inhibited to a degree, causing a reduction in their specific yield (in this study from 0,10 to 0,024 mol glucose/mol glucose processed). Calculation of the N requirement for "normal" acidogen growth in the high $\overline{p}H_2$ zone of a UASB system indicates a minimum influent TKN/COD ratio of about 0,0086 mg($NH_3$-N)/mgCOD.

- Irrespective of whether acidogen synthesis is inhibited or not, if there is insufficient N, growth of the hydrogenotroph *M.* Strain *AZ* is inhibited and, in consequence, also $H_2$ substrate uptake. The decrease in *M.* Strain *AZ* activity is ascribed to the high intracellular ATP/ADP level due to high concentration of the substrate $H_2$, generated by the acidogens. The hydrogenotroph species cannot decrease their ATP/ADP level, through generation of amino acids and polypeptides, because $NH_3$-N is limiting. This prevents pellet production and the hydrogen generated is lost from the bed as a gas. For unimpeded pellet formation it is estimated that the influent TKN/COD should be about 0,02 mgN/mg influent COD.

The above two effects cause a decrease in VSS specific yield. In this particular study with influent TKN/COD of 0,0038 mgN/mgCOD, the N deficiency resulted in a decrease from 0,47 to 0,08 mgVSS/mgCOD removed.

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