UNIVERSITY OF CAPE TOWN
Department of Civil Engineering

FINAL REPORT
to the
WATER RESEARCH COMMISSION
on the contract

PELLETIZATION IN UPFLOW ANAEROBIC SLUDGE BED (UASB) SYSTEMS

(January 1988 to December 1991)

by

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Research supported by the
Water Research Commission
and the Foundation for Research Development

WRC Report No. 249/1/93
ISBN 1 874858 57 8

Research Report No. W 75
Contract No. K5/249
June 1992

SYNOPSIS

Anaerobic digestion using the upflow anaerobic sludge bed (UASB) system has great potential application for many wastes, the principal benefits being:

- Loading rates up to seven times greater than those for completely mixed systems; this implies that smaller reactor volumes are required.

- High nitrogen removal; in fact the UASB system is the only anaerobic system that can remove significant concentrations of nitrogen.

- No artificial mixing of the mixed liquor is required; this can be an expensive and difficult operation in completely mixed systems.

- No separate gravity sedimentation tanks are required.

Successful application of UASB technology hinges on the generation of sludge aggregating into pellets, to facilitate retention of the sludge in the reactor. However, by the mid 1980's the causes and mechanisms for pelletization in UASB systems were ill–defined and not understood.

In 1985, the University of Cape Town (UCT) Water Research Group were approached by a consultant to conduct a feasibility study of pelletization in a UASB system treating apple juicing wastewaters. This investigation proved very successful in that this wastewater generated a clearly defined pelletized sludge bed in the UASB system (Dold *et al.*, 1987). The investigation also exposed the need for further research on UASB systems for potential application to other wastewaters. In 1988, a three year contract (later extended by one year) was set up between UCT and the Water Research Commission with the following principal objectives:

- Develop an hypothesis for pelletization in UASB systems.
- Verify the pelletization hypothesis.
- Develop a mathematical model describing the kinetic behaviour of UASB systems.
- Assess H_2CO_3*alkalinity requirements and pH control in UASB systems.
- Develop process design, operation and control strategies to optimize the UASB

system.

- Investigate the feasibility of effecting pelletization by introducing hydrogen from an external source into UASB systems treating wastes that do not generate bio–pellets.
- Formulate models for mineral precipitation in both completely mixed reactors and UASB systems, e.g. calcium carbonate, metal sulphides and struvite.

During the course of the research, a further objective was identified:

- Develop a simple experimental method for measurement of H_2CO_3*alkalinity and determination of short–chain fatty acids (SCFA).

To meet these objectives, a number of research tasks had to be completed, as set out in Fig 1.1 (see Chapter 1).

This report summarizes the research undertaken to address the tasks and objectives. For greater detail on the research, the reader is referred to the technical reports and publications listed in the section "PUBLICATIONS ON UASB SYSTEMS".

DEVELOPMENT OF HYPOTHESIS FOR PELLETIZATION

From an extensive experimental investigation into the behaviour of UASB systems treating apple juicing wastewaters, it was concluded that the pellets are formed by the generation of an extracellular polypeptide polymer. From the literature and experimental observations, the hydrogenotrophic methanogen *Methanobacterium* strain *AZ* (*M.* Strain *AZ*), now classified as *Methanobrevibacter arboriphilicus*, was implicated in the polypeptide formation; its characteristics provided the basis for formulating an hypothesis on pellet formation:

> When the *M.* Strain *AZ* is surrounded by excess substrate i.e. high $\bar{p}H_2$, the ATP/ADP ratio will be high. 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.

The hypothesis for pelletization could be used to explain all the observations made

on the UASB systems treating apple juicing wastewater.

HYPOTHESIS VERIFICATION

Having developed an hypothesis for pelletization, the hypothesis was tested by comparing the criteria for pelletization identified from the hypothesis and their implications against observations on a range of UASB systems. Observations on UASB systems were obtained from the literature and from experimental investigation, using the pure substrates glucose (carbohydrate), casein (protein) and oleate (lipid).

All observations conformed to the hypothesis, and the hypothesis could be used to identify the characteristics that a waste must possess to induce pelletization.

KINETIC MODEL

The pelletization hypothesis formed the basis for development of a kinetic model describing the behaviour of the UASB system receiving carbohydrate as substrate. Simulation of the behaviour of experimental systems gave good correlation for COD, SCFA, organic N and NH_3–N for flow–through systems. For systems with recycles the experimental response indicated better performance than the simulated response, apparently due to the presence of organisms in the recycle which were not included in the model.

H_2CO_3*ALKALINITY AND pH CONTROL

An important aspect in the design and operation of UASB systems is to ensure that the minimum pH in the sludge bed > 6,6, to maintain methanogenic organism growth. In the UASB system, due to the partial phase separation of the acidogenic and methanogenic phases, (1) in the lower region of the sludge bed SCFA accumulate (via acidogenesis) reducing H_2CO_3*alkalinity which causes the pH to decline, and (2) in the upper region of the sludge bed SCFA are converted to methane (via methanogenesis) regenerating H_2CO_3*alkalinity which causes the pH to rise. Thus, in the UASB system the mass of H_2CO_3*alkalinity required to maintain the minimum pH > 6,6 is controlled by the transient high concentration of SCFA in the lower part of the sludge bed.

In a study of the H_2CO_3*alkalinity requirements with apple juicing wastewaters, it was found that with a *flow–through* system the alkalinity requirements were so high that it could render the UASB system uneconomic. The high H_2CO_3*alkalinity

requirement prompted an extensive enquiry into operational procedures to reduce this requirement, and to assess the H_2CO_3*alkalinity requirements and system performance for apple juicing, lauter tun (brewery), wine distillery and proteinaceous (casein) wastes. From this investigation, the following conclusions were drawn:

- All the wastes listed above were amenable for treatment in UASB systems; the wastes develop pelletized sludges.

- For flow–through systems, the H_2CO_3*alkalinity/COD of the influent to maintain minimum bed pH > 6,6, differed for the wastes; H_2CO_3*alkalinity had to be added to all the influents in order to control the minimum bed pH.

- For all wastes, the H_2CO_3*alkalinity addition could be reduced significantly by recycling from the effluent to the influent. For the wine distillery waste, significant H_2CO_3*alkalinity was generated internally, and with recycle of this alkalinity to the influent, self sufficiency in alkalinity could be achieved.

- With external addition of H_2CO_3*alkalinity, this should be added to the recycle.

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

- Effluent H_2CO_3*alkalinity, influent COD and recycle ratio can be used to control the minimum sludge bed pH.

MEASUREMENT OF H_2CO_3*ALKALINITY AND SCFA

During the course of the research, the need arose for a simple practical method for measuring H_2CO_3*alkalinity and SCFA; no such method was available in the literature. A 5 pH point titration was developed for determining H_2CO_3*alkalinity and SCFA (as acetic acid) in aqueous solutions also containing known concentrations of other weak acid/bases such as the phosphate, ammonium or sulphide weak acid/bases.

The method was tested extensively, on made–up solutions of sodium bicarbonate and acetic acid, and on UASB effluents containing phosphate and inorganic nitrogen by adding known concentrations of acetic acid; both the H_2CO_3*alkalinity and

SCFA concentrations always correlated closely to the known input values.

For monitoring system performance and for pH control of anaerobic systems, the 5 pH point titration method has decided advantages over existing methods in, (1) attainable accuracy, (2) testing time required, and (3) simplicity of testing procedure. The 5 pH point titration method should find ready application for monitoring and control of all types of full-scale anaerobic digestion systems. The algorithms for the titration method have been encoded in a computer program which will be available from the Water Research Commission (Moosbrugger *et al.*, 1992a).

PROCESS DESIGN, OPERATION AND CONTROL STRATEGIES

From the research tentative guidelines could be delineated, for:

- Waste selection; which wastes will generate a pelletized sludge bed and therefore be amenable for treatment in a UASB system.

- Process design; mixing régimes, loading rates, balancing tanks, provision of recycles.

- Operation and control; strategies for using the measured effluent H_2CO_3*alkalinity, influent COD and recycle ratio to control the minimum sludge bed pH.

CONCLUSIONS AND RECOMMENDATIONS

The research summarized in this report has focussed on the formation of *bio-pellets* in UASB systems, in particular pellets composed of a polypeptide matrix binding the anaerobic organisms into aggregates. In this regard, an hypothesis for the bio-pellet formation has been developed and validated against literature and experimental observations. From the hypothesis the characteristics that a waste must possess to stimulate bio-pellet formation could be identified. Also, a mathematical model describing the kinetic behaviour of the pelletized UASB system could be developed.

An intensive experimental investigation was undertaken to identify strategies for operation and control of the pelletized UASB system. In this regard, provision of H_2CO_3*alkalinity in adequate concentrations to ensure the minimum bed pH > 6,6, was identified as essential. Guidelines for provision of this alkalinity requirement

were set out, e.g. recycles, point of alkalinity addition, etc. As part of the control strategy, a simple titration method was developed to measure both H_2CO_3*alkalinity and SCFA concentrations in aqueous solutions containing also known concentrations of other weak acid/bases.

The research work summarized in this report has addressed and met all the objectives set out at the start of the contract, except for:

- Investigate the feasibility of effecting pelletization by introducing hydrogen from an external source into UASB systems treating wastes that do not generate bio–pellets.

- Formulate models for mineral precipitation in both completely mixed reactors and UASB systems, e.g. calcium carbonate, metal sulphides and struvite.

With regard to hydrogen introduction, preliminary experiments in which pure hydrogen gas was bubbled through the sludge appeared to be completely ineffective. In the biological production and utilization of hydrogen, the transfer of hydrogen probably is at an interspecies level, a condition that could not be created artificially.

With regard to mineral precipitation, this objective was not addressed. The research effort was focussed on the formation of bio–pellets, and in the systems investigated mineral precipitation did not appear to play a significant role. However, this aspect should receive future attention. There is evidence that for wastes that do not stimulate bio–pellet formation (e.g. acetate), mineral precipitation can be used to form a nucleus onto which organisms can aggregate (Ahring and Schmidt, 1992; Ahring, 1992), in effect to constitute a type of pellet. This would imply that, by control of the mineral precipitation process, wastes identified in this report as not forming biopellets could still be treated in UASB systems.

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ACKNOWLEDGEMENTS

The writers wish to express their gratitude to the following persons for their contribution to the research reported here:

- Mr Taliep Lakay – Technical Assistant, for his invaluable help in running the experimental units and the laboratory.
- Mrs Heather Bain – Clerical Assistant, for so cheerfully typing and retyping papers and reports.

Thanks also are due to the members of the Steering Committee who guided the research work during the contract period:

- Dr J E McGlashan – Water Research Commission (Acting Chairman, 1988)
- Dr W H J Hattingh – Water Research Commission (Chairman, 1989)
- Dr H M Saayman – Water Research Commission (Chairman, 1990; Member 1991)
- Dr S A Mitchell – Water Research Commission (Chairman, 1991)
- Mr G Offringa – Water Research Commission (Member, 1989–91)
- Mr H A Nicholls – Johannesburg City Health Department (Member, 1988)
- Mr A R Pitman – Johannesburg City Engineer's Department (Member, 1989–1991)
- Dr L H Lötter – Johannesburg City Health Department (Member, 1990–1991)
- Mr W R Ross – DWT,CSIR (1988); Ross cc (1989–1991) (Member, 1988–1991)
- Mr H A de Villiers – DWT,CSIR (Member, 1989–1991)
- Ms H Louw – Water Research Commission (Acting Committee Secretary, 1988)
- Mr P W Weideman – Water Research Commission (Committee Secretary, 1988–1991)

The writers also would like to express their gratitude to the Water Research Commission and the Foundation for Research Development for financial support of the research.

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2. Sam-Soon, P A L N S, Loewenthal R E, Dold P L and Marais GvR (1987). Hypothesis for pelletization in the upflow anaerobic sludge bed reactor. Water SA, 13, 69-80

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W 72 Sam–Soon P A L N S, Loewenthal R E, Wentzel M C and Marais GvR (1989). Pelletization in the upflow anaerobic sludge bed (UASB) reactor.

W 74 Moosbrugger R E, Wentzel M C, Ekama G A and Marais GvR (1992). Simple titration procedures to determine H_2CO_3*alkalinity and short chain fatty acid concentrations in aqueous solutions containing known concentrations of ammonium, phosphate and sulphide weak acid/bases.

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LIST OF SYMBOLS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
C	Constant for H_2CO_3*alkalinity requirements per influent COD to maintain minimum pH > 6,6 [mg H_2CO_3*alkalinity (as $CaCO_3$)/mg influent COD]
$CaCO_3$	Calcium carbonate
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
COD_e	Effective influent COD = base influent COD/(1+recycle ratio)
H_2	Dimolecular hydrogen gas
HAc	Acetic acid
HBr	Butyric acid
HPr	Propionic acid
H_2CO_3*alkalinity	Proton accepting capacity (alkalinity) relative to the H_2CO_3* reference state and can be measured as the mass of H^+ (or OH^-) that must be added to titrate from the solution pH to the H_2CO_3* reference state pH – the H_2CO_3* reference state pH is the pH established on addition of H_2CO_3* (CO_2) reference species to pure water
N	Nitrogen
NaOH	Sodium hydroxide
NH_3–N	Ammonia nitrogen
orgN	Organic nitrogen
P	Phosphorus
$\bar{p}H_2$	Hydrogen partial pressure
SCFA	Short chain fatty acids
SO_4^{2-}	Sulphate
TKN	Total Kjedahl Nitrogen
UASB	Upflow Anaerobic Sludge Bed
UCT	University of Cape Town
VSS	Volatile Suspended Solids
WRC	Water Research Commission

CHAPTER 1

INTRODUCTION

Anaerobic digestion using the upflow anaerobic sludge bed (UASB) system has great potential application for many wastes, the principal benefits being:

- Loading rates up to seven times greater than those for completely mixed systems; this implies that smaller reactor volumes are required.

- High nitrogen removal; in fact the UASB system is the only anaerobic system that can remove significant concentrations of nitrogen.

- No artificial mixing of the mixed liquor is required; this can be an expensive and difficult operation in completely mixed systems.

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Successful application of UASB technology hinges on the generation of sludge aggregating into pellets, to facilitate retention of the sludge in the reactor. However, by the mid 1980's, the causes and mechanisms for pelletization in UASB systems were ill-defined and not understood.

In 1985, the University of Cape Town (UCT) Water Research Group were approached by a consultant to conduct a feasibility study of pelletization in a UASB system treating apple juicing wastewaters. This investigation proved very successful in that this wastewater generated a clearly defined pelletized sludge bed in the UASB system (Dold *et al.*, 1987). The investigation also exposed the need for further research on UASB systems for potential application to other wastewaters. In 1988, a three year contract (later extended by one year) was set up between UCT and the Water Research Commission with the following principal objectives:

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During the course of the research, a further objective was identified:

- Develop a simple experimental method for measurement of H_2CO_3*alkalinity and short–chain fatty acids (SCFA).

To meet these objectives, a number of research tasks had to be completed, as set out in Fig 1.1.

This report summarizes the research undertaken to address the tasks and objectives. For greater detail on the research, the reader is referred to the technical reports and publications listed in the section "PUBLICATIONS ON UASB SYSTEMS".

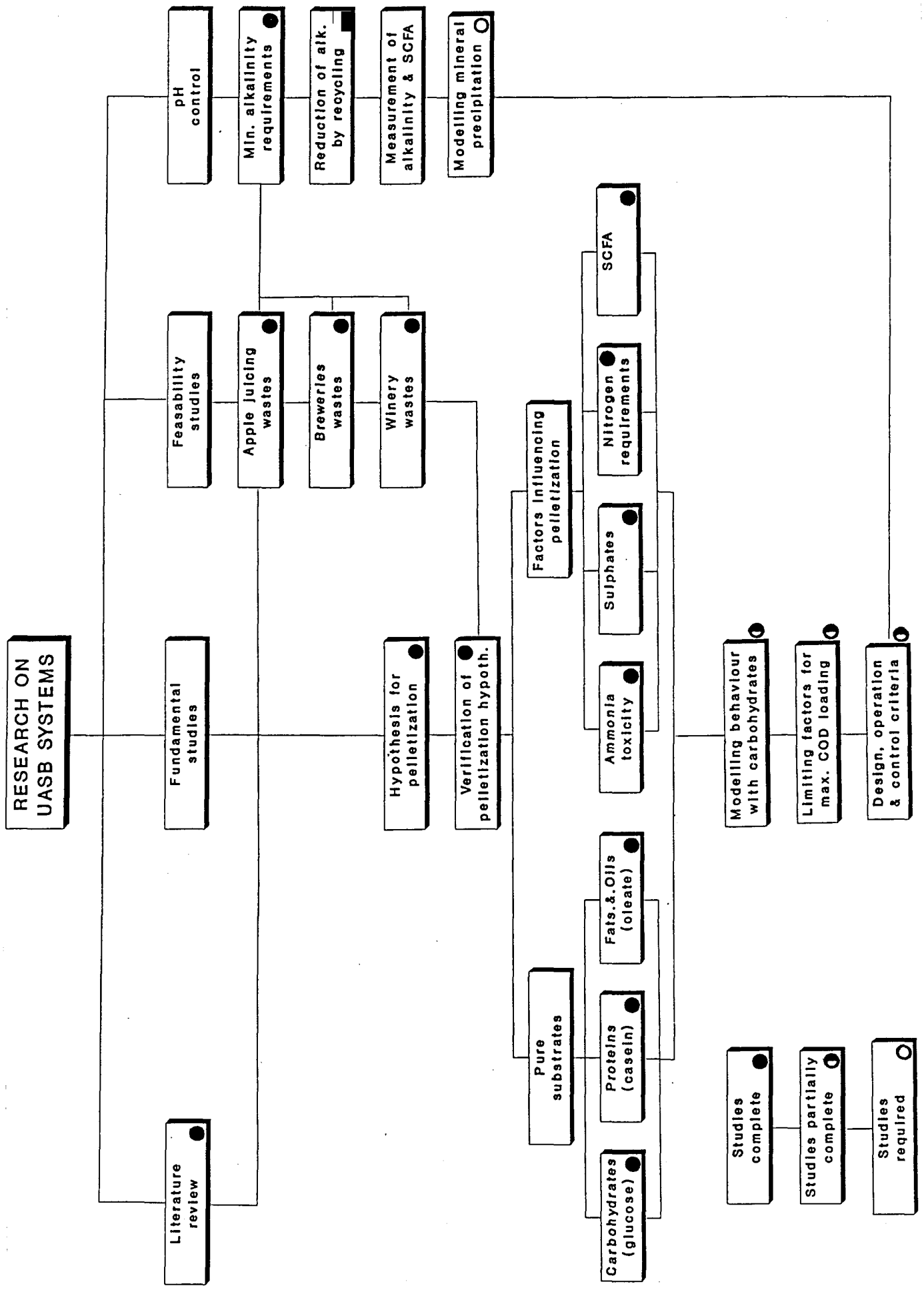


Fig 1.1: Research 'flow chart' showing tasks that had to be undertaken to meet the objectives, and the state of completion of the tasks.

CHAPTER 2

DEVELOPMENT OF HYPOTHESIS FOR PELLETIZATION

2.1 Experimental observations

To enquire into the causes for pelletization, an extensive investigation was undertaken into the behaviour of a UASB system treating apple juicing wastewaters (Sam-Soon *et al.*, 1987;1989). The apple juicing wastewater was about 99 per cent soluble, consisted principally of carbohydrates (sugars), was acidic (pH 4,5 to 5,5) and deficient in nitrogen and phosphorus. For the study, the influent COD concentration was diluted to about 2500 mg/ℓ. Trace elements and macro nutrients were added to give an influent COD:N:P ratio of approximately 100:4:1. Sufficient H_2CO_3*alkalinity was added to the influent to ensure the minimum bed pH > 6,6. The following pertinent observations were made:

(1) The pelletization process started when the loading was about 5 kgCOD/(m^3 reactor.d) and once started, was very rapid – over a period of six to seven days roughly half the sludge bed was transformed into pellets.

(2) From visual observations the pellets showed only slight movement with time; there was very little intermixing of bed material and the flow in the bed approached a plug flow regime.

(3) Along the axis of the reactor the following parameters were measured; short chain fatty acids (SCFA), soluble COD, TKN, NH_3-N, alkalinity and pH. From the bed concentration profiles (one example is given in Fig 2.1) 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, NH_3-N reduced to a minimum, organic nitrogen (orgN) increased to a maximum and alkalinity and pH declined to minimum values.

- *An upper active zone* in which the propionate and acetate concentrations decreased to minima, soluble COD decreased to a near minimum, NH_3-N remained near constant, orgN decreased to a minimum, alkalinity increased

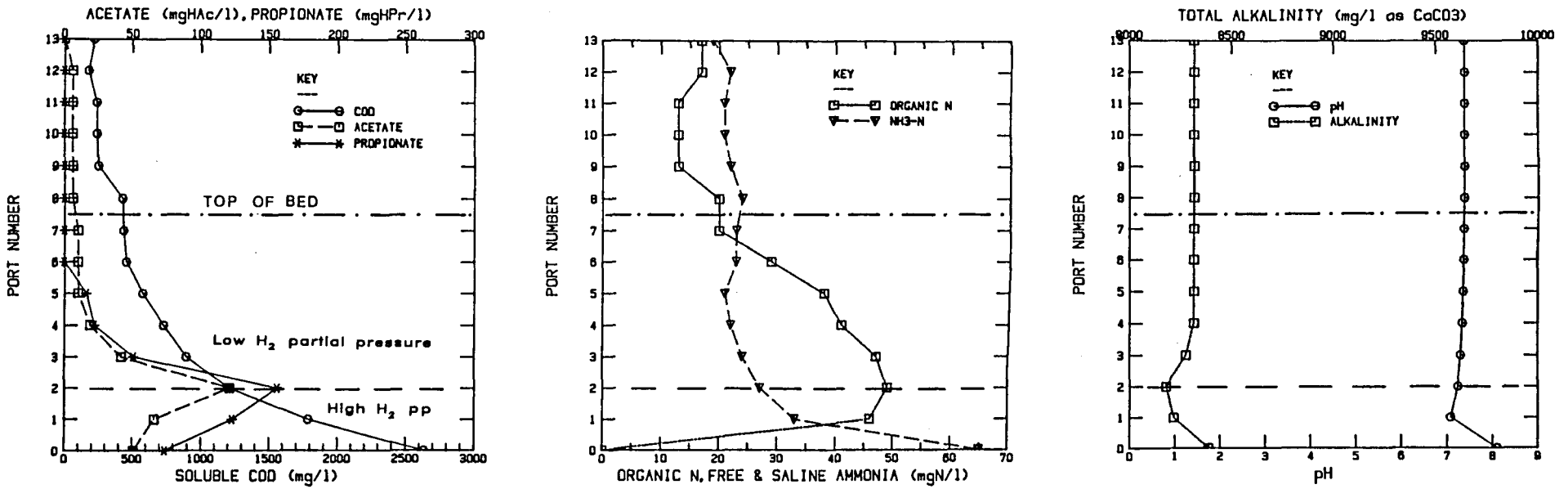


Fig 2.1: Concentration and pH profiles observed in single reactor UASB system treating apple juicing wastewater. (Influent COD 2 600 mg/ℓ; influent flow rate 92 ℓ/d; loading rate 26,6 kgCOD/m³ reactor/d; recycle ratio 0:1).

to near its value in the influent and pH increased to a stable value.

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

From the biochemistry of fermentation processes, the action of anaerobic microorganisms in the three zones could be identified:

- *In the lower active zone*

– acidogens generate short–chain fatty acids (SCFA), principally acetic (HAc) and propionic (HPr), carbon dioxide (CO_2) and hydrogen (H_2). The hydrogen is generated at such a rate that *a high hydrogen partial pressure* (*high* $\bar{p}H_2$) *is created*;

– hydrogenotrophic methanogens generate methane (CH_4) from H_2 and CO_2; and

– acetoclastic methanogens convert some HAc to CH_4 and CO_2.

- *In the upper active zone*, the $\bar{p}H_2$ is reduced to, and maintained at, such low values due to the action of hydrogenotrophs that

– acetogens convert HPr to HAc, H_2 and CO_2; and

– acetoclastic methanogens convert all the HAc to CH_4 and CO_2.

- *In the upper inactive zone*

– no observable biological activity takes place.

From the above, it is apparent that the differentiation of the sludge bed into lower and upper active zones is based on whether HPr oxidation to HAc occurs (upper active, low $\bar{p}H_2$) or not (lower active, high $\bar{p}H_2$).

(4) 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. To obtain more information on the pellet formation-break up, a two-in-series reactor UASB system was set up with the first reactor having a bed volume equal to the lower active zone of a single reactor 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. It was concluded that pellet *growth took place principally in the lower active zone of the sludge bed and pellet break up in the higher zones.*

(5) In the two-in-series UASB reactor system [(4) above], the specific pellet yield in the first reactor was estimated to be about 0,42 mgVSS/mgCOD removed which is an abnormally high yield value, on a par with that for aerobic growth (≈ 0,45 mgVSS/mgCOD removed).

(6) COD/VSS ratio for the pelletized sludge, was 1,23 against 1,42 mgCOD/mgVSS for sludges from "normal" anaerobic processes.

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

(8) In the lower active (high $\bar{p}H_2$) zone, there was a high production of organic nitrogen.

2.2 Hypothesis for pelletization

From observations (5) and (6) above, it was postulated that the high VSS yield was due to the generation of extracellular polymer which enmeshed the organism mass into pellets (Sam-Soon *et al.*, 1987); this conformed with electron microscopy results in the literature in which polymer matrices binding the pellets together had been observed (Dolfing *et al.*, 1985). From (7) and (8), it was postulated, *inter alia*, that the polymer was composed predominantly of peptides, and from (4) that generation of the peptide polymer took place principally in the lower active (high $\bar{p}H_2$) zone.

Attention now focused on developing an hypothesis to explain the generation of the pellet forming peptide polymer (Sam-Soon *et al.*, 1987;1989). Since generation of the peptide polymer took place principally in the lower active (high $\bar{p}H_2$) zone, it

was concluded that the polymer must be generated by the action of acidogens, hydrogenotrophic methanogens or acetoclastic methanogens. From a literature survey of these organism groups, the characteristics of one species (Zehnder and Wuhrmann, 1977) appeared to be directly relevant – a methanogen, *Methanobacterium* strain *AZ* (*M.* Strain *AZ*), now classified as *Methanobrevibacter arboriphilicus.* 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 a limitation of cysteine, the species in pure culture secretes high concentrations of amino acids (orgN) to the surrounding medium.

In terms of the behavioural pattern of M. Strain AZ, if cysteine is supplied in increasing concentrations polypeptide formation should correspondingly decrease. This was tested by supplementing the influent to the high $\bar{p}H_2$ reactor in the two in–series reactor UASB system with a trace concentration of cysteine – immediately (within 24h) there was a reduction in the specific pellet yield, of about 50 per cent (Fig 2.2).

Having identified the hydrogenotrophic methanogen *M.* Strain *AZ* as the organism most likely mediating pelletization, its characteristics provided a basis for formulating an hypothesis on pellet formation:

When the *M.* Strain *AZ* is surrounded by excess substrate i.e. high $\bar{p}H_2$, the ATP/ADP ratio will be high. 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.

The hypothesis for pelletization could be used to explain all the observations made on the UASB systems treating apple juice waste.

2.3 Criteria for pellet formation

From the hypothesis, conditions necessary for pellet formation in anaerobic systems could be formulated (Sam-Soon *et al.*, 1987;1989;1990a):

- An environment with a high $\bar{p}H_2$.

- A nitrogen source, in the free and saline ammonia form, well in excess of the metabolic requirement of the organisms.

- A limited source of cysteine either from the feed or becoming available from the action (e.g. death) of other organisms.

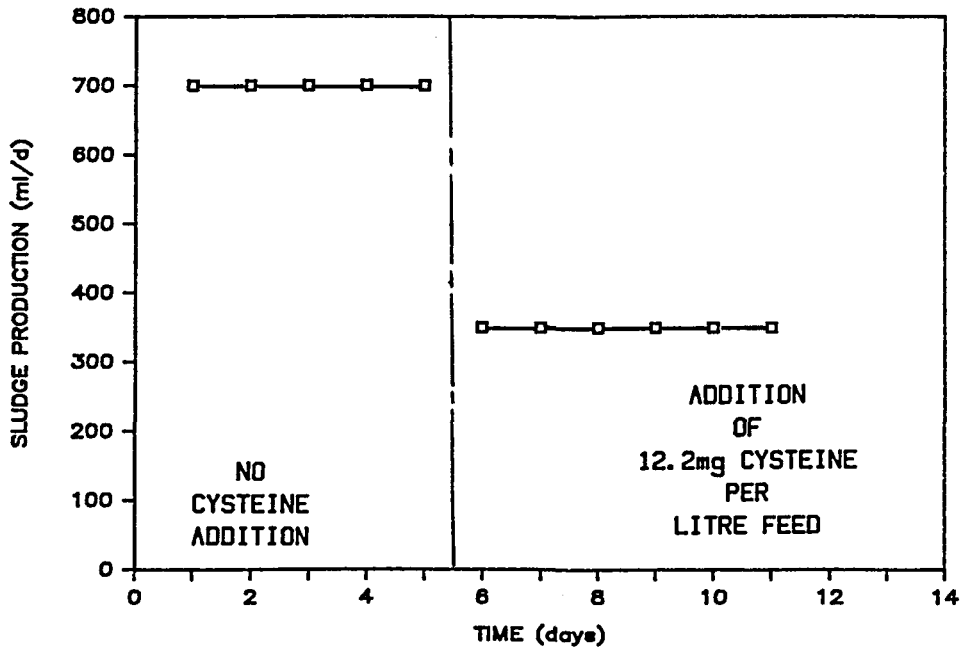


Fig 2.2: Effect of cysteine addition on pelletized sludge production in the high H_2 partial pressure reactor of a UASB system treating apple juicing wastewater.

CHAPTER 3

HYPOTHESIS VERIFICATION

Having developed an hypothesis for pelletization, the hypothesis was tested by comparing the criteria for pelletization identified from the hypothesis (listed in Chapter 2) and their implications against observations on a range of UASB systems. Observations on UASB systems were obtained from the literature and from experimental investigation. To obtain reliable quantitative information, the experimental investigation was undertaken using the pure substrates glucose (carbohydrate), casein (protein) and oleate (lipid).

3.1 Criterion 1: An environment with a high $\bar{p}H_2$

To create an environment with a high $\bar{p}H_2$ in an anaerobic fermentation system, the influent substrate must be able to generate H_2 at a high rate during acidogenesis, and reactor operation such that there is a partial phase separation of the acidogenic and methanogenic fermentation phases. From an examination of the biochemistry, thermodynamics and kinetics of acidogenic breakdown of different substrates, and of reactor mixing regimes, the following situations were identified under which one could expect pelletization or not, and literature or experimental observations used to test for pelletization:

(1) *Pellets not generated from polymer in influent*: In the literature, presence of a polymer matrix has been ascribed to polymer present in the influent, incorporated in the pellets by an agglutination process (Ross, 1984). To ascertain unambiguously the origin of the pellet polymer, a non-polymer carbohydrate substrate, glucose, was tested as influent (Sam-Soon *et al.*, 1990a). Excellent pellet formation was observed in the UASB system.

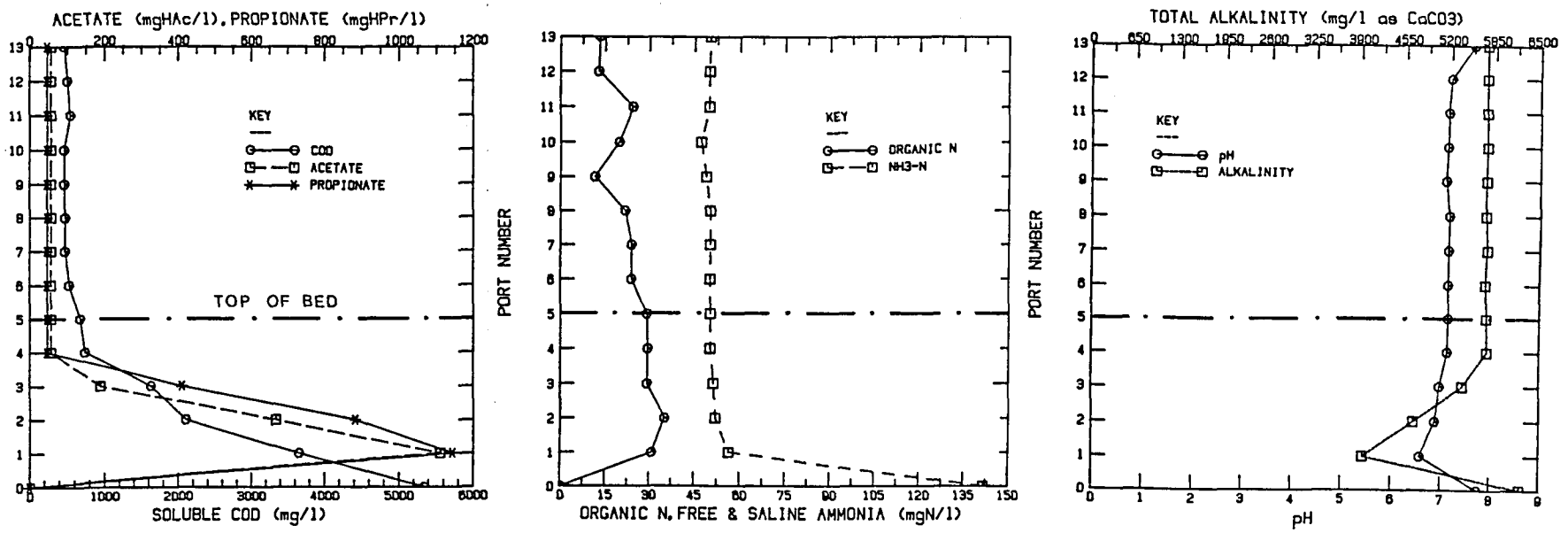
(2) *Pelletization in systems where the substrate yields hydrogen and the operation allows a zone of high* $\bar{p}H_2$ *build up*: *e.g. carbohydrates and proteins in plug flow reactors*: With regard to carbohydrates, an extensive investigation was undertaken into the response of a UASB system treating the carbohydrate glucose (Sam-Soon *et al.*, 1989;1990a). Excellent pellet formation was observed in a single reactor (high + low $\bar{p}H_2$ zones) UASB system. Product profiles along the axis of flow through the reactor, exhibited behavioural patterns that conformed to the observations with apple juicing waste as

influent (detailed earlier), e.g. excess NH_3–N uptake, organic–N production, etc. (for example, see Fig 3.1). The single reactor system then was separated into two in–series UASB reactor system with the first operating in the high $\bar{p}H_2$ phase. In the high $\bar{p}H_2$ reactor, from mass balance considerations, the gross specific yield of the hydrogenotrophic methanogens was determined to be 0,21 to 0,24 mgVSS/mgCOD(H_2), a value approximately 6 times that normally observed for these organisms [0,043 mgVSS/mgCOD(H_2), Shea *et al.*. 1968].

With regard to proteins, pellet formation was also investigated using the proteinaceous substrate casein (Moosbrugger *et al.*, 1990;1991). Biological pellet formation was readily established in a laboratory–scale upflow anaerobic sludge bed (UASB) reactor. Pellets were small (<2mm), fragile and black. Product profiles along the line of flow through the reactor conformed to observations with apple juicing waste and glucose as substrate, e.g. excess N uptake, pellet production in high $\bar{p}H_2$ zone, etc. (for example, see Fig 3.2). To obtain further information on pellet production, the bed was divided and operated as a two in–series reactor system, the first reactor operating in a high and the second in a low $\bar{p}H_2$ state. The first reactor produced (virtually) all the pellets with a sludge yield of 0,26 mgVSS/mgCOD removed (COD removal 3 500 mg/ℓ for influent COD 10 000 mg/ℓ). However, two thirds of the VSS generated was lost to the second reactor due to pellet break–up.

(3) ***No pelletization in systems where the substrate yields hydrogen but the system is operated with low hydrogen partial pressure, e.g. carbohydrates and proteins in completely mixed reactors:*** Low $\bar{p}H_2$ will be present in completely mixed reactors in which no propionate accumulates. In the literature there is no report on pelletization in completely mixed reactors that satisfy this condition. No information could be found on behaviour of completely mixed reactors with propionate in the effluent and hence with high $\bar{p}H_2$.

(4) ***No pelletization in systems where the influent substrate does not yield hydrogen in the fermentation process, e.g. acetate as sole substrate:*** The literature reports no biological pelletization in anaerobic systems with acetate only as substrate even if the system is operated with a plugflow mixing regime, e.g. UASB.



<u>Fig 3.1</u>: Concentration and pH profiles observed in single reactor UASB system with glucose as substrate. (Influent COD 5 345 mg/ℓ; influent flow rate 45 ℓ/d; loading rate 26,7 kgCOD/m³ reactor/d; recycle ratio 0:1).

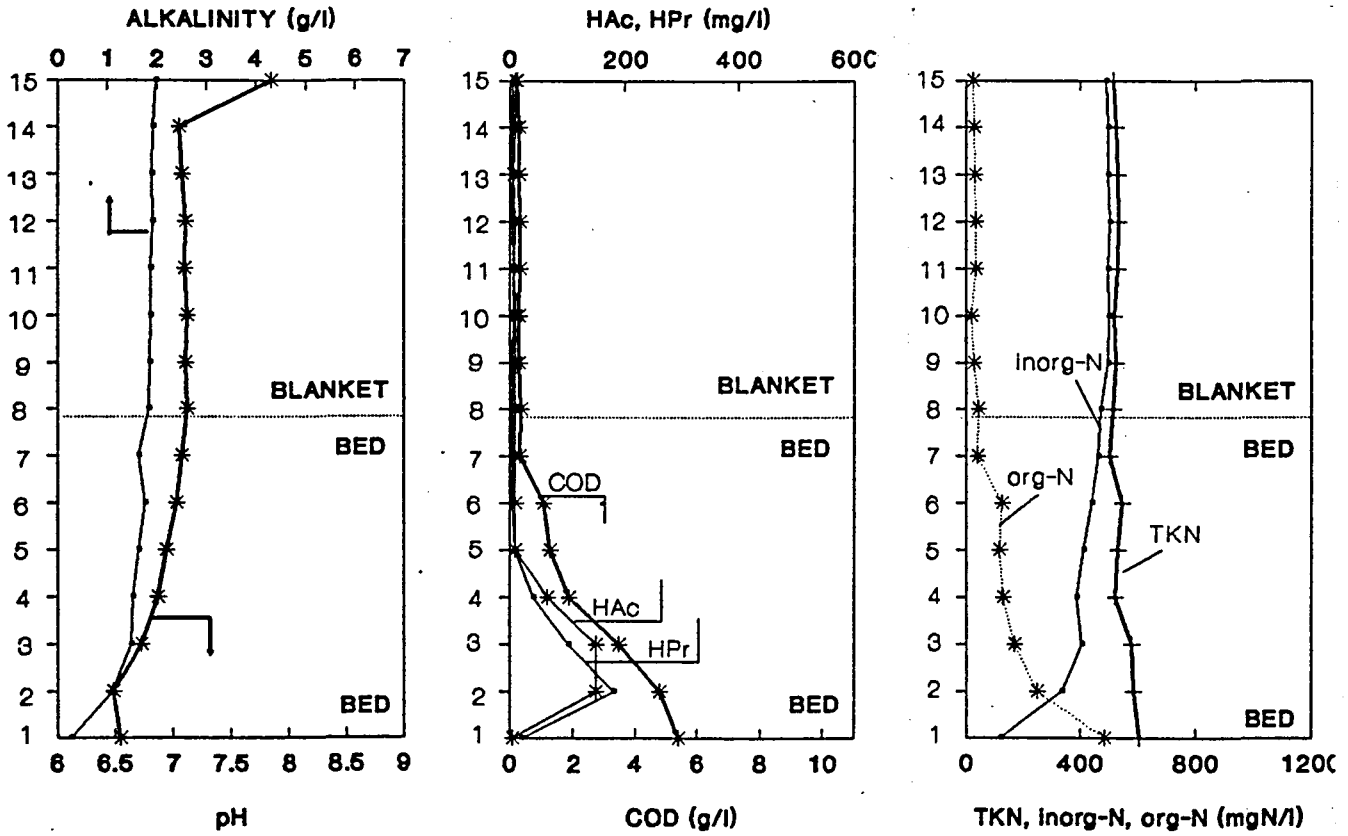


Fig 3.2: Concentration and pH profiles observed in single reactor UASB system with casein as substrate. (Influent COD 5 320 mg/ℓ; influent flow rate 25 ℓ/d; loading rate 48 kgCOD/m³ reactor/d; recycle ratio 0:1).

(5) *Limited pelletization where the substrate can generate a high* $\bar{p}H_2$ *but some of the* H_2 *generated is preferentially utilized by other organisms such as sulphate-reducers*: This was verified in a study on a UASB system with glucose as substrate and sulphate (SO_4^{2-}) ions added to the influent feed (Sam-Soon *et al.*, 1989;1991c). Pellet formation was reduced. Sulphate reducers appeared to utilize hydrogen preferentially thereby reducing $\bar{p}H_2$ and hence limiting pelletization. As SO_4^{2-} concentration increased in the influent from trace to excess, decreases were observed in (1) NH_3-N uptake, (2) orgN generation, (3) pellet size, from 2-3 mm to 1-2 mm, (4) nett pellet yield, to 1/5 of that observed with trace SO_4^{2-} concentrations. The pellet yield did not reduce to zero even when the SO_4^{2-} supplementation was in excess, i.e. above a certain influent SO_4^{2-} concentration the SO_4^{2-} removal was constant (see Fig 3.3). (This behaviour was apparent also when paper pulping waste was treated in a UASB system, Russo and Dold, 1989).

(6) *No pelletization in systems where the influent substrate can be broken down only under low* H_2 *partial pressure conditions, e.g. lipids:* A typical long chain fatty acid monomer of lipids, oleate, was used as sole substrate to a UASB system (Sam-Soon *et al.*, 1989;1991a). Oleic acid can be broken down to SCFA only under low hydrogen partial pressure conditions. Pelletization did not take place; the three characteristic zones did not develop in the sludge bed; both NH_3-N uptake and orgN generation were low and the SCFA acetate only was detected (see Fig 3.4). A well defined sludge bed developed but it was of a gelatinous nature.

3.2 Criterion 2: A nitrogen source, in the free and saline ammonia form, well in excess of the metabolic requirements of organisms.

With glucose as substrate, a UASB system with bed volume limited to constitute only the lower active (high $\bar{p}H_2$) zone was set up (Sam-Soon *et al.*, 1989;1990b). With excess nitrogen in the influent (0,03 mgN/mg influent COD), 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_2) or expressed differently 0,4 to 0,5 mgVSS/mgCOD removed. Nitrogen requirements were calculated to be 0,017 mgN/mg influent COD. Reducing the NH_3-N in the influent from an excess amount to slightly more than that required for cell synthesis (0,004 mgN/mg influent COD, based on requirements for completely mixed anaerobic systems), resulted in a decrease in the overall gross specific pellet yield

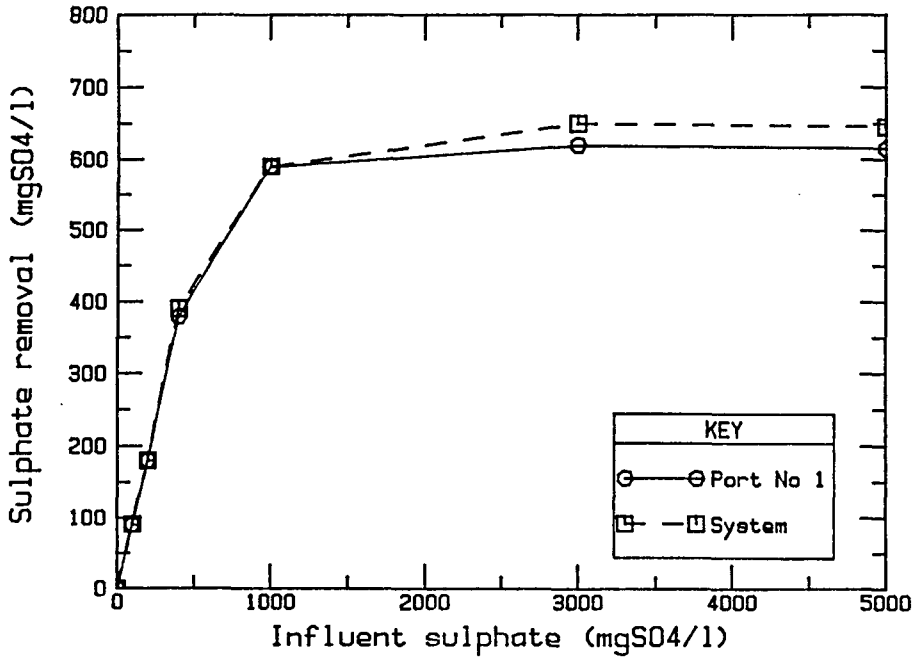


Fig 3.3: Sulphate (SO_4^{2-}) removal, by port No.1 and through the system versus influent SO_4^{2-} concentration for single reactor UASB system with glucose as substrate.

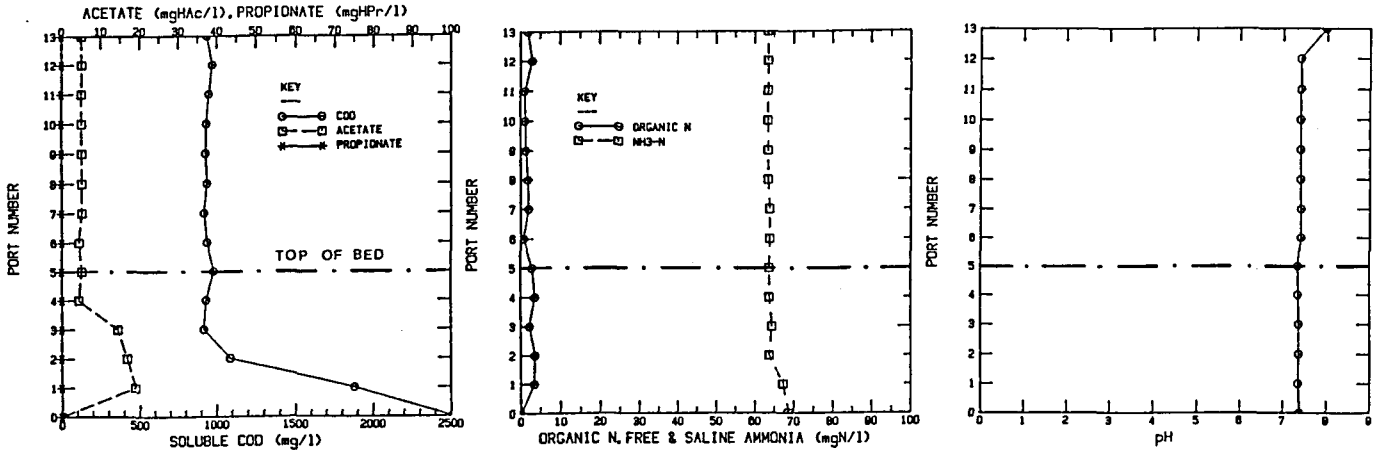


Fig 3.4: Concentration and pH profiles observed in single reactor UASB system with sodium oleate substrate. (Influent COD 2 518 mg/ℓ; influent flow rate 15 ℓ/d; loading rate 4,2 kgCOD/m³ reactor/d; recycle ratio 0:1).

from 0,47 to 0,08 mgVSS/mgCOD removed. From mass balance calculations it was concluded that at the lower yield of 0,08 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_2 substrate concentration) which the species cannot decrease through the generation of amino acids and polypeptides, when NH_3–N is limiting; accordingly the H_2 leaves the high $\bar{p}H_2$ as gas. From the two experiments, from mass balance on N uptake, it was concluded that the influent TKN/COD must be greater than 0,02 mgN/mg influent for unimpeded pellet formation.

The N requirements for pellet formation were investigated also for a UASB system receiving the proteinaceous substrate casein (Moosbrugger *et al.*, 1990;1991). N removal was more than 5 times higher than that normally expected. However, it was found that deamination of organic N generated NH_3–N in excess of N requirements for pellet formation, that is, the N source in the influent does not have to be in the free and saline ammonia form. Accordingly, Criterion 2 was modified to;

an available nitrogen source well in excess of the metabolic requirements of organisms.

3.3 Criterion 3: A limited source of cysteine either from the feed or becoming available from the action (e.g. death) of other organisms

As reported earlier, with apple juicing wastewater as influent to a two in–series reactor UASB system, the influent was supplemented with a trace concentration of cysteine (12,2 mg/ℓ) – immediately (within 24h) there was a reduction in the specific pellet yield, of about 50 per cent (Fig 2.2).

CHAPTER 4

KINETIC MODEL

4.1 Introduction

Mathematical models are widely used in wastewater treatment, for the following purposes: A model

- gives expression to conceptual ideas, to account for major events of interest occurring within a system, and allows evaluation of these; by comparing the simulated and the observed response, attention may be drawn to deficiencies in the conceptual structure;
- provides information not apparent from pilot–scale studies; this can be particularly useful if the system being modelled is a complex one;
- allows potentially feasible solutions to be explored that are not covered by the pilot–scale and other studies, thereby giving guidance for selecting the more promising ones for testing;
- assists in identifying the parameters that significantly influence the system response and thereby gives guidance for the establishment of design criteria; and
- assists in identifying possible causes for system malfunction or failure, and in devising remedial measures.

It is unlikely that any particular model will fulfill all of these uses. Simpler models usually are developed to satisfy specific uses in the list above; these have restricted use but also usually require less input than the more complex models, with their greater range.

Research into upflow anaerobic sludge bed (UASB) reactor systems developing pelletised sludge beds had progressed to the extent that development of a relatively complex mechanistically based kinetic model was feasible, to describe their behaviour (Sam–Soon *et al.*, 1989;1991d).

4.2 Modelling tasks

To set up a model for the UASB system a number of tasks needed to be completed:

- Describe the conditions within which the model is to operate.
- Identify the essential compounds utilised and formed.

- Identify the processes acting on these compounds.
- Conceptualise a mechanistic model that qualitatively describes the kinetic and stoichiometric behavour of the processes and compounds.
- Formulate mathematically the process rates, stoichiometry and transport relationships.
- Calibrate the model and test its response against that observed experimentally.

4.3 Model conditions

The kinetic model was limited to a UASB system producing a pelletized sludge bed. The conditions for pelletization (see earlier) restricted the kinetic model to a UASB system treating a soluble carbohydrate or proteinaceous waste, with adequate influent nitrogen and alkalinity, and no oxidising agent or cysteine in the influent. The model was further restricted to deal with soluble carbohydrate waste only, e.g. apple juicing waste, glucose. Other restrictions set on the model are the following system and operating conditions:

- Sludge bed volume is fixed.
- Temperature is fixed at 30° C
- The liquid passes in a plug flow fashion through the bed; no vertical mixing between sludge bed layers (Sam-Soon *et al.*, 1987;1990a).
- Loading rate is less than the maximum rate determined by experiment (Sam-Soon *et al.*, 1991b).

4.4 Compounds

16 essential compounds directly involved in a UASB system treating a carbohydrate substrate were identified. Some of these compounds were directly observable. With others the means for measuring these were not available in the laboratory; their existence had to be inferred, either from the hypothesised biochemical behaviour, or from the requirement of mass balances. Six compounds were directly observable, viz. concentrations of:

- soluble unbiodegradable COD;
- acetic acid (HAc);
- propionic acid (HPr);
- ammonium nitrogen (NH_3-N);
- soluble organic nitrogen (orgN); and

- methane (CH_4).

Ten compounds were inferred, viz. concentrations of:

- molecular hydrogen (H_2);
- glucose;
- polymer mass;
- nitrogen in polymer;
- nitrogen in biomass;
- unbiodegradable particulate COD;
- acidogens;
- acetogens;
- acetoclastic methanogens; and
- H_2–utilising methanogens.

In modelling the behaviour in the compounds identified above, only changes of the compounds in the bulk liquid were described. The compound concentrations within the pellet may differ from the bulk liquid, but modelling these was not practical; techniques to measure concentrations within the pellet were not available.

4.5 Processes

The processes that act on the compounds were identified by observing changes in the compounds under a variety of conditions, e.g. different influent COD concentrations, flow rates and NH_3–N concentrations. Twelve essential processes were identified:

Growth:
- Acidogens on glucose under high $\bar{p}H_2$
- Acidogens on glucose under low $\bar{p}H_2$
- H_2–utilising methanogens on hydrogen
- Acetoclastic methanogens on acetic acid
- Acetogens on propionic acid under low $\bar{p}H_2$.

Death:
- Acidogens
- H_2–utilising methanogens
- Acetoclastic methanogens
- Acetogens

Other Processes:
- Ammonification of soluble organic nitrogen
- Pellet break-up
- Adsorption/enmeshment of soluble organic nitrogen.

4.6 Conceptual model

The conceptual model was based on the biochemical model for pelletisation (Sam-Soon *et al.*, 1987). However, the conceptual model (and the mathematical model developed from this) takes a more macroscopic approach than the microscopic approach used for the biochemical model. A microscopic approach would require modelling each biochemical reaction and the mechanisms controlling it; there is no experimental data for structuring a model to incorporate these. Accordingly, the conceptual model was structured considering only the net effects as present in the bulk liquid.

4.7 Model presentation

In the mathematical model, the process rates and the stoichiometric relationships between the processes and compounds were formulated mathematically. The large number of complex interactions between compounds and processes necessitated that these be clearly presented. Following the proposals of the IAWPRC Task Group (Henze *et al.*, 1987) on "Mathematical Modelling of Waste-water Treatment", the processes and compounds are set out in a compound-process matrix (Table 4.1). This format facilitates clear and unambiguous presentation of the compounds and processes and their interaction.

4.8 Model constants

Kinetic and stoichiometric constants were obtained from the literature or from trial curve fitting of simulated data to experimental observations; only three constants were obtained by curve fitting.

4.9 Model verification

Simulation of the system behaviour at COD loadings below the maximum gave good correlation for COD, SCFA, orgN and NH_3-N profiles for flow-through systems (for examples, see Figs 4.1 and 4.2). For systems with a recycle from the settled effluent to the influent the experimental responses indicated better performances than the simulated responses (for example, see Fig 4.3); this appears to be due to pellet debris in the recycle. The model accepts the debris as inert but there are indications that the debris is biologically active.

Table 4.1: Matrix for UASB system with soluble carbohydrate as influent.

COMPONENT → i	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	PROCESS RATE, ρ_j
j PROCESS ↓	$X_{B,A}$	$X_{B,MH}$	$X_{B,AM}$	$X_{B,AP}$	$X_{B,P}$	$X_{N,H}$	$X_{N,B}$	$S_{I,D}$	S_G	S_A	S_P	$S_{I,P}$	S_{NH}	S_{ND}	S_H	S_{CH_4}	M L^{-3} T^{-1}
1 Acidogen growth on S_G (at high $\tilde{p}H_2$)	1						$+i_{XBN}$		$\frac{-(1+Y_A)}{Y_A}$	$1/3 \cdot 1/Y_A$	$7/12 \cdot 1/Y_A$		$-i_{XBN}$		$1/12 \cdot 1/Y_A$		$\hat{\mu}_A \frac{S_G}{K_A+S_G} \frac{S_H}{(K_H+S_H)} X_{B,A}$
2 Acidogen growth on S_G (at low $\tilde{p}H_2$)	1						$+i_{XBN}$		$\frac{-(1+Y_A)}{Y_A}$	$2/3 \cdot 1/Y_A$			$-i_{XBN}$		$1/3 \cdot 1/Y_A$		$\hat{\mu}_A \frac{S_G}{K_A+S_G}\left(1-\frac{S_H}{(K_H+S_H)}\right) X_{B,A}$
3 H_2-utilizing methanogen growth		1			Y_P/Y_{MH}	$\frac{Y_P \cdot i_{XPN}}{Y_{MH}}$	$+i_{XBN}$						$\frac{-i_{XBN}}{-Y_P \cdot i_{XPN}}$ / Y_{MH}		$-1/Y_{MH}$	$\frac{(1-Y_{\tilde{p}}-Y_{MH})}{Y_{MH}}$	$\hat{\mu}_{MH} \frac{S_H}{(K_{MH}+S_H)} X_{B,MH}$
4 Acetoclastic methanogen growth			1				$+i_{XBN}$			$\frac{-(1+Y_{MA})}{Y_{MA}}$			$-i_{XBN}$			$\frac{(1-Y_{MA})}{Y_{MA}}$	$\hat{\mu}_{MA} \frac{S_A}{(K_{MA}+S_A)} X_{B,AM}$
5 Acetogen growth on HPr				1			$+i_{XBN}$			$4/7 \cdot 1/Y_{AP}$	$\frac{-(1+Y_{AP})}{Y_{AP}}$		$-i_{XBN}$		$3/7 \cdot 1/Y_{AP}$		$\hat{\mu}_{AP} \frac{S_P}{K_{AP}+S_P}\left(1-\frac{S_H}{(K_H+S_H)}\right) X_{B,AP}$
6 Death acidogens	-1						$-i_{XBN}$	1						i_{XBN}			$b_A \cdot X_{B,A}$
7 Death H_2-utilizing methanogens		-1					$-i_{XBN}$	1						i_{XBN}			$b_{MH} \cdot X_{B,MH}$
8 Death acetoclastic methanogens			-1				$-i_{XBN}$	1						i_{XBN}			$b_{MA} \cdot X_{B,AM}$
9 Death acetogens				-1			$-i_{XBN}$	1						i_{XBN}			$b_{AP} \cdot X_{B,AP}$
10 Ammonification													+1	-1			$K_{ND}\left(\sum X_B \text{ except } X_{B,P}\right)$
11 Pellet Break up					-1	$-i_{XPN}$						+1		$+i_{XPN}$			$K_{BP}\ (0,1\rho_1 + 0,1\rho_2 + 0,1\rho_3 + 0,7\rho_4)$
12 Adsorption/Enmeshment of soluble orgN					+1	i_{XPN}						-1.		$-i_{XPN}$			$K_{EP} \cdot S_{ND}$
	Biological active acidogen mass -M(COD)L^{-3}	Biological active H_2-utilizing methanogen mass -M(COD)L^{-3}	Biological active acetoclastic methanogen mass -M(COD)L^{-3}	Biological active acetogen mass -M(COD)L^{-3}	Polymer mass -M(COD)L^{-3}	Nitrogen content of polymer -M(N)L^{-3}	Nitrogen content of biomass -M(N)L^{-3}	Inert mass (organism death) -M(COD)L^{-3}	Soluble glucose substrate -M(COD)L^{-3}	Soluble acetic acid substrate -M(COD)L^{-3}	Soluble propionic acid substrate -M(COD)L^{-3}	Soluble inert polymer mass -M(COD)L^{-3}	Soluble ammonia nitrogen -M(N)L^{-3}	Soluble organic nitrogen -M(N)L^{-3}	Soluble hydrogen -M(COD)L^{-3}	Methane -M(COD)L^{-3}	

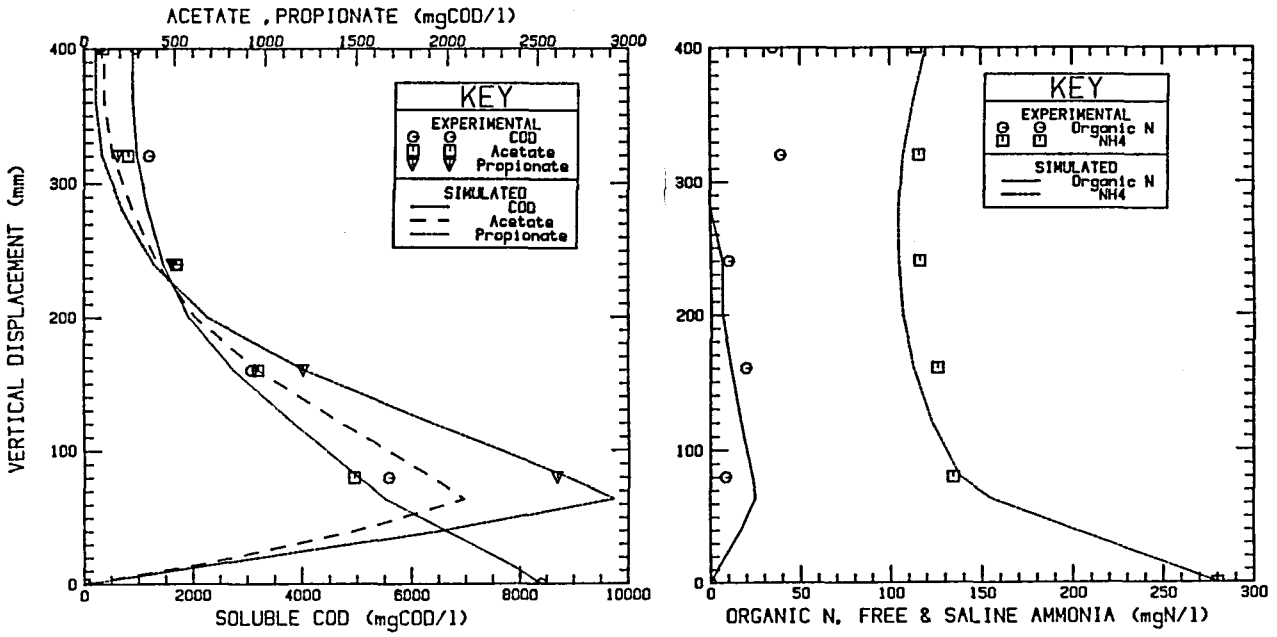


Fig 4.1: Experimentally observed and simulated concentration profiles in the pelletised sludge bed of a single reactor UASB system (substrate apple juice; influent COD = 8 397 mg/ℓ; flow rate = 15 ℓ/d; recycle ratio = 0:1).

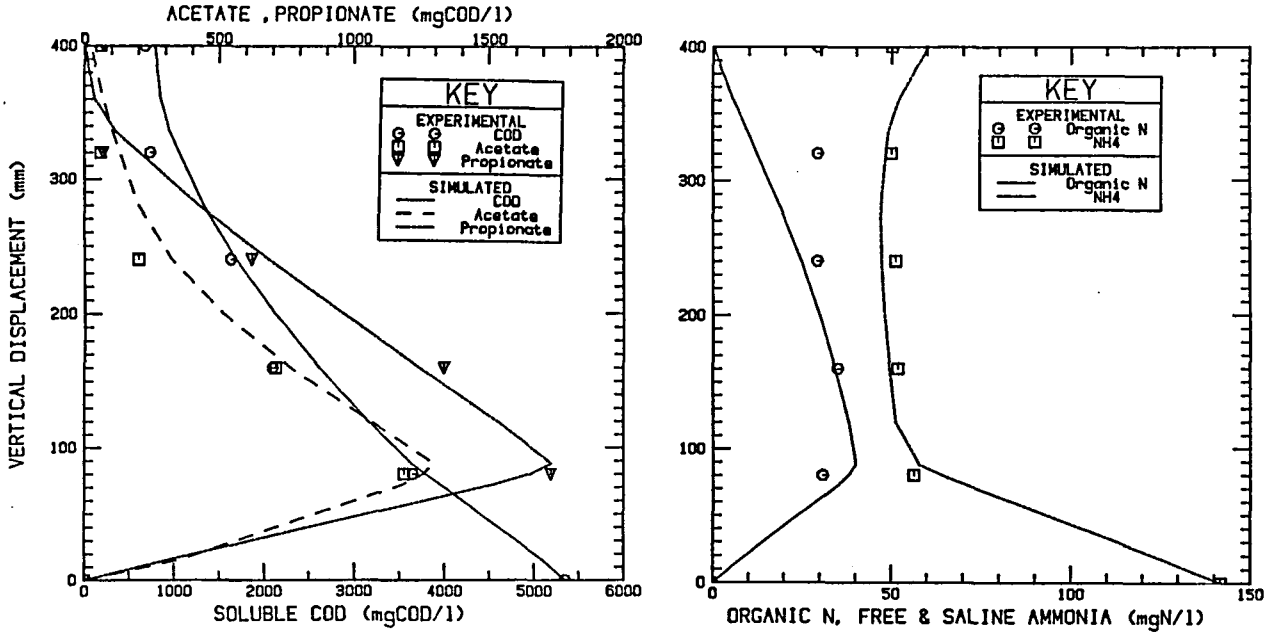


Fig 4.2: Experimentally observed and simulated concentration profiles in the pelletised sludge bed of a single reactor UASB system (substrate glucose, influent COD = 5 345 mg/ℓ; flow rate = 45 ℓ/d; recycle ratio = 0:1).

4.10 Discussion

A significant feature in this model is 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 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 only three constants were obtained by trial and error curve fitting. The model therefore is much broader based, on fundamental aspects or independently obtained data, than it would have been if the constants were all derived by curve fitting.

Omissions in the model are that:

- It does not simulate pH and alkalinity changes in the sludge bed; the model assumes alkalinity provision is sufficient to maintain minimum bed pH > 6,6.

- It does not simulate pellet generation.

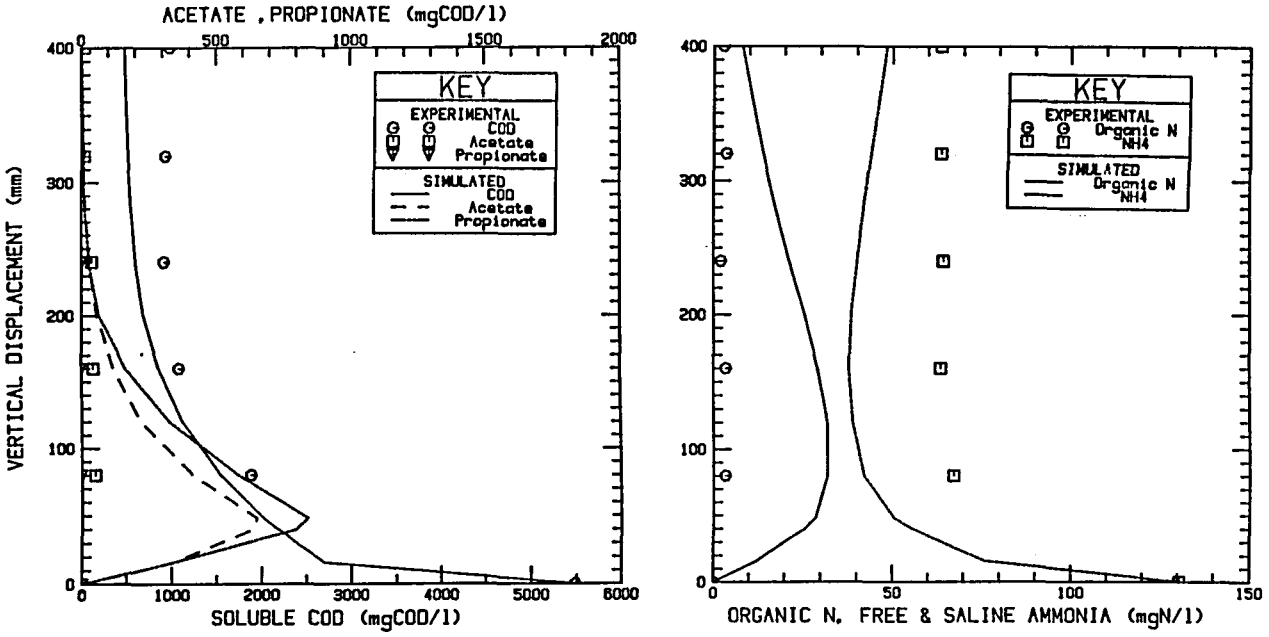


Fig 4.3: Experimentally observed and simulated concentration profiles in the pelletised sludge bed of a single reactor UASB system (substrate apple juice, influent COD = 5 481 mg/ℓ; flow rate = 15 ℓ/d; recycle ratio = 1:1).

CHAPTER 5

H_2CO_3*ALKALINITY REQUIREMENTS AND pH CONTROL IN UASB SYSTEMS

5.1 Introduction

As noted in Chapter 2, in anaerobic fermentation a number of different microbial species contribute to the breakdown of soluble organic compounds to carbon dioxide and methane (Mosey and Fernandes, 1989; Sam-Soon *et al.*, 1987). The main group of bacterial species and the reactions they mediate are:

- acidogens; convert influent COD to HAc, HPr, HBr, CO_2 and H_2,
- acetogens; convert HPr and HBr to HAc, CO_2 and H_2,
- hydrogenotrophic methanogens; convert H_2 and CO_2 to CH_4,
- acetoclastic methanogens; convert HAc to CH_4 and CO_2.

Each of these groups has a specific pH region for optimal growth; for acidogens a pH $\simeq$ 6, for acetogens, hydrogenotrophic and acetoclastic methanogens a pH $\simeq$ 7 (Gujer and Zehnder, 1983). The relative rates of growth of these groups change with pH. Under 'normal' operating conditions, in anaerobic digestion (see below), the following average doubling times have been reported (Mosey and Fernandes, 1989): acidogens – 30 min, acetogens – 1,4 days, hydrogenotrophic methanogens – 6 hours, acetoclastic methanogens – 2,6 days. To ensure optimal breakdown one condition that must be satisfied is to provide optimal pH conditions for the slowest growing organism group. From the above, the acetoclastic methanogens are the rate limiting group; their growth rate is at its maximum at pH $\simeq$ 7,0 but falls sharply at pH $<$ 6,6. Consequently, it is essential to maintain the pH $>$ 6,6. Thus, information on the pH and on the factors causing/resisting change in pH is essential to ensure pH $>$ 6,6 for the successful operation and control of the anaerobic system.

The pH established in an anaerobic fermentation system is a result of the interaction of the weak acid/bases present, the main ones being the short chain fatty acids (SCFA) and the carbonate weak acid/bases (the latter characterized by the H_2CO_3*alkalinity and pH, or, the total carbonate species and pH). The SCFA reduce the H_2CO_3*alkalinity and induce a decline in pH. In normal anaerobic fermentation, i.e. completely mixed systems, the SCFA generated are utilized immediately and SCFA are low throughout the reactor with the result that the net H_2CO_3*alkalinity reduction is relatively small – consequently the H_2CO_3*alkalinity

required to maintain a near neutral pH also is relatively small. In a UASB system, profiles of propionate and acetate up the sludge bed (e.g. Figs 2.1, 3.1 and 3.2) indicate that with carbohydrate and a protein substrate, along the line of flow there develops partial separation of the anaerobic fermentation reactions acidogenesis, acetogenesis, acetoclastic and hydrogenotrophic methanogenesis. In particular the acidogenic phase dominates in the lower part of the bed leading to an increase in SCFA and the development of a high hydrogen partial pressure, the latter creating an environment conducive to pelletization. However, the presence of substantial concentrations of SCFA generated in the lower region of the sludge bed reduce the H_2CO_3*alkalinity – accordingly the H_2CO_3*alkalinity required to maintain a near neutral pH is relatively large. In the upper part of the bed the SCFA are converted to methane and CO_2, and H_2CO_3*alkalinity is regenerated; in effect the H_2CO_3*alkalinity supplied to maintain a near neutral pH in the lower part of the bed is now in excess and wasted in the effluent. Thus, in the UASB system the mass of H_2CO_3*alkalinity to be supplied is controlled by the transient high concentration of SCFA in the lower part of the sludge bed.

5.2 Preliminary study

In a semiquantitative study into the H_2CO_3*alkalinity requirements of apple juicing waste (Sam-Soon *et al.*, 1989;1991c) it was found that with a *flow-through UASB system* the alkalinity requirement was so high that it could render the UASB system uneconomic. The high H_2CO_3*alkalinity requirement prompted enquiry into operational procedures to reduce this requirement: In passing up the bed there is an H_2CO_3*alkalinity loss in the lower active zone but this H_2CO_3*alkalinity is recovered in the upper zones so that only a small nett H_2CO_3*alkalinity loss from influent to effluent is observed. Thus, in effect any H_2CO_3*alkalinity added in the influent is wasted in the effluent.

Conceptually the H_2CO_3*alkalinity in the effluent can be recovered partially by instituting a recycle from the effluent to the influent. In this way the H_2CO_3*alkalinity per influent COD is increased and accordingly H_2CO_3*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 preliminary study was initiated with apple juicing waste as influent to determine the effects of recycle on H_2CO_3*alkalinity requirements, maximum loadings and process performance (Sam-Soon *et al.*, 1991c). The following findings were obtained:

- In a flow-through system, the minimum H_2CO_3*alkalinity supplementation for a pure carbohydrate waste with zero alkalinity, should not be less than about 1,2 mg (as $CaCO_3$)/mg influent COD.

- With a recycle, H_2CO_3*alkalinity supplementation [mg (as $CaCO_3$)/mg influent COD] is reduced to a value given by C·flow/(flow + recycle flow), with C = 1,2 mg alkalinity (as $CaCO_3$)/mg influent COD.

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

The preliminary study with apple juicing waste exposed the alkalinity problem, but the solution found applied only to wastes consisting nearly totally of carbohydrates. In practical situations, the waste to be treated may contain a low carbohydrate fraction, the balance of the COD being organic acids etc. Accordingly, it was decided to investigate the H_2CO_3*alkalinity requirements for such wastes. In selecting wastes for study two basic categories of wastes may be distinguished; (1) wastes which *do not* generate significant amounts of H_2CO_3*alkalinity during anaerobic fermentation (such as the apple juicing waste), and (2) wastes that generate significant amounts of H_2CO_3*alkalinity during fermentation, e.g. due to deamination of proteins to inorganic nitrogen or the uptake of H^+ in the degradation of organic weak acid/base salts to methane and CO_2. Accordingly, in this investigation the following wastes were selected to represent these two categories: Lauter tun (brewery) waste which generates very little internal H_2CO_3*alkalinity, wine distillery waste and the pure protein substrate casein which generate a substantial amount of internal H_2CO_3*alkalinity.

Prior to the investigation into H_2CO_3*alkalinity, each waste (substrate) was studied with respect to its potential for pellet formation.

5.3 Further tasks

Against the background described above, the following further tasks were set:

- Assessment of H_2CO_3*alkalinity requirements for lauter tun (brewery) waste to maintain a near neutral minimum bed pH in UASB systems. Because this waste generates very little H_2CO_3*alkalinity internally, virtually all H_2CO_3*alkalinity has to be supplied externally.

- Assessment of H_2CO_3*alkalinity requirements for wine distillery waste to maintain a near neutral minimum bed pH in UASB systems. This waste generates a substantial mass of H_2CO_3*alkalinity internally due to deamination of proteins to ammonium/ammonia, and removal of organic acid salts such as potassium bitartrate.

- Assessment of a pure proteinaceous waste, casein: This substrate provides the opportunity to study the H_2CO_3*alkalinity generation from deamination and the effect of pH changes on process performance of systems with high levels of inorganic nitrogen. With high levels of inorganic nitrogen generated in the reactor liquid due to deamination the likelihood increases of inhibitory effects developing due to increased ammonia (NH_3) levels at higher than neutral pH values.

Because of the differing nature of these tasks each will be dealt with separately, describing the problems encountered and the solutions achieved.

5.4 Lauter tun (brewery) waste

The study of lauter tun waste in a laboratory-scale UASB reactor at 30 °C was undertaken with three principal objectives in mind (Moosbrugger *et al.*, 1991;1992f):

- to investigate the potential for pelletization in a UASB system in a feasibility study,

- to study the H_2CO_3*alkalinity requirements to maintain a near neutral minimum sludge bed pH when recycling the reactor effluent back to the influent and,

- to investigate the effect of recycling on process performance, i.e. SCFA concentration in the effluent and COD removal.

The feasibility study was done on a single reactor UASB system operated in flow-through mode with an influent COD concentration of 4 000 mg/ℓ and seeded with pelletized sludge from a UASB system treating wine distillery waste. During the feasibility study the COD loading rate was increased from 2 to 25 kg COD/(m^3 sludge bed.d).

Studies involving the recycle ratios were done at a presumed operational COD load-

ing rate of 9 kg/(m^3 sludge bed.d). This COD loading rate was substantially lower than the maximum COD loading rate applied during the feasibility study to ensure stable operating conditions throughout this experimental period.

From the experimental study the following conclusions were drawn:

- Lauter tun waste is amenable to treatment in a UASB system and the waste develops a pelletized sludge bed. The pattern of product formation along the line of flow of the reactor (for example, see Fig 5.1) is very similar to that observed under similar conditions when treating a pure carbohydrate type substrate, e.g. glucose or apple juicing waste.

- The pellets produced were smaller and less compact than with glucose. This contributed to the pellets being lifted by the escaping gas to the gas separator and the settler at higher COD loading rates. The maximum COD loading rate at which the pellet loss became unacceptable was 15 kg/(m^3 sludge bed.d), i.e. the maximum rate was set by the physical rather than biochemical behaviour. The operational COD loading rate was accepted at 9 kg/(m^3 sludge bed.d).

- The TKN/COD ratio of the lauter tun waste was 0,011 mgN/mgCOD; for unimpeded pelletization when treating glucose in a UASB system, a TKN/COD ratio of 0,02 mgN/mgCOD was suggested. In this study the feed was supplemented with NH_4Cl to give a TKN/COD ratio of 0,024; the observed TKN uptake was 0,015 mgN/mg undiluted influent COD. Thus it appears that lauter tun waste needs to be supplied with nitrogen when treated in UASB system to achieve unimpeded pelletization. The lower TKN/COD uptake compared to glucose substrate may be due to the fact that in lauter tun waste carbohydrates form only a fraction of the COD whereas in glucose the carbohydrates constitute 100 percent of the COD.

- Lauter tun waste generates no, or only insignificant, internal pH buffer measured as H_2CO_3*alkalinity; pH buffer needs to be supplied from an external source to control the minimum pH in the reactor to acceptable levels (approx. $6,8 < pH < 7,2$). When supplying H_2CO_3*alkalinity via a strong base (e.g. NaOH) to the base feed flow, the pH in the influent increased to such high levels that apparently some of the trace elements precipitated and became unavailable to the microorganisms, giving rise to complete failure of the process. Hence, the

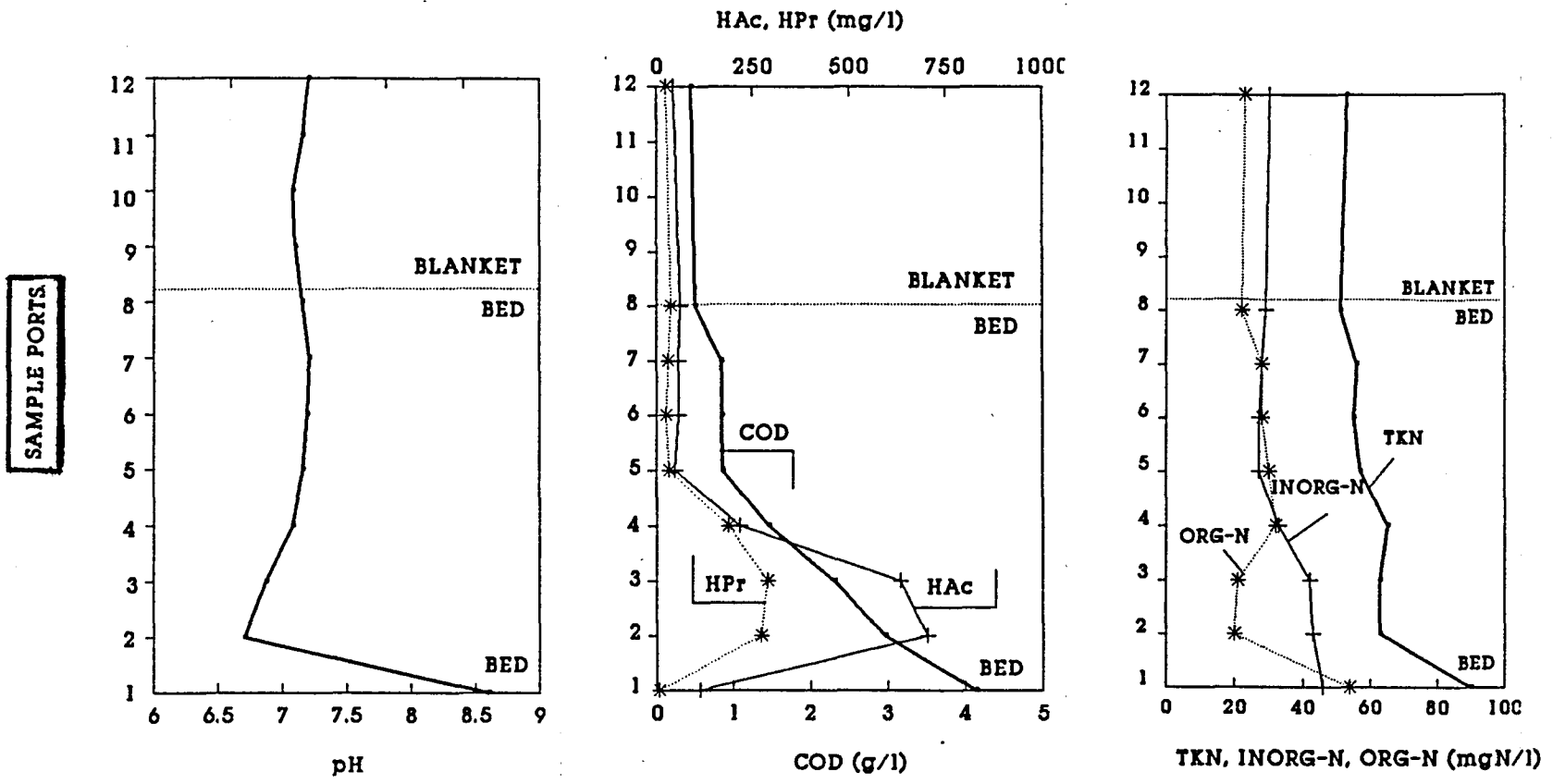


Fig 5.1: Concentration and pH profiles in single reactor UASB system treating diluted lauter tun (brewery) waste. (Influent COD = 4 000 mg/ℓ; flow rate = 25 ℓ/d; loading rate 25 kgCOD/m³ sludge bed/d; recycle ratio = 0:1).

dosing point needed to be selected such that a drastic pH increase at the dosing point was avoided. An appropriate dosing point was found to be the recycle stream; the presence of dissolved CO_2 and H_2CO_3*alkalinity in the recycle stream buffered the pH downstream of the point of strong base addition to a pH < 8,5, instead of a pH of > 11 when NaOH was added to the base influent flow.

- The alkalinity requirement (mg H_2CO_3*alkalinity/ℓ base influent) to maintain a selected minimum pH in the bed, was reduced by imposing a recycle. Earlier, with apple juicing waste the alkalinity requirements/ℓ influent were formulated as C · (base influent)/(1 + recycle ratio) where C was accepted to be more or less constant for all recycle ratios, at 1,2 mgH_2CO_3*alkalinity (as $CaCO_3$)/mg influent COD. This study showed that for a specific COD loading rate, C was not constant but increases as the recycle ratio, r, increased, from C = 1,5 (r = 6) to C = 2,4 (r = 22) for the selected operational COD loading rate of 9 kg/(m^3 sludge bed.d) and the selected minimum pH ≃ 7.

- With the target minimum bed pH of ≃ 7 the pH profile in the bed exhibited only a slight depression to its minimum value for a recycle ratio of 6:1 (base influent COD = 13 000 mg/ℓ diluted by the recycle to 1 860 mg/ℓ) and no significant depression at higher recycle ratios. This tendency to smooth out the "dip" in the pH profile when the effective influent COD is reduced by increasing the recycle, conforms with the observations with apple juicing waste.

- Dilution of the base influent COD from 13 000 mg/ℓ to an effective influent COD of 570 mg/ℓ, by applying a recycle ratio of 22:1, appeared to have no adverse effect on process performance, in COD removal and SCFA conversion to methane and carbon dioxide (the percentage COD removal never declined below 90 percent). Thus it would seem that the lower limit of the effective influent COD of 1 000 mg/ℓ, found with apple juicing waste, can be substantially lowered.

- To ensure that a micro nutrient deficiency was not present, trace elements were added throughout the experiment. Consequently no pronouncement can be made as to whether the UASB system treating lauter tun waste would operate effectively without trace element supplementation.

5.5 Wine distillery waste

The study of wine distillery waste in a laboratory scale UASB reactor at 30^0 C,

similar to the study on lauter tun waste, was undertaken with three principal objectives in mind (Moosbrugger *et al.*, 1991;1992g):

- to investigate the potential for pelletization in a UASB system in a feasibility study,

- to study the H_2CO_3*alkalinity required to maintain a near neutral minimum sludge bed pH when recycling the reactor effluent back to the influent and,

- to investigate the effect of recycling on process performance, i.e. SCFA concentration in the effluent and COD removal.

The feasibility study was done on a single reactor UASB system operated in flow-through mode with an influent COD concentration of 5500 mg/ℓ and seeded with pelletized sludge from a UASB system treating glucose substrate. During the feasibility study the COD loading rate was increased from 27 to 41 kg COD/(m^3 sludge bed.d). At 41 kgCOD/(m^3 sludge bed.d) the pellets were being lifted into the gas separator and settling section and in this manner established the maximum COD loading rate. The bed behaviour did not show any signs of biochemical failure.

Studies involving the recycle ratios and H_2CO_3*alkalinity requirements were done at a presumed operational COD loading rate of 9 kg/(m^3 sludge bed.d). This COD loading rate was substantially lower than the maximum COD loading rate applied during the feasibility study to ensure stable operating conditions throughout this experimental period.

From the experimental study the following conclusions were drawn:

- Wine distillery waste is amenable to treatment in a UASB system and develops a pelletized sludge bed. The pattern of product formation along the line of flow of the reactor (for example, see Fig 5.2) was very similar to that observed under similar conditions treating a pure carbohydrate type substrate e.g. glucose or apple juicing concentrate.

- The pellets produced were smaller, less compact than with glucose and appeared to have a sightly filamentous surface texture. This contributed to the pellets being lifted by the escaping gas to the gas separator and the settler (see above).

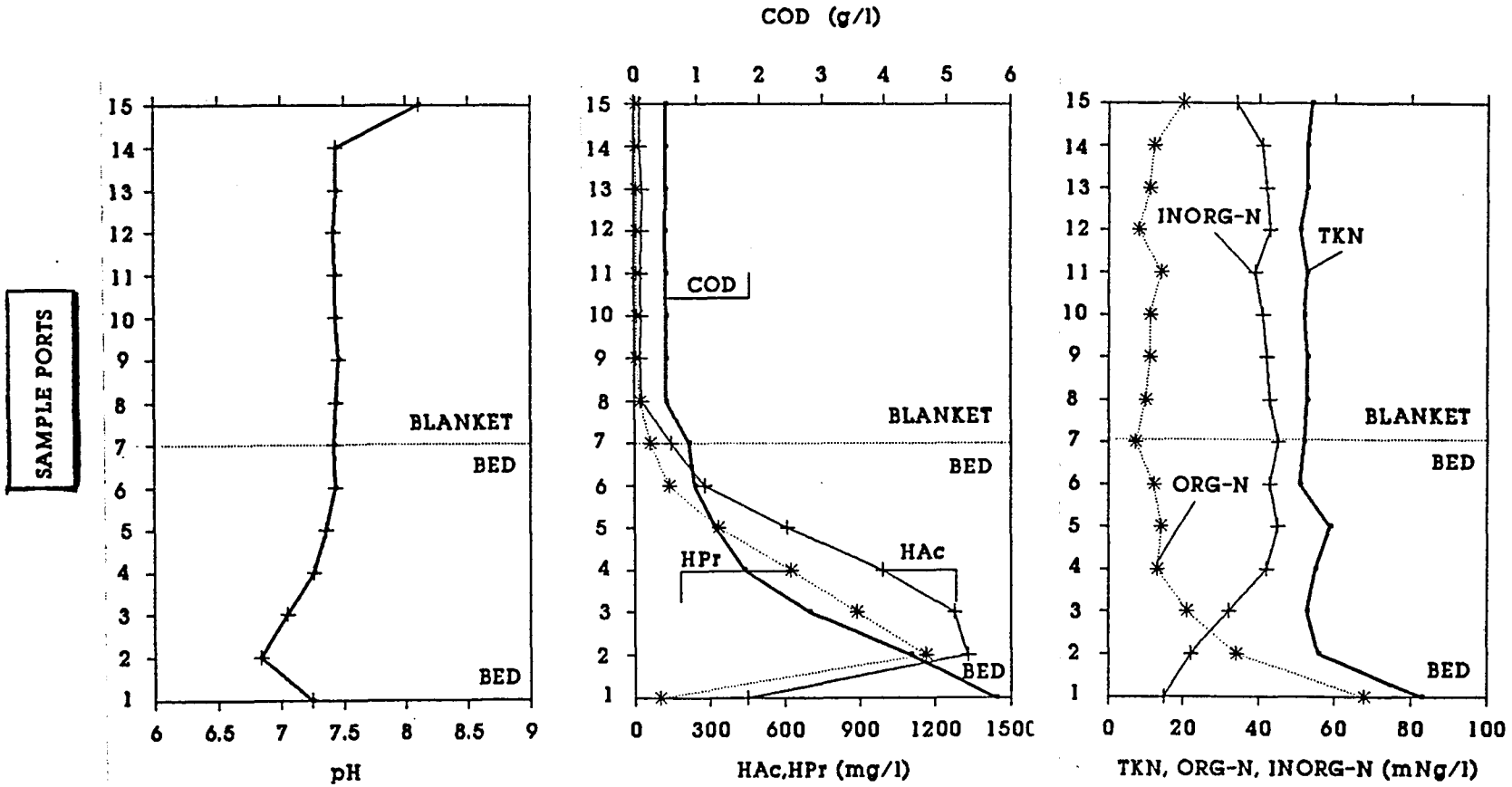


Fig 5.2: Concentration and pH profiles in single reactor UASB system treating diluted grape wine distillery waste. (Influent COD = 5 500 mg/ℓ; flow rate = 30 ℓ/d; loading rate 41 kgCOD/m³ sludge bed/d; recycle ratio = 0:1).

- The TKN/COD ratio of the wine distillery waste was about 0,014 mgN/mgCOD; unimpeded pelletization when treating glucose in a UASB system required a TKN/COD ratio of 0,02 mgN/mgCOD. However, in this study the average mass of TKN uptake per mass of COD for wine distillery waste was 0,01 mgN/mgCOD. This reduced TKN uptake may be ascribed to the nature of the waste: Part of the COD (short chain fatty acids and other organic acids) did not induce high hydrogen partial pressure conditions; hence, only reduced biopolymer production would take place. From the measured TKN uptake of about 0,01 mgN/mgCOD it would appear that in most cases wine distillery waste would require no addition of nitrogen.

- Pellet production in the high hydrogen partial pressure region of the reactor was 0,14 mgVSS/(mgCOD removed). This pellet yield is significantly lower than that reported, when treating apple juicing wastes. This observation is in agreement with the reduced TKN uptake due to reduced pelletization.

- Wine distillery waste generated significant internal buffer, i.e. H_2CO_3*alkalinity. This alkalinity was generated due to removal of H^+ ions during deamination of proteins and the uptake of H^+ in the degradation of organic weak acid/base salts. The mass of H_2CO_3*alkalinity generated internally could not be predicted *ab initio* because the concentrations of the proteins and various organic acid/base salts could not be determined. Experimentally, by measuring the alkalinity in the effluent and in the influent, the H_2CO_3*alkalinity generated could be estimated, at about 0,06 to 0,14 mgH_2CO_3*alkalinity (as $CaCO_3$) per mg base influent COD. Imposing a recycle from the effluent to the influent, the H_2CO_3*alkalinity generated in the bed, and appearing in the effluent, is recycled to the influent; the dilution due to the recycle reduces the base influent COD to an effective influent COD, COD_e [COD_e = base influent COD/(1 + recycle ratio)]. In the measure the recycle ratio increases, the effective influent COD concentration decreases, but the effluent (i.e. recycled) H_2CO_3*alkalinity remains constant (because the H_2CO_3*alkalinity generated per base influent COD remains constant). Consequently the H_2CO_3*alkalinity/COD_e ratio increases, causing the minimum pH to increase.

- The base influent COD concentration ranged from 20 000 to 30 000 mg/ℓ. Dilution of the base influent COD to an effective influent COD (COD_e) as low as 800 mg/ℓ, by applying a recycle ratio of 33:1, appeared to have no adverse effect

on the process performance. Thus it would seem that the lower limit of the effective influent COD of 1 000 mg/ℓ, tested for apple juicing waste with satisfactory operation, can be lowered.

- In a UASB system with a recycle, in assessing the H_2CO_3*alkalinity supplementation to maintain a selected minimum bed pH, the *effluent H_2CO_3*alkalinity* must serve as a reference parameter because this parameter includes any H_2CO_3*alkalinity generated in the bed.

- The effect of different *effective* influent COD (COD_e) concentrations on the minimum bed pH was evaluated at the constant operational COD loading rate (constant base flow rate x constant base influent COD). By changing the COD_e via the recycle the minimum bed pH could be controlled; by increasing the recycle the minimum bed pH was raised and *vice versa.* However, a relatively large change in recycle was required to induce a significant change in minimum bed pH.

- The effect of lowering the effluent H_2CO_3*alkalinity (i.e. the H_2CO_3*alkalinity generated or present per litre of base influent), by adding HCl to the base influent flow, was evaluated at a constant COD loading rate. It was found that:

  (1) for the same COD_e the minimum bed pH increases with increase of effluent H_2CO_3*alkalinity, and,

  (2) for the same minimum bed pH, the higher the COD_e (i.e. the lower the recycle ratio) the higher the effluent H_2CO_3*alkalinity requirements.

- The effect of different COD loading rates on the minimum bed pH was evaluated. It was found that for a constant influent COD *concentration*, under different COD loading rates (by changing the base influent flow rate), provided the recycle ratio remains the same (i.e. recycle flow rate increases proportionally to base influent flow rate) the minimum bed pH remained relatively stable despite an almost threefold change in COD loading rate.

- The pH profiles in the bed exhibited only a slight depression (to the minimum pH) for high recycle ratios of 33:1 and 20 :1 (base influent COD of 27 000 mg/ℓ and COD_e values of 790 and 1 290 mg/ℓ). This tendency to smooth out the "dip"

in the pH profile at low effective influent CODs, conforms with the observations on apple juicing and lauter tun wastes. It would seem, therefore, that provided the effective influent COD is maintained in the range, say, 1 000 to 1 500 mg/ℓ the pH up the bed will be substantially constant and can be monitored at any point in the bed.

- The base influent COD should be diluted by the recycle to an effective influent COD range of about 1 500 to 2 000 mg/ℓ. Within this range of COD_e, for a COD loading rate of about 10 kg/(m^3 sludge bed.d) the different batches of wastes generated sufficient internal H_2CO_3*alkalinity to maintain a near neutral minimum sludge bed pH.

5.6 Pure proteinaceous substrate, casein

To investigate the behaviour of a proteinaceous substrate in a UASB system, casein was fed to laboratory scale reactors (Moosbrugger *et al.*, 1990;1991). The objectives of the study were to:

- study the feasibility of treatment of casein in a UASB system, i.e. to investigate its potential for pelletization,

- measure sludge production,

- assess H_2CO_3*alkalinity requirements to maintain a new neutral minimum bed pH, and,

- investigate the response of the process to changes in pH. Depending on the concentration of proteins, deamination may generate high concentrations of ammonium/ammonia. The species concentration of ammonium and ammonia is dependent on the pH, the concentration of ammonia increasing with an increase in pH. From the literature, methanogenic organisms are inhibited even at low ammonia concentrations. It was of interest therefore to study possible inhibition effects due to increased levels of ammonia at pH levels above neutral.

The feasibility study was done on a single reactor UASB system, that is, the sludge bed that included both the high and low $\bar{p}H_2$ regions. The study on sludge production and inhibition effects was done on a two in-series reactor UASB system, the first reactor containing the high $\bar{p}H_2$ region, and the second reactor containing

the low $\bar{p}H_2$ region of the sludge bed respectively. From the study on the treatment of the proteinaceous substrate, casein, in these two laboratory UASB systems the following conclusions were formed:

- A UASB system treating the proteinaceous substrate casein developed a pelletized bed.

- The profiles of product formation along the line of flow of the reactor were similar to those when treating a carbonaceous substrate in a UASB reactor (for example, see Fig 3.2).

- Uptake of nitrogen was well in excess of that observed in "normal anaerobic fermentation".

- The specific sludge yield obtained in a high hydrogen partial pressure reactor, under the prevailing low pH conditions (minimum pH $\simeq$ 6,2) was 0,26 mgVSS/mgCOD utilized. In the first reactor the VSS retained as pellets was 38 per cent of the VSS produced; the remaining 62 per cent was lost from the bed to the effluent of the first reactor.

- The overall sludge yield of the high and low hydrogen partial pressure reactors combined (with a minimum bed pH $\simeq$ 6,2 in the first reactor) was estimated at 0,11 mgVSS/mgCOD utilized.

- The system could be operated without H_2CO_3*alkalinity addition to the influent.

- When the pH in the system was raised (minimum pH $\simeq$ 7,0) by addition of H_2CO_3*alkalinity to the influent, the specific sludge yield in the high hydrogen partial pressure reactor declined to 0,17 mgVSS/COD utilized. The VSS retained as pellets was 11 percent and the remaining 89 per cent was lost to the effluent.

- The lower specific sludge yield measured under near neutral pH conditions was contradictory to the hypothesis on pelletization, which predicts a higher sludge yield at neutral pH levels because of increased activity of the hydrogenotrophs. The decrease in sludge production at near neutral pH levels was ascribed to inhibitory effects on the hydrogenotrophic organisms of the increased NH_3 species concentration as pH neutrality was approached, see below.

- On addition of H_2CO_3*alkalinity to raise the minimum pH from 6,2 to 7 in the first reactor, the concentration of HAc decreased, the COD removal increased but there was now an increase in HPr in the profile and decrease in VSS production from 0,26 to 0,17 mgVSS/mgCOD utilized in the high $\bar{p}H_2$ reactor. Accepting the hypothesis for pelletization, i.e. increased VSS production due to the action of the hydrogenotroph M strain *AZ*, it was concluded that the reduced VSS production was due to inhibition of the hydrogenotrophs. Inhibition of the hydrogenotrophic microorganisms is further supported by the fact that the HPr advanced higher up the sludge bed. HPr can be converted to HAc and H_2 only at low $\bar{p}H_2$. Inhibition of hydrogenotrophs reduced the rate of H_2 utilization thereby extending the region of high $\bar{p}H_2$ up the sludge bed, in this manner retarding the conversion of HPr to HAc and H_2.

- The cause of the inhibition appeared to be the increase in NH_3 species concentration when the pH was raised to pH 7. The higher NH_3 concentrations appeared to inhibit primarily the hydrogenotrophs, not acetoclastic organisms.

- With time the hydrogenotrophs appeared to adapt, to a large degree, to the increased NH_3 concentrations. When the ammonium/ammonia concentration was raised in steps from 900 to 1400 to 2400 mgN/ℓ influent with pH maintained within the reactor between 7,0 to 7,5, the system's overall COD removal showed only temporary loss in COD conversion at each step. On termination of ammonium and H_2CO_3*alkalinity addition the system reverted rapidly back to the response observed before these additions were made.

CHAPTER 6

MEASUREMENT OF H_2CO_3*ALKALINITY AND SCFA

6.1 Introduction

In assessing the H_2CO_3*alkalinity requirements for the lauter tun and winery wastes, a reliable practical method for measuring H_2CO_3*alkalinity and SCFA was required. In the literature a number of methods had been proposed to measure, (1) some form of alkalinity which approximates the H_2CO_3*alkalinity only, (2) the SCFA concentration only, and (3) some form of alkalinity approximating the H_2CO_3*alkalinity *and* the SCFA by using strong acid/base titrations. Since the objective was to measure H_2CO_3*alkalinity *and* SCFA concentration, only methods measuring both these parameters were considered (Moosbrugger *et al.*, 1992b). One approach to quantify the SCFA *and* approximate H_2CO_3*alkalinity in anaerobic digester liquids involves strong acid *and* strong base titrations over a large pH range which imposes a rather cumbersome titration procedure, increases uncertainty in the correctness of the pH readings, and may give rise to precipitation phenomena, all these resulting in loss of accuracy of the derived values.

6.2 5 pH point titration

To overcome these problems, a 5 pH point acid titration was developed for determining the SCFA (as acetic acid) and H_2CO_3*alkalinity in aqueous solutions also containing known concentrations of other weak acid/bases such as the phosphate, ammonium or sulfide weak acid/bases (Moosbrugger *et al.*, 1992a;1992c;1992d;1992e). The method requires only an acid titration over the middle range of pH (initial pH to pH 6,7; 5,9; 5,2 and 4,3) so that the problem of adequate calibration of the pH probe is overcome and precipitation phenomena are unlikely. If the initial pH is below 6,7 strong base addition is required to reach pH 6,7 before the strong acid titration can be commenced; however, the requirement is only to increase the pH to 6,7, i.e. there is no need to standardize the strong base. The need for initial adjustment of pH to 6,7 with strong base in anaerobic digester liquids should be the exception rather than the rule because pH neutrality is required for optimal operation of anaerobic processes.

Besides the carbonate and SCFA weak acid/bases the most common additional weak acid/bases in anaerobic digestion are phosphate and ammonium. These can be

accounted for in the 5 pH point titration method if the total species concentration of each one is known. In some instances the the total species concentrations of these two weak acid/bases might not be known: It was shown that if the concentration of the ammonium weak acid/base is neglected, the errors induced in the determination of the SCFA and H_2CO_3*alkalinity are very small and usually negligible. However if the concentration of the phosphate weak acid/base is neglected, the error in the determination of the SCFA always will be minor but the error in the H_2CO_3*alkalinity can be substantial, being high if the phosphate concentration is high and small if the phosphate concentration is small.

Besides estimating the H_2CO_3*alkalinity and SCFA, the 5 pH point method allows a check on the pH probe in that it provides an estimate of the systematic pH error where this may be present due to poor calibration, residual liquid junction effect or any other influences on the glass electrode. The estimate of the systematic pH error, however, requires that the carbonate subsystem dominates over the SCFA subsystem, i.e. the total species concentration of the SCFA subsystem must not exceed half that of the carbonate subsystem. In anaerobic digestion the carbonate subsystem will dominate over the SCFA subsystem unless the system is failing; in this event accurate measurements are not crucial for control.

The method was tested extensively on made up solutions of acetic acid and H_2CO_3*alkalinity (sodium bicarbonate) (for example, see Figs 6.1 and 6.2) and on UASB effluents, containing phosphate and inorganic nitrogen by adding known concentrations of acetic acid (for example, see Figs 6.3 and 6.4; both the SCFA and H_2CO_3*alkalinity concentrations were always within a standard deviation of 8 percent of the input values.

For monitoring the system performance via SCFA and H_2CO_3*alkalinity and for pH control of anaerobic systems, the 5 pH point titration method has decided advantages over existing methods in, (1) attainable accuracy, (2) testing time required, and (3) simplicity of testing procedure.

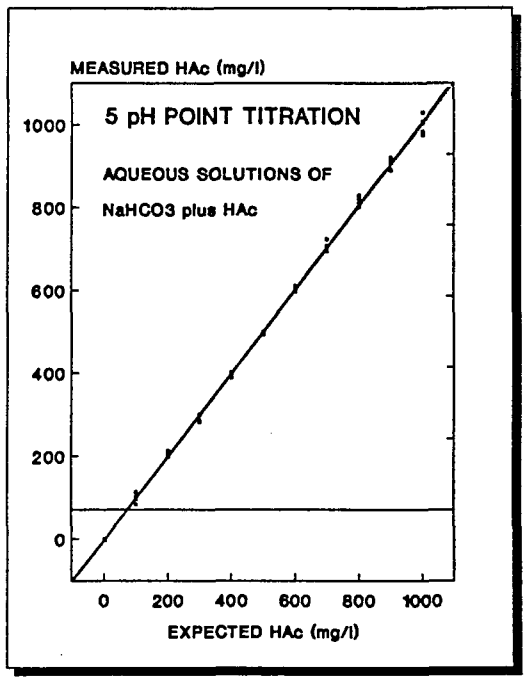


Fig 6.1: Measured versus expected acetic acid (HAc) concentrations in made up aqueous solutions of varying HAc concentrations and 1 990 and 2 488 mg$NaHCO_3/\ell$ as $CaCO_3$; measured HAc values were determined using the 5 pH point titration method.

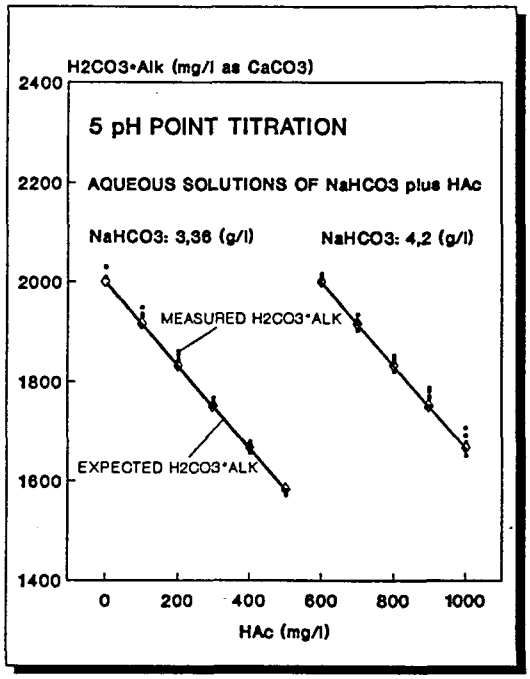


Fig 6.2: Measured and expected H_2CO_3*alkalinity versus expected acetic acid (HAc) concentrations in made up aqueous solutions of varying HAc concentrations and 1 990 and 2 488 mg$NaHCO_3/\ell$ as $CaCO_3$; measured H_2CO_3*alkalinity values were determined using the 5 pH point titration method.

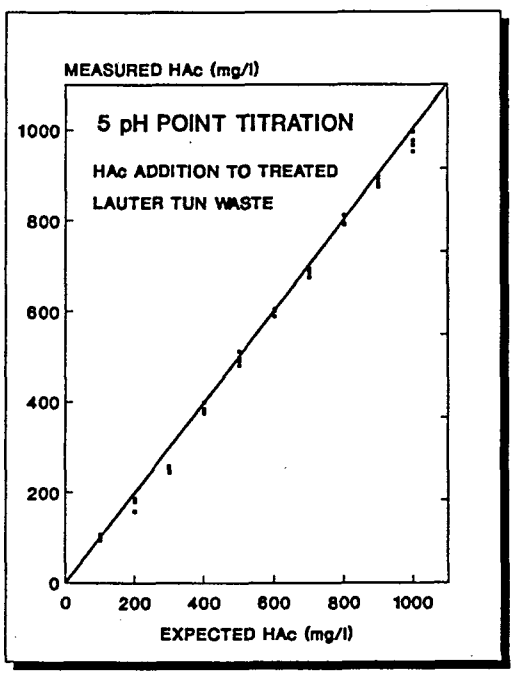


Fig 6.3: Addition of HAc to treated (in laboratory UASB reactor) lauter tun waste and measurement of added (expected) HAc concentration by subtracting 5 pH point titration method results before and after HAc addition.

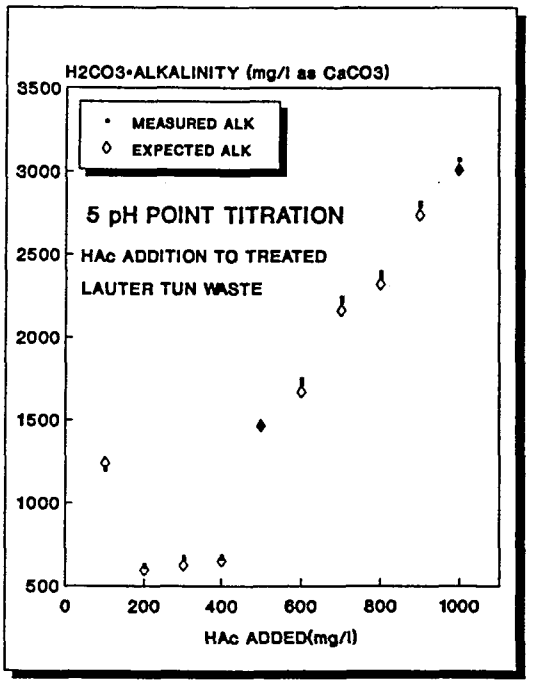


Fig 6.4: Addition of HAc to treated (in laboratory UASB reactor) lauter tun waste and measurement of H_2CO_3*alkalinity after addition of HAc using the 5 pH point titration method. These results were obtained over a period of time under different operating conditions, i.e. different H_2CO_3* alkalinities in the UASB reactor effluent.

CHAPTER 7

PROCESS DESIGN, OPERATION AND CONTROL STRATEGIES

The research can be used to provide tentative guidelines for waste selection, process design, operation and control of upflow anaerobic sludge bed (UASB) reactors (Moosbrugger *et al.*, 1992h). Successful application of UASB technology hinges on the generation of sludge aggregating into pellets, to facilitate retention of the sludge in the bed. An hypothesis for pelletization has been presented, and from the hypothesis, characteristics that a waste must possess for treatment in a UASB system can be identified and evaluated against observations in the literature:

- Wastes that contain an appreciable fraction of carbohydrate or protein in soluble (and, possibly colloidal) form should generate a pelletized sludge bed and be suitable for treatment in a UASB system.

- Wastes that contain principally short-chain fatty acids (SCFA) and/or organic acids or their salts very likely will generate little or no pellets and hence not be suitable for treatment in a UASB system.

- Wastes consisting principally of lipids (e.g. oleic acid) will not generate a pelletized sludge. Such wastes might generate a sludge bed, but of a gelatinous nature.

- For unimpeded pellet formation, the waste must contain an excess of nitrogen. Guidelines for nitrogen requirements for a number of different wastes are given (Sam-Soon *et al.*, 1990b; Moosbrugger *et al.*, 1990;1992f;1992g).

- Wastes must contain adequate macro- and micro-nutrients (e.g. phosphorus) for cell growth.

- Wastes must not contain the amino acid cysteine.

- Wastes that contain sulphates will give rise to limited pelletization due to the preferential utilization of H_2 by sulphate reducing organisms.

With regard to UASB process design, operation and control, from the research the

following guidelines can be identified:

- The UASB system must be operated in a plug-flow or semi plug-flow mode so that there is partial phase separation of the acidogenic and methanogenic phases.

- Pre-acidification of the waste should be avoided and a single reactor system used to ensure retention of the hydrogenotrophic methanogens in the region of high $\bar{p}H_2$.

- The loading rate on the pelletized bed ($kg/m^3/d$) should be about $\frac{1}{2}$ the maximum where such information is available; where not available, the research provides guidelines for laboratory or pilot-scale studies to obtain the information (Sam-Soon *et al.*, 1990a).

- With fluctuating discharges of mixtures of influent, provision should be made for adequate balancing of flow and quality.

- An important aspect is to ensure the minimum pH in the sludge bed > 6,6, to maintain methanogenic organism growth. In the UASB system, due to the partial phase separation of the acidogenic and methanogenic phases, (1) in the lower region of the sludge bed SCFA accumulate (via acidogenesis) reducing H_2CO_3*alkalinity which causes the pH to decline, and (2) in the upper region of the sludge bed SCFA are converted to methane (via methanogenesis) regenerating H_2CO_3*alkalinity which causes the pH to rise. The extent to which the pH will decline in the lower region of the bed will differ for each waste. Should the minimum bed pH decline below 6,6 (usual situation), the pH must be raised by increasing the influent H_2CO_3*alkalinity/COD; this can be done by either adding H_2CO_3*alkalinity to the influent, or recycling from the effluent to the influent, or both. With recycling, H_2CO_3*alkalinity generated in the sludge bed and appearing in the effluent is introduced to the influent and at the same time the influent COD is diluted; in this manner the influent H_2CO_3*alkalinity/COD is increased. This research provides guidance for H_2CO_3*alkalinity addition and recycle ratio selection for a number of different wastes (Sam-Soon *et al.*, 1991b; Moosbrugger *et al.*, 1992f;1992g).

- Having made design provision for pH control by recycle/H_2CO_3*alkalinity addition, a practical control strategy is presented (Moosbrugger *et al.*, 1992g)

that provides guidance on using the measured effluent H_2CO_3*alkalinity, influent COD and recycle ratio to control the minimum sludge bed pH.

- As a part of the control strategy, the concentrations of H_2CO_3*alkalinity in the effluent need to be determined. However, when this research project was initiated reliable practical measurements of the H_2CO_3*alkalinity in an anaerobic system had been solved only approximately and required separate measurement of the SCFA concentration. The research in this project produced an elegant simple practical 5 pH point titration method which gives reliable estimates of both the H_2CO_3*alkalinity and the SCFA concentration (Moosbrugger *et al.*, 1991e). From a practical point of view, the method has a particularly favourable attribute in that if a slight error should be made in pH probe calibration, this is automatically compensated for and an estimate of the error is given. The 5 pH point titration method should find ready acceptance for monitoring and control of all types of full-scale anaerobic digester systems. The solution algorithms for the 5 pH point method have been encoded in a computer program; listed (Turbo Pascal) and executable versions of the computer program are available from the Water Research Commission (Moosbrugger *et al.*, 1992a).

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

The research summarized in this report has focussed on the formation of *bio–pellets* in UASB systems, in particular pellets composed of a polypeptide matrix binding the anaerobic organisms into aggregates. In this regard, an hypothesis for the bio–pellet formation has been developed and validated against literature and experimental observations. From the hypothesis the characteristics that a waste must possess to stimulate bio–pellet formation could be identified. Also, a mathematical model describing the kinetic behaviour of the pelletized UASB system could be developed.

An intensive experimental investigation was undertaken to identify strategies for operation and control of the pelletized UASB system. In this regard, provision of H_2CO_3*alkalinity in adequate concentrations to ensure the minimum bed pH > 6,6, was identified as essential. Guidelines for provision of this alkalinity requirement were set out, e.g. recycles, point of alkalinity addition, etc. As part of the control strategy, a simple titration method was developed to measure both H_2CO_3*alkalinity and SCFA concentrations in aqueous solutions containing also known concentrations of other weak acid/bases.

The research work summarized in this report has addressed and met all the objectives set out at the start of the contract, except for:

- Investigate the feasibility of effecting pelletization by introducing hydrogen from an external source into UASB systems treating wastes that do not generate bio–pellets.

- Formulate models for mineral precipitationin both completely mixed reactors and UASB systems, e.g. calcium carbonate, metal sulphides and struvite.

With regard to hydrogen introduction, preliminary experiments in which pure hydrogen gas was bubbled through the sludge appeared to be completely ineffective. In the biological production and utilization of hydrogen, the transfer of hydrogen probably is at an interspecies level, a condition that could not be created artificially.

With regard to mineral precipitation, this objective was not addressed. The research effort was focussed on the formation of bio-pellets, and in the systems investigated mineral precipitation did not appear to play a significant role. However, this aspect should receive future attention. There is evidence that for wastes that do not stimulate bio-pellet formation (e.g. acetate), mineral precipitation can be used to form a nucleus onto which organisms can aggregate (Ahring and Schmidt, 1992; Ahring, 1992), in effect to constitute a type of pellet. This would imply that, by control of the mineral precipitation process, wastes identified in this report as not forming biopellets could still be treated in UASB systems.

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