ANAEROBIC TREATMENT OF THERMO-MECHANICAL PULP MILL WASTE WATER.

A thesis presented in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy in
Process and Environmental Technology
at Massey University.

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1994

ACKNOWLEDGEMENTS

I would like to thank the following:

- Dr A.H.J. Paterson, Dr A.J. Mawson and Dr Rao Bhamidimarri for their supervision and assistance.

- Tony Paterson and Neville Dow for their late night work and encouragement.

- Mr J.T Alger, Mr R.B. Collins, Mr D. McLean, Mr P. Shaw and Mr W. Mallet for their skills, help and experience in building and maintaining equipment.

- John especially for his patience, advise and the extensive use of his workshop tools for many activities.

- Mike Stevens for his advise and help with the University administrative system.

- Mrs A-M Jackson, Mrs J. Collins, Mr J. Sykes and Mr M. Sahayam for their assistance and patience with equipment dissapearing overnight.

- Prof R.L Earle and the staff of the P.E.T. department for their assistance and encouragement.

- Dr P.N. MacFarlane, Dr T. Clarke and the N.Z.F.R.I. for their assistance.

- P.A.P.R.O.N.Z, the University Grants Committee, the extramural bar and rock and roll for financial assistance.

- Pan-Pacific Forest Industries for their eager provision of 20 tonnes of TMP wastewater.

and especially Janine, Katrina, Bella and Veronica for their support, patience for the extreme demands of anaerobic digestion work and for providing an existance outside Massey.

ABSTRACT

The operation and performance of anaerobic digesters treating thermomechanical pulping wastewater was investigated. The aim of this work was to develop a reactor design that could successfully treat the wastewater with minimal pretreatment.

Six reactors were trialed, all modifications of the upflow anaerobic sludge blanket [UASB] reactor design. Seed sludge source, start-up regime and suspended solids retention were varied to determine effective operation methodology. A screw press effluent from Pan Pacific Forestry Industries Ltd thermomechanical pulp [TMP] mill at Whirinaki, North Island, New Zealand was used as feed for all of the reactors. Seed sludge was obtained in granular form from UASB reactors treating dairy industry wastewaters and in non-granular form from the aerated lagoons of the New Zealands central North Island pulp mills. Operation of all six experimental reactors was difficult due to many feed blockages caused by the relatively high suspended solids concentration in the feed. Inhibition was frequently observed after feed interruptions with slow recovery of performance, possibly due to the lack of a co-metabolite soon after the feed supply ceased. Many modifications to the reactor inlet and feed system greatly reduced blockage problems.

In the later reactors a low suspended solids retention allowed granulation to be achieved from a non-granular forestry industry sludge. With the final reactor configuration total COD removals of 50-60% and dissolved COD removals of 70-85% were achieved at organic loading rates up to 40 $kgCOD.m^{-3}.d^{-1}$. A 20 to 50 % conversion of feed suspended solids to methane was calculated on a COD basis.

Granulation was achieved without a gas-solids-separator. This was attributed to the need for severe selection against poorly settling wood suspended solids, and the presence and precipitation of iron. The granules were approximately 37%

iron on a dry weight basis and had densities averaging 2000 $kg.m^{-3}$. Scanning electron microscope work indicated that extensive precipitates, presumed to be iron based complexes, were responsible for the structural integrity of the granule. An abundant layer of bacteria of predominantly *Methanothrix* morphotypes was found beneath the surface of the granules. The granules have been demonstrated to provide some protection from inhibition, probably by diffusional gradients.

After five years exposure to TMP wastewater a dissolved extract of feed resin acids exerted an inhibitory effect on granules at similar concentrations to that reported for dehydroabietic acid with unacclimated granules. Thus no acclimation to soluble resin acids was evident. Changes in the distribution of resin acids suggested that some degree of resin acid dissolution occurs within the reactors but degradation of the total concentration of resin acids is poor, averaging 10 % reduction as total acids.

Overall, the final reactor design has proved to be an effective treatment of TMP wastewater. Suspended solids removal rates are not high but equally the suspended solids do not threaten the viability of the reactor system. Changes in the nature of the suspended solids passing through the reactor are such that subsequent suspended solids removal will be more efficient and have a lower loading rate than for the untreated wastewater. The reactor has demonstrated a high degree of ability to accept large variations in feed rate and strength and still function efficiently.

The work has produced the basis for a successful primary reactor design for the treatment of a problem wastewater and the necessary information on which a pilot scale plant could be designed for high suspended solids wastewaters. A possible method for the cultivation of granules in difficult wastewaters has been identified.

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CHAPTER ONE INTRODUCTION

The forest industry in New Zealand is a significant export earner and a large employer in provincial area and thus is of major economic importance in these areas. However government and public awareness of the effects of pollution is increasing to the extent that the continued operation of most New Zealand pulp mills is questioned. An increase in the harvest of logs is expected from the late 1990s and onwards into the next century. Two of the most favoured options for use of this increased production are expansion of the thermomechanical pulping (TMP) and the medium density fibreboard (MDF) capacities of the industry. These options produce significant amounts of effluents of similar composition. To ensure public and governmental acceptance of the increase in TMP and MDF mills effective treatment of the wastewaters must be demonstrated.

A meeting of forest industry leaders and wastewater treatment research providers in 1985 discussed the problems of increased forestry industry wastewater output and the public pressure for a reduction in the pollution load from the industry. The contention of many at this meeting was that at least some of the problems arose from collecting all of the various forestry industry mill effluents together into one high flow effluent and then treating this effluent. It was felt that more effective overall treatment would be achieved by treating the high strength problem wastewaters individually in purpose designed systems. This would also reduce the loadings upon the aerobic ponds allowing more effective treatment of the remaining wastewater streams. Out of this meeting arose the principle of a co-ordinated plan of research into the wastewater treatment problems of the industry. This project was initiated as part of that plan to examine the reactor type and design parameters for the anaerobic digestion of TMP wastewaters. Aerobic treatment was to be examined in a separate effort. The consensus of the meeting was that, given the integrated nature of most New Zealand's forestry mills, a successful reactor would be used as an "add in" between the TMP plant and the usual clarifier and aerobic ponds treating the rest of the mills various

wastewater streams. The ponds would serve as an aerobic polishing step for the effluent from the TMP wastewater reactor.

The initial project plan reflected the above intentions and other concerns of the industry. The aims were to :

1) produce a compact, low cost, anaerobic digestion system capable of treating TMP wastewaters,

2) degrade if possible the resin acids in the wastewater, some of which had been reported to pass through the aerobic lagoons of the Central North Island pulp mills and to;

3) degrade the wood biomass fraction of the wastewater.

A real industrial wastewater rather than a synthetic wastewater was used to examine the effect of the predominantly *Pinus radiata* pulp stock upon treatment systems compared to the wide mix of hardwood and softwood species pulped overseas, where most of the forestry industry related anaerobic research originated.

The initial decision to treat the screw press effluent (SPE) rather than the whole mill effluent was taken from predominantly economic considerations of full scale operation and upon the advice of forest industry waste treatment experts. Wash and cooling waters which were used to dilute the SPE at the Pan-Pacific Forest Industries TMP pulp mill were regarded as easily treatable and only added at this mill to meet the specifications for ocean discharge. The SPE flow rate was half that of the whole mill effluent and separate treatment would allow the use of smaller reactor volumes, reducing capital expenditure.

The suspended solids concentration of the first batches of SPE and whole mill effluent obtained were considerably higher, at 4,100 mg.ℓ^{-1} and 3,400 mg.ℓ^{-1}

respectively, than figures commonly quoted for thermo-mechanical pulping effluents, of less than 400 $mg.\ell^{-1}$ (Jurgenson, 1985). The screw press effluent suspended solids were predominantly fine in nature. Approximately 25 % of these solids did not settle in an Imhoff cone even after 3 months. The treatment situation envisaged for any full scale plant would have a considerable investment in suspended solids removal for the treatment of other mill wastewater streams placed after the anaerobic digester. It was considered that the fine nature of most of the SS in the effluent would necessitate the use of high efficiency solids removal equipment further increasing investment in solids removal and possibly negating the cost advantages of anaerobic digestion. Subsequent feasibility assessments and suspended solids removal trials conducted by fourth year technology students indicated that dissolved air flotation or flocculant assisted sedimentation were needed to provide effective removal of the fine wood fibre fragments (Bird, 1990; Brizzle, 1991b; Darwoto and Paterson, 1992). Thus SS removal could become a major cost factor. Also of impact was the close association of the resin acids with the suspended solids. To degrade the resin acids they had to enter the reactor. Removal of the SS would simply shift the problem to another waste. Finally, disposal of the SS fraction removed from forestry industry wastewaters was becoming another major problem of the forestry industry.

A description in the literature of UASB treatment of two screw press effluents from fibreboard manufacture, an effluent similar to TMP effluents, showed 80 % total COD removal. Only 32 -35 % of the total COD was soluble (M^{c}Farlane and Tan, 1985). Thus degradation of the TMP wastewater SS in UASB reactors looked promising. The decision was made to treat the screw press effluent with only light screening to remove large particles (a 1 mm screen was used).

Once results from the experimental work undertaken showed methane yields greater than 0.30 $m^3.kg\ COD_{removed}$ a re-examination of the above paper was made using the theoretical methane yield of 0.351 $m^3.kg\ COD_{removed}$ (at STP) rather than the commonly quoted yields in forestry waste treatment literature of the

time of 0.20 - 0.24 m^3.kg $COD_{removed}$ (at STP) (Jurgensen, 1985). This recalculation of theoretical COD removal showed that the dCOD removal alone was sufficient to provide the methane generated. Thus the apparent suspended solids removal can be attributed to accumulation of the suspended solids within the reactor rather than any degradation of those solids. If this inference had been made earlier in the project it is probable that a more extensive SS removal would have been adopted.

Batch digestion trials of the wood fibre from the TMP wastewater both with the dairy industry sludge used to seed reactors A and B and with the forestry industry sludges used for reactor C failed to show any significant evidence of suspended solids degradation. This was not conclusive however as many of these trials failed to even remove all of the dissolved COD. Difficulties in differentiating wood fibre biomass, active bacterial biomass and inactive bacterial biomass made any conclusions about wood fibre degradation problematical. The lack of conclusive evidence of wood suspended solids degradation one way or the other in batch tests versus the reported successful degradation in a UASB reactor with MDF effluent only emphasized the need for continuous reactor trials. Suggestions of some co-metabolism mechanism from scientists at the New Zealand Forestry Research Institute subsequently reported (McFarlane and Clark, 1988) and some similar indications from the performance of reactors A and B supplied a possible explanation for the failure of the early batch tests. Later biological methane potential tests using granules from reactors C and D gave results of 70 - 90 % of the theoretical methane yield. The dissolved COD fraction was 61 % of the total feed COD suggesting that up to 75 % of the wood fibre added in the feed was degraded.

1.1 Reactor selection

The decision to use a UASB type of reactor, for the first trials, rather than any of the other anaerobic reactor designs was taken in consideration of a number of factors reported as characteristic of this style of design. These were:-

1) successful history of application to a variety of wastewaters in laboratory to full scale operations (Speece, 1983; Lettinga and Hulshoff Pol, 1991)

2) reported lower capital cost and often running costs of this design compared to the other high-rate anaerobic reactor designs

3) reported successful treatment of a similar wastewater type (M^{c}Farlane and Tan, 1985)

4) long solids retention times (SRT) and short hydraulic retention times (HRT) allowing compact reactor sizes compared to the conventional and contact anaerobic reactors.

5) the anaerobic contact design gives proven solids degradation but not necessarily of wood suspended solids and, especially with poorly settling suspended solids, requires relatively high HRTs compared to the other high rate designs

6) an anaerobic filter would be very prone to plugging and may not function effectively

7) the down flow stationary fixed film reactor eliminates the plugging problem but would not retain any wood solids unless they impacted with and were adsorbed onto the fixed film

8) the fluidised bed and expanded bed designs also eliminate the solids plugging problems but would not retain any wood solids

9) the UASB design does retain suspended solids, although this may become a problem, but the degree of retention can be controlled to some extent by the design of or absence of a gas solids separator.

Further to these points was the consideration of the degree of solids degradation likely to occur especially at higher loading rates than those used by M°Farlane and Tan, (1985). It was considered that, if solids were only poorly degraded then wash out of SS would occur once the solids in question reached the top of the sludge bed. Thus the UASB, whilst it has advantages of its own was seen as a compromise in that it was likely to retain wood solids sufficiently long to allow degradation to occur but may allow the release of enough undegraded wood solids to prevent reactor clogging.

The objectives of the initial trials were to:

1) assess the suitability of the UASB reactor design for treatment of the SPE,

2) provide an anaerobic sludge acclimated to the SPE for further reactor design trials,

3) identify and overcome the operational problems of dealing with the suspended solids of the SPE,

4) assess the start-up of a UASB with granular non-forestry industry sludge. Little granular anaerobic sludge was available at the time, especially not for forestry industry wastewaters. No reports of granulation in UASB reactors treating forestry industry wastewaters were available but granulation was reported as aided by the use of a granular seed and,

5) develop if possible a well granulated UASB sludge.

Two reactors (A and B) were started up with granular dairy industry sludge. Reactor A was subjected to more severe rates of change than Reactor B to assess the more suitable start-up regime. Reactor B had a much larger settling zone at the top of the reactor than reactor A to assess the affect of the degree of solids retention upon reactor and sludge performance. The feed was a mixture of lactic acid casein whey permeate to maintain the sludge activity and dilute whole mill TMP effluent. After a short period of operation the TMP effluent was replaced with SPE. The fraction of SPE in the feed was slowly increased and that of whey was decreased until 100% SPE was being fed to the two reactors. Numerous problems with pump and reactor inlet blockages occurred and modifications were made to the reactors and feed systems to reduce the problem.

The original granular sludge was lost after 100 % SPE feed was achieved, in both reactors A and B, leaving floc based growth. The high retention of the wood fibre may have been detrimental to the granules and granular formation as has been suggested in the literature (Hulshoff Pol *et al*, 1982). Granulation was lost in reactor B considerably sooner than in reactor A and the resultant floc based sludge was of a looser nature than that typically seen in reactor A. It was thought that the larger size of the expanded diameter chamber (volume $\approx$ 10 ℓ) at the top of reactor B, shown in Figure 3.2, was giving greater retention of SS than the smaller chamber (volume $\approx$ 2.5 ℓ) of reactor A.

Reactors A and B showed good apparent suspended solids and chemical oxygen demand (COD) removal (subsequently shown to be poor by gas yield analysis described in Sections 4.2.3.2 and 4.3.1.1). Literature reports for other reactor types indicated good treatment of dissolved COD only. The experience gained in handling the wastewater suggested that the known disadvantages of the other high-rate anaerobic reactor types would make these designs unsuitable for TMP wastewaters. The decision was taken to stay with the apparently successful UASB reactor concept.

Thus reactor C was also built as a UASB type. No settling chamber was installed in reactor C as shown in Figure 3.4. This was expected to put a greater selection pressure upon the SS of this reactor so that only well settling particles were retained in the reactor. Granulation was achieved and maintained over more than 3 years in this reactor despite many upsets and a long period of severe inhibition apparently from high zinc levels. This reactor was further refined in response to blockage problems in the inlet to eliminate this problem.

1.2 Summary of early work

The above early work, whilst not particularly successful at producing hard data, produced a workable reactor configuration that avoided most of the blockage problems of the initial reactors. From this work it was concluded that:-

1) The collected TMP screw press effluent was degradable anaerobically especially the soluble fraction, which showed good removals.

2) Extensive granulation in a UASB reactor was possible with TMP wastewaters.

3) The UASB reactor design apparently gave good treatment of the screw press effluent. Suspended solids and resin acids accumulated within reactors A, B and C giving apparent reductions of 40 - 60 % but calculations showed that there was no significant degradation of SS or resin acids in the floc based reactors A and B. In the well granulated reactor C 40 - 50 % reduction in SS was calculated for organic loading rates (OLR) of 5 - 11 kg $COD.m^{-3}.d^{-1}$. Conclusions about the degradation of resin acids could not be drawn at this time. Resin acids are discussed in Chapter 5.

4) Start-up of UASB reactors treating TMP type wastewaters are far more successful with the use of an acclimated sludge and a relatively high

OLR (10 kg $COD.m^{-3}.d^{-1}$) compared to non acclimated sludge and low OLR (<1 kg $COD.m^{-3}.d^{-1}$). Allowance must be made for the adhesive nature of the wood resin components. In combination with wood fibre a structure similar to fibre board can build up and block flow tubes in small scale work.

5) There is strong evidence of inhibition of the reactors after a short period (> 18 hours) of no feed. A much longer period of low feed rate does not give this effect. The effect was worsened by two periods with no feed in close proximity (< 7-8 days). This may be evidence of the co-metabolism of resin acids reported by M^{c}Farlane and Clark, (1988).

6) The suspended solids in the effluents were different in nature to those of the feed in that their settling ability was greatly enhanced. 75 % of effluent SS settled out within 15 minutes rather than 16 hours for feed SS. Thus any SS removal process after the reactor will exhibit far greater efficiency.

This final configuration proved to be successful at degrading at least some wood solids and so was duplicated in the form of reactor D. The final reactor design could no longer be said to be representative of the UASB design but combined aspects of the UASB design with some of those of both the expanded bed and anaerobic filter. Reactors C and D were used to speed up the collection of steady state data to characterise the reactor system. Both reactors were seeded identically by splitting the original reactor C sludge. The granular portion of each reactor's seed consisted of 1.1 kg of granules larger than approximately 1 mm in diameter. Fine granules plus other sludge from reactor C were added in a suspension of reactor liquor and fresh reactor effluent.

Reactors C and D were then put through a number of "pseudo steady state" periods to assess the performance of this reactor design. Following the "steady state" analysis, a series of tracer studies were performed to investigate the type

of flow in the reactors. Reactors C and D were then used as a source of sludge for a series of serum bottle assays to determine granular activity and the effect of resin acid toxicity upon well acclimatised granules.

CHAPTER TWO LITERATURE REVIEW

2.1 FORESTRY INDUSTRY WASTEWATERS

2.1.1 Introduction

A wide variety of forestry industry wastewaters are produced. The most significant in terms of quantity and pollution load are the various pulping and bleaching liquors. These are categorised by the pulping methods used and range from the mechanical pulping wastewaters, in which only a small fraction (2-7%) of the wood input to pulping is lost, through to the chemical pulping wastewaters in which a significant fraction (45-50%) of the wood is dissolved (Sierra-Alvarez, 1990). Thus the composition and character of the various forestry industry wastewaters differ quite markedly and from a wastewater treatment point of view cannot be effectively regarded as a related group. Traditional wastewater treatment systems have consisted of some primary suspended solids removal step followed by aerobic treatment, usually in aerated lagoons. If designed and operated properly, reasonable treatment results, with the notable exception of halogenated organics and colour (Sierra-Alvarez, 1990). However increased organic loads and energy costs, and the relatively high operating costs of conventional aerobic systems have resulted in increasing application of anaerobic treatment technologies (Sierra-Alvarez *et al*, 1991). From the early 1980s reports of application of anaerobic digestion to pulp and paper industry wastewaters have been appearing with increasing frequency. Installation of large-scale anaerobic digesters for pulp and paper wastewaters has been increasing from 1983 with a large increase in 1988 (Lettinga *et al*, 1991(a)). There are great differences in effluent characteristics between individual mills due to the mix of operations performed, species mix of raw material pulped and operating conditions utilised. This means that treatment systems need to be carefully tailored to each mill (Rintala, 1991) . Discussion in this section will generally be limited to thermomechanical pulp (TMP) mill effluent.

2.1.2 Thermomechanical pulping wastewaters

In the thermomechanical pulping process wood chips are steamed in pressurised vessels immediately prior to entry to the rotatory grooved disk refiners via screw press feeders. Typically the chips are steamed for two to four minutes at a pressure of 200-300 kPa (Ducker, 1980). This softens the lignin matrix holding the cellulose fibres together making the separation of the fibres easier. The resulting coarse pulp may be passed through two or three refiner stages. Pre-steaming may or may not be applied before the second and third stages. The prime source of the waste water from these mills is from the screw press feeders where considerable amounts of water are expressed from the chip or pulp stream. Chemi-thermomechanical (CTMP) pulping utilises a chemical pretreatment as well to gain additional softening/dissolution of the lignin.

2.1.3 Anaerobic biodegradability of TMP wastewaters

The anaerobic degradability of TMP wastewaters has been reported as high (30-60% of total COD; 40-80% of dissolved COD [dCOD]) and the toxicity as low (Jurgensen *et al*, 1985; Rintala *et al*, 1991; Sierra-Alvarez *et al*, 1991). These degradation values are low compared to that achieved for sorghum biomass where degradabilities over 90% where achieved (Jerger *et al*, 1987). The difference probably represents the lignin fraction of the TMP wastewaters. Carbohydrates and lignin have been reported to be present in TMP effluents in much the same ratio as they are found in wood (Jurgensen *et al*, 1985). Carbohydrate and lignin have been reported as 71% and 27% respectively of oven dry wood (Kininmonth and Whitehouse, 1993). Low molecular weight lignins are partially degraded anaerobically (Colberg and Young, 1985) accounting for the 80% degradation. Free cellulose and hemi-cellulose are expected to be degraded but limited by the rate of saccharification. Lignocellulose units not separated in the pulping process should be partially

degraded and the long chain fatty acids are known to be degraded. (Jurgensen *et al*, 1985). The resin acids and related alcohols, representing the rest of the extractives, have been reported as recalcitrant anaerobically (Sierra-Alvarez *et al*, 1991; Sierra-Alvarez *et al*, 1993). However abietic acid has been reported as being removed under anaerobic conditions, probably by conversion to other resin acids such as dehyroabietic and 13-abieten-18-oic (McFarlane and Clark, 1988; Zender *et al*, 1993).

Most of those working with TMP or CTMP effluents have identified the wastewater's suspended solids as a potential or actual problem for successful treatment of these wastwaters (McFarlane and Tan, 1985; Habets and de Vegt, 1991). Those that did not overtly mention any problem with suspended solids showed very low suspended solids in the feed or even used filter paper to remove them (Rintala and Lepisto, 1992; Vinas *et al*, 1993; Rintala, 1991). A general assessment of the position of the above authors is that good removal of the influent suspended solids is mandatory for good anaerobic digestion performance due to its effect upon sludge settleability, the reactor bed volume and flow patterns within the reactor. However given the degradability of the cellulose fibres and the action of the TMP disk refiners upon the fibres such that the ligno-cellulose structure is disrupted, it seems possible that provided sufficient retention time of the suspended solids is provided, significant solids removal would be achieved.

2.1.4 Toxicity

The toxicity of resin acids in TMP effluents has been reported as being very low as stated above. The explanation given is that the acid conditions of the TMP process does not dissolve the resin acids. Thus any resin acids present in the wastewater are not in solution and therefore have no effect upon the methanogenic consortia (Sierra-Alvarez *et al*, 1991). Most of the resin acids present in TMP and, to a somewhat lesser extent, CTMP effluents appear to be closely associated with the very fine wood fibre fragments characteristic of these

wastewaters (Habets and de Vegt, 1991; Richardson *et al*, 1991). After accumulation of these fines in the methanogenic stage of UASB reactors in two-phase systems, resin acid concentrations had risen to 300-1500 $mg.l^{-1}$ (Richardson *et al* 1991). The effluent resin acid concentration was greater than that of the feed and was almost completely soluble compared to the approximately 50% soluble value for the CTMP feed. Richardson *et al* (1991) postulated that the accumulated resin acids had undergone solubilisation and subsequent leaching into the effluents. It is probable that this will occur in TMP treating reactors as well. Methanogenic inhibition by a screw press effluent from a medium density fibre board plant was reported (McFarlane and Tan, 1985). This wastewater is similar in composition to TMP screw press effluents and resin acids were thought to be responsible for the inhibition. A gradual acclimation to this toxicity was possible. Elsewhere, TMP and CTMP effluents were reported to be inhibitory to anaerobes unless diluted (Vipat and Branion, 1990). Either other compounds in these wastewaters are inhibitory or there is some dissolution of resin acids in at least some TMP wastewaters.

2.1.5 Suspended solids removal

With suspended solids removal having been reported as important for good anaerobic treatment of TMP wastewaters, the degree and cost of solid removal become important factors in the overall wastewater treatment system. The choice of solids separation method and equipment design is dependent upon the size distribution and type of the solids to be removed, and the flowrate and solids content of the wastewater (Tchobanoglous and Burton, 1991).

The suspended solids of the Pan Pacific Forest Industries TMP mill after solids removal by a "contra-shear" was reported to be up to 2000 $mg.l^{-1}$ and to comprise solids of less than 150 μm diameter (Brizzle, 1991 b). Gravity settling of this wastewater was reported to remove 30-60% of these solids, and flocculation and sparged air flotation 90-97% , at room temperatures (Darwoto and Paterson, 1992; Rae, 1993). At the effluent temperature of approximately

70 ^{o}C at the mill gravity settling was found to be quite variable and somewhat lower than that at ambient temperatures. The decrease in performance of flocculation and dissolved or sparged air flotation was even greater (Flynn, 1993; Rae, 1993). An initial feasibility study for suspended solids removal from this pulp mill's effluent found gravity settling to be the lowest cost option and even this would be a significant cost. The existing "contra-shear" screens were not a costed option (Bird, 1990).

A potential problem with the use of flocculants as a pretreatment for anaerobic digestion is that a number of the flocculants used in the above flocculation work, although not tested for anaerobic bacterial inhibition, may exhibit some inhibitory effects upon the anaerobic bacteria.

2.2 ANAEROBIC REACTOR DESIGNS

2.2.1 Introduction

For the successful use of anaerobic technology for the treatment of thermo-mechanical pulp mill wastewaters in New Zealand a number of requirements must be met:

1) The flowrate of waste water is high and thus a high rate reactor design is needed to lower capital costs.
2) The resin acids and other extractives present potential toxicity problems and so require that the anaerobic biomass be protected from inhibitory effects, at least to some extent.
3) Significant quantities of wood suspended solids are contained within the wastewater. Much of the suspended solids is very fine wood fibre fragments not easily removed from the wastewater and if removed it's separate disposal is another problem. Ideally the reactor design should degrade the influent suspended solids. To do so would require long solids retention times for the influent suspended solids. Some control

over the degree of wood solids retention is needed to avoid clogging the reactor with undegraded wood suspended solids and/or displacement of the active anaerobic biomass.

4) If wood suspended solids are not degraded at all or the rate of degradation is slow compared to the rate of inflow of the wood suspended solids to the reactor, a design that allows some or all of the wood suspended solids to flow through the reactor with little effect upon other reactor performance criteria is desirable.

The successful application of anaerobic digestion reators depends upon careful matching of the reactor configuration to the wastewater to be treated. Essential functions of an anaerobic digester have been listed (Baader, 1981) as:

1) to continuously provide the bacteria with nutrients and to remove the metabolic products from the biomass,

2) to ensure a detention time for the organic matter adapted to the different digestion rates of the organic components,

3) to prevent uncontrolled accumulation of solids in the digester and blockages in the material flowing through the digester and

4) to distribute equally the heat in the digester.

The high-rate anaerobic digestion reactor designs are based upon three fundamental aspects (Iza *et al*, 1991).

1) Accumulation of biomass within the reactor by means of settling, attachment or by recirculation. This allows the separation of solids retention time and hydraulic retention time, thus low reactor volumes and high degradation rates are possible.

2) Improved contact between biomass and wastewater, to overcome diffusional limitations.

3) Enhanced activity of the biomass, due to adaptation and growth.

A number of reactor designs have been developed. The basic reactor units are illustrated in Figure 2.1. These units can be combined to give a number of different hybrid types.

2.2.2 Conventional single tank process

This is still the most common design and are often used to treat municipal sludge or animal wastes where the solids content is of the order of 2-10%(w/v). Solids retention time (SRT) and hydraulic retention time (HRT) are generally the same and typically range from ten days to several months. These are usually stirred and heated to 30-40 ^{o}C but may be operated at thermophilic temperatures of 55-65 ^{o}C. Some mixing is provided by sludge recirculation from the heating heat exchangers but additional mechanical or gas recirculation mixing is often provided.

The major advantages of this design are simple construction and operation and high solids handling. The treatment of more dilute wastes is not economic because of the large tank needed.

2.2.3 The anaerobic contact process

This design is similar in principle to the aerobic activated sludge system. A conventional stirred tank is used but SRT is increased by recycle of solids separated from the effluent, usually by settling or centrifugation. This allows reduction of HRT for the more economical treatment of dilute wastes. Gas evolution by the cells in the separation stage has caused failure of solids separation (Mawson, 1986). Thus in this situation the design becomes similar to the conventional design with the same limitations.

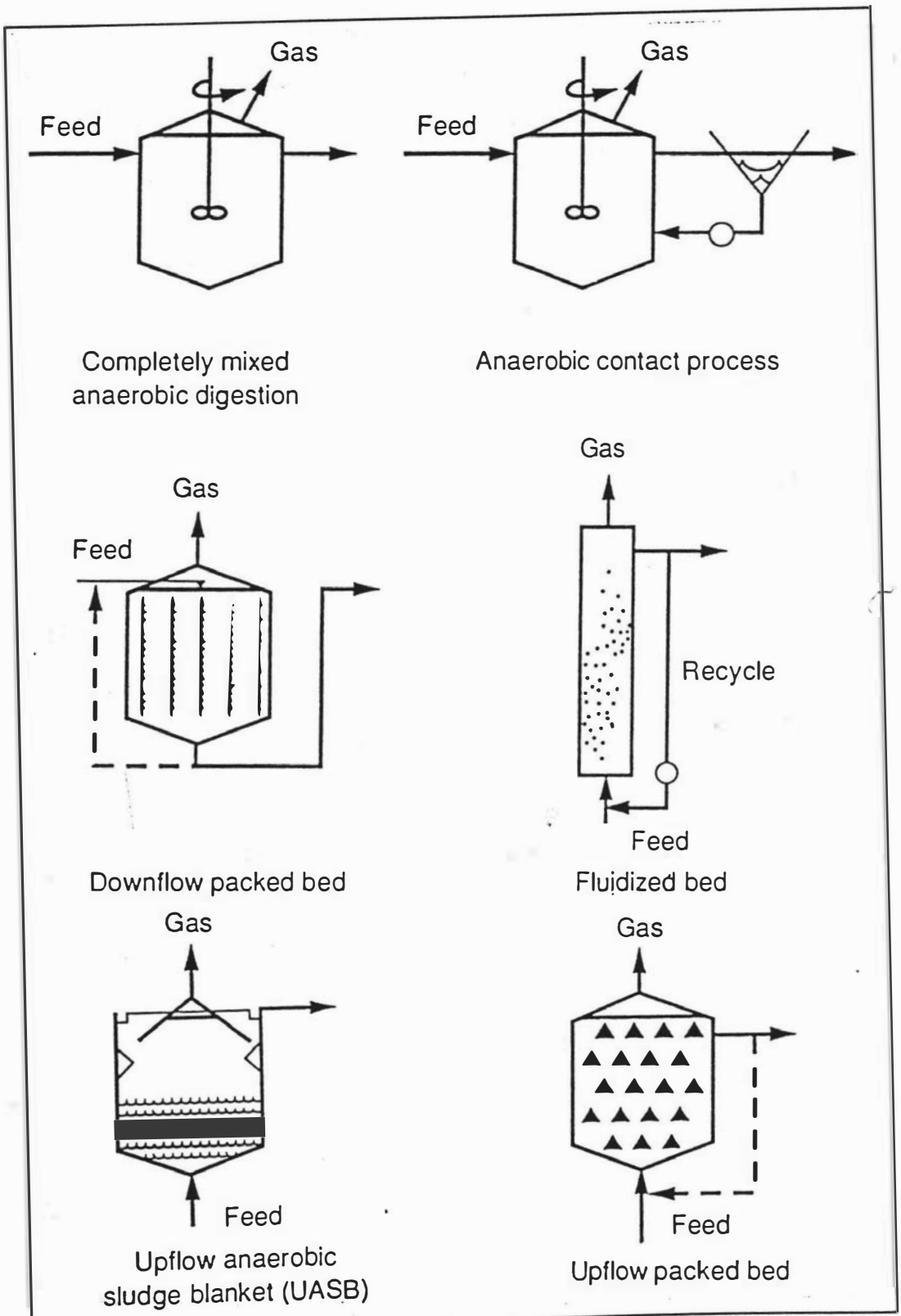

Figure 2.1: Configurations for the anaerobic reactor design units.

2.2.4 The upflow anaerobic sludge blanket (UASB) reactor

Wastewater is introduced at the bottom of the reactor and passes through a blanket of active, often granular, sludge. A gas solids separator (GSS) near the top of the tank separates gas bubbles from the solids and allows the solids to settle. Clarified effluent is withdrawn at the top of the reactor. Mixing is provided by the rising gas bubbles and the upflow of feed. The design has gained rapid acceptance because of its ability to operate efficiently at high loading rates with low suspended solids and its low construction costs (Lettinga *et al*, 1980; Frostell, 1983). The design has been reported as successful in treating high suspended solids wastewaters but this is dependent upon the rate of suspended solids accumulation versus the rate of liquefaction (Sayed *et al*, 1987). This design relies upon the tendency of anaerobic bacteria to form flocs or granules that settle efficiently. Granular sludge is not necessarily a requirement for successful treatment of a wastewater. A potential problem is the occasional loss of flocculation or granulation as a response to reactor upset.

2.2.4.1 Granulation in UASB reactors

Granulation in UASB reactors has been reported as being dependent upon the type of wastewater treated (Iza, 1991; Hickey *et al*, 1991). This may be overcome by the use of a granular seed sludge. In some cases disintegration of the granules resulted, presumably as a result of the change in feed type. Where granulation is likely to occur, a granular seed sludge is optimal for fast start-up (Hickey *et al* , 1991) although some care must be taken that granules capable of treating the wastewater in question are chosen.

Granulation from non-granular seed sludges has been observed over the range of temperatures used for anaerobic digestion and over a range of wastewaters such as brewery, citrate, slaughterhouse, distillery, sugar molasses, glucose, acetate plus yeast extract and a VFA mixture (Hickey *et al*, 1991). Reports of

granules in forestry industry wastewater UASB reactors have been made but it is uncertain if nongranular seed sludge was used (Driessen and Wasenius, 1993).

Strategies for the development of granules fall into two categories, those involving the addition of particulate matter, such as ground up granules, fine sand or hydro-anthracite, to serve as initiators for granulation, and those aimed at optimising *Methanothrix* type bacteria, which are normally the predominant bacteria in granules (Chang *et al*, 1993; Uemura and Harada, 1993; Morgan *et al*, 1991). Mixed results have been reported for the addition of particulates except for ground up granules, where supplying granulating bacteria was probably responsible for the success obtained (Hickey *et al* 1991). Those strategies aimed at increasing *Methanothrix* type bacteria used low acetate concentrations (<200 $mg.l^{-1}$) to favour the low Ks of these acetate users or high acetate concentrations (>1500 $mg.l^{-1}$) to decrease growth of non-filamentous bacteria by substrate inhibition (Morvai *et al*, 1992).

The structure of granules is quite variable. Two broad types were identified, one predominantly *Methanothrix* morphotypes in short fragments, the other predominantly *Methanothrix* morphotypes in long filaments. The first, type one, were denser and showed slightly lower activity than the type two granules, which had a more open spaghetti-like structure that may not be as diffusionally limited. The type two granules still settled well (Hulshoff Pol *et al*, 1982; Dolfing, 1986). The microbiological distribution within granules has been variously reported, ranging from a near homogeneous mixture of acetogens and methanogens, through distributed micro-colonies (Morgan *et al*, 1991), to a layered structure of acetate consuming *Methanothrix* at the centre surrounded by a mixture of hydrogen producing acetogens and hydrogen consuming methanogens (plus NRB and SRB) ,this layer again surrounded by acidogens and hydrogen consuming organisms (Guiot *et al*, 1992). This is further complicated by the variability in non-bacterial matter in the granules; some are predominantly bacteria, others contain various mixtures of extracellular polymers, metal organic and inorganic complexes in amorphous forms and distinct crystals. It is generally postulated

that these materials play some role in the strength and stability of the granules (Shen *et al*, 1992; Macario *et al*, 1991). The precipitates and crystals also contribute to a higher density, and thus a better settling granule.

2.2.5 The anaerobic filter (AF) reactor

This design is also referred to as packed bed or fixed film reactors. An inert support material, in sheet, ring or sphere conformation, is packed randomly or in a modular configuration within a reactor. Feed may be introduced in upflow or downflow mode. The downflow reactors are discussed in Section 2.3.6. While regarded as fixed film reactors a significant portion of the biological activity is due to biomass in suspension, entrapped in the interstities of the media (Hickey *et al*, 1991; Young, 1991). It has been reported that HRT is the most important design and performance parameter (Young, 1991) and that waste strength, reactor height, media surface area and orientation have only minor effects. The advantages of this design are a high rate of treatment for soluble wastes and a low suspended solids effluent. Disadvantages are channelling, poor mixing, clogging by influent solids, loss of reactor volume and high capital costs.

2.2.6 The downflow stationary fixed film (DSFF) reactor

This design, a variant of the anaerobic filter, was developed to solve the channelling and clogging problems noted above. All biomass is attached to relatively large diameter supports through which the wastewater passes downwards. The large flow channels allow influent suspended solids to pass straight through the reactor preventing clogging. Downwards flow and the wide channels prevent large gas bubble evolution from drawing large slugs of untreated wastewater through the reactor (Van den Berg and Lenz, 1979; Van den Berg and Kennedy, 1983).

A wide variety of materials have been tested as support media for the anaerobic filter reactor types. Their performance appears directly related to the ease of

adhesion and/or entrapment of the bacteria. Three major factors can be used to characterise the media: surface roughness, porosity and leaching of trace elements. The relative importance of these factors is determined by the reactor configuration (Hickey *et al*, 1991).

2.2.7 The expanded bed (EB) reactor and the fluidised bed (FB) reactor

These designs are characterised by the growth of the biomass on small inert carrier particles such as sand, activated carbon, anthracite and plastic. The bed of these particles is expanded or fluidised by upflow influent plus extensive recycle. The carrier particle may be chosen to suit the specific waste treated, for example granular activated carbon for removal of inhibitory compounds. A small number of full scale plants are in existence. Advantages are high specific surface area, no clogging, high mass transfer efficiency and high biomass concentration. The major disadvantages appear to be pumping costs and the current first generation technology resulting in many flow distribution and growth control problems. These problems do not appear to be inherent to the system design. Good flow distribution is critical to the success of the design and thus screening to remove large influent solids that may clog the inlet system is needed.

2.2.8 Combined systems

These designs consist of two or more of the above reactor types combined in one reactor or linked sequentially.

2.2.8.1 Two phase reactor systems

These designs are intended to separate the acidogenic and methanogenic phases of anaerobic digestion to allow establishment of near optimum conditions for both phases. The acidogenic reactor is usually small with a low HRT taking advantage of the acidogens fast growth rate and the reactor type may be chosen

to match the influent characteristics. The second reactor is usually at least twice as large. Advantages are optimisation of both phases, lowering of alkalinity addition costs and high rates of treatment. Disadvantages are high capital and control costs and loss of interspecies hydrogen transfer with resultant loss of potential methane generation (van Andel and Breure, 1984; Dinopoulou and Lester, 1989; Ghosh *et al*, 1985 a).

2.2.8.2 Hybrid reactors

These designs incorporate two or more reactor types in one reactor usually to gain the advantages of both types. Most commonly reported are UASB/AF hybrids where the anaerobic filter replaces or combines with the gas-solids separator to entrap suspended solids to improve effluent quality. Another type is the baffled reactor, essentially a series of UASB reactors. These reactor designs, while potentially more complex than the single types, appear to have great promise for specific wastewaters.

2.3 ANAEROBIC DIGESTION

2.3.1 Introduction

Anaerobic digestion is a waste treatment process increasingly utilised for a variety of municipal and industrial wastes and it is based on the fermentation of organic compounds to methane. This fermentation plays a vital role in organic matter recycling in nature in such diverse environments as the rumen, marsh lands, lake sediments, soils and rotting tree stumps (Zeikus, 1977). During this process organic matter is degraded to methane and carbon dioxide by an interlinked series of chemical pathways mediated by direct and indirect symbiotic associations of several groups of bacteria. This network of linked pathways and bacterial groups, and the final end product, methane, are responsible for many of the advantages and disadvantages of the anaerobic digestion process compared to aerobic treatment processes. It was thought that the utilisation of these

anaerobic pathways may allow degradation of components of the TMP wastewaters that were only poorly degraded when the wastewaters were treated aerobically.

Methane retains about 90% of the substrate energy and is easily separated from the waste so it is a useful energy source. Only a small amount of the substrate energy is used by the various bacterial cell groups which must share this energy along the specific pathway utilised. Low cell yields result, causing considerably lower generation of waste sludge and lower nutrient requirements. However this low cell yield is a disadvantage during reactor start-up or recovery from upset when extensive cell growth is desirable. The number of pathways available for anaerobic degradation often means that compounds such as chlorinated organics which are not effectively degraded aerobically, are degraded anaerobically (Lettinga *et al*, 1991; Sierra-Alvarez *et al*, 1993). Other advantages have been reported as:

1) no aeration requirements resulting in low power requirements,
2) generally low space requirements,
3) long dormant periods are possible,
4) very high cell concentrations can be attained depending upon reactor type.

Disadvantages have been reported as;

1) low specific substrate removal rates, although this is usually more than compensated for by the high cell numbers of the high rate digesters,
2) cell retention is critical,
3) generally not a complete treatment process, i.e., a further, usually aerobic, polishing step is often required,
4) sensitivity to some inhibitory compounds.

While toxicity comparisons between aerobic and anaerobic bacteria reveal that this statement is not justified (Blum and Speece, 1991), the impact of toxicity is greater, owing to the slower recovery rates alluded to above.

With the present state of knowledge, the time taken to recover from toxicity of various xenobiotics is the remaining major drawback of the anaerobic process. However much more is now known about the extent and the reasons for the toxicity and the measures necessary to avoid the problem. This, in combination with the wide variety of configurations of reactor available with their range of cost and technology requirements, is resulting in the application of the anaerobic digestion process for treatment of a wide range of effluents.

The objectives of this literature review are to briefly background the methane fermentation and reactor configurations, and to survey the recent developments in anaerobic treatment of forestry industry wastewaters, with an emphasis upon thermomechanical pulping effluents.

2.3.2 Microbiology and biochemistry

The microbiology and biochemistry of anaerobic microbial processes are much more complicated than for aerobic processes. This is a result of the many interdependent, consecutive and parallel pathways available to the anaerobic community for substrate utilization. In the absence of sulphate, complete anaerobic degradation of organic matter to methane and carbon dioxide requires the coordinated involvement of four trophic groups:

1) Hydrolytic- fermentative organisms that hydrolyze and ferment complex organic matter to simple organic compounds (such as volatile fatty acids and ethanol), hydrogen and carbon dioxide;
2) syntrophic acetogenic organisms that, in combination with hydrogen utilising methanogens, convert the C3 or greater metabolic products of the first group mainly into acetate and hydrogen (or formate); and

3) hydrogen-utilising acetogenic bacteria which convert hydrogen and carbon dioxide to acetate
4) methanogenic bacteria which convert acetate, or hydrogen/carbon dioxide and formate to methane (and carbon dioxide in the case of acetate degradation).

In the presence of sulphate and/or nitrogen, the sulphate-reducing bacteria (SRB) and the nitrogen-reducing bacteria (NRB) can partially or completely mineralize the fermentation products to hydrogen sulphide and/or ammonia and carbon dioxide. The functions and interrelationships of the above groups of organisms are illustrated in Figure 2.2.

Nine recognisable steps, each mediated by a specific group of bacteria, can be identified, including:

1) hydrolysis of organic polymers to organic monomers such as sugars, organic acids and amino acids;
2) conversion of organic monomers to hydrogen, bicarbonate, acetic propionic and butyric acids and other products such as ethanol and lactic acid;
3) oxidation of reduced organic products to hydrogen, bicarbonate and acetic acid by obligate hydrogen producing acetogens (OHPA);
4) acetogenic respiration of bicarbonate by homoacetogens;
5) oxidation of reduced organic products to bicarbonate and acetate by SRB and NRB;
6) oxidation of acetate to bicarbonate by SRB and NRB;
7) oxidation of hydrogen by SRB and NRB;
8) aceticlastic methane fermentation; and
9) methanogenic respiration of bicarbonate.

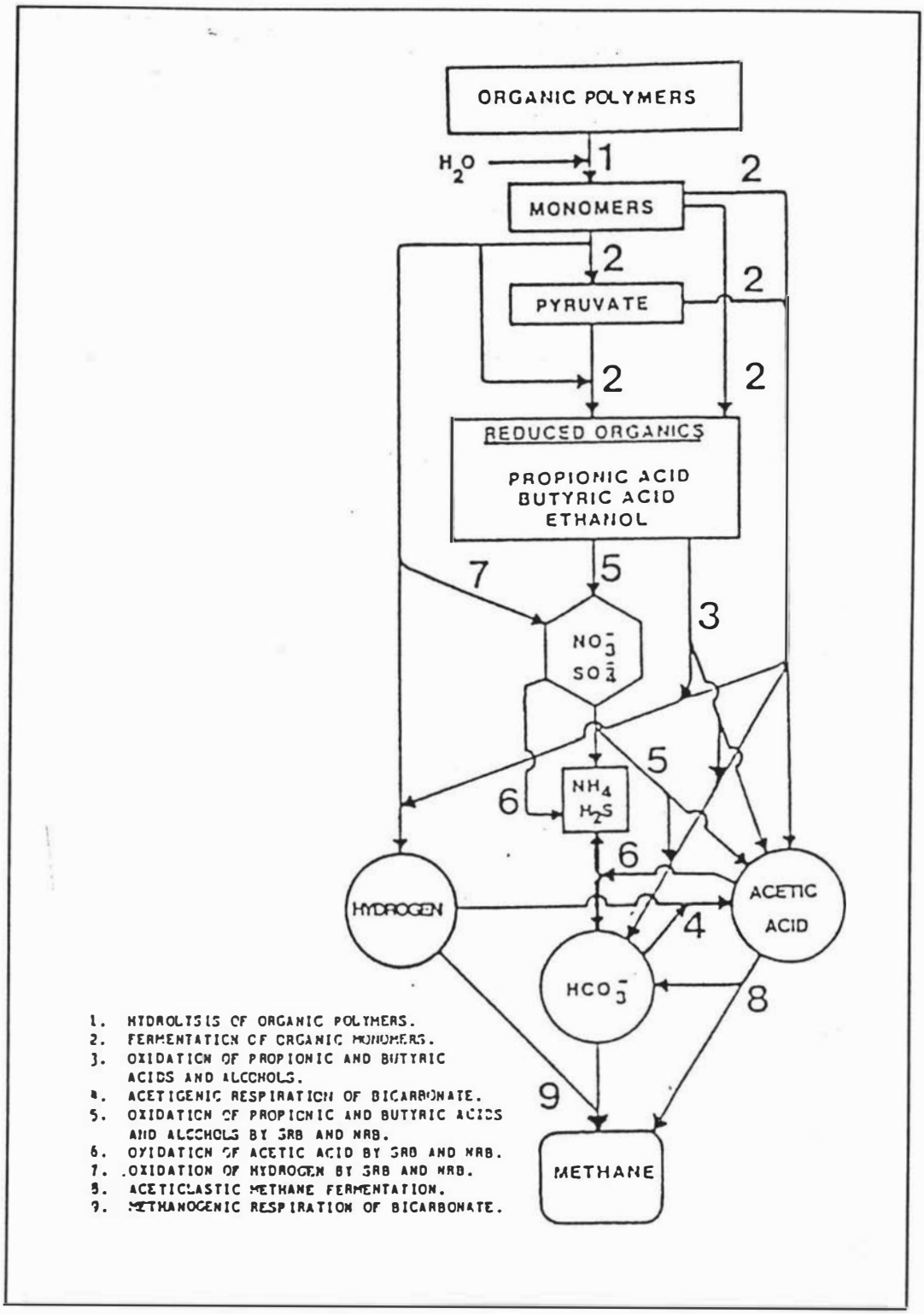

Figure 2.2: Schematic representation of the substrate-linked redox processes operative during the microbially mediated conversion of organic wastes to methane (from Harper and Pohland, 1986)

The populations of the various bacteria are dependent upon the composition of the influent, the conditions of operation (such as temperature and pH) and the optimal conditions for growth for the bacteria themselves. Changes in any of these result in the formation of a new mix of species (Henze and Harremoes, 1983; Speece, 1983). This adaptation process may be slow and complex, but providing species from all the metabolic groups are able to grow a stable methanogenic consortia will result. The species mix may be very different but the trophic functions and interrelationships will be intact (Zeikus, 1977; Hattingh *et al*, 1967). Many of the wastes treated by anaerobic digestion are time variable and the reactor population can be in a constant state of flux. This makes characterisation of the microbiology and biochemistry of the process difficult.

The hydrolytic and fermentative organisms are a diverse group whose mix is predominantly determined by the influent composition. In-flow of organisms in the waste stream can also affect this species balance. Hydrolysis is a rather slow process brought about by extracellular enzymes. The hydrolysis of non-polar lipids and cellulose has been reported as rate limiting (Henze and Harremoes, 1983; Tsao, 1984; Brummeler and Koster, 1990). The hydrolysis of cellulose is affected by the degree of its association with lignin. Lignin has been reported as essentially nondegradable under anaerobic conditions, although low molecular weight lignin derived molecules are reported as being degraded (Colberg and Young, 1982; and 1985; Kalra and Panwar, 1986; Odier and Monties, 1983).

The fermentation of organic monomers has been widely reported as being regulated by the hydrogen partial pressure (Harper and Pohland, 1986; Speece, 1983). An explanation has been proposed (Mosey, 1983 and others), as can be seen in Figure 2.3 for the catabolism of glucose. Hydrogen appears to regulate the acidogenic reactions at several points in the glycolytic pathway. In order for catabolism to proceed the NADH produced must be regenerated to NAD. This is accomplished by the reduction of protons to hydrogen gas which is removed via interspecies hydrogen transfer to the hydrogenotrophs (methanogens, SRB and NRB). When hydrogen production exceeds the capacity of these

hydrogenotrophs to utilise it, an alternative method of NAD regeneration is needed. This results in the fermentation of pyruvate to propionate, lactate and ethanol and/or the fermentation of acetyl-CoA to butyric acid. These products are not assimilated directly by the methanogens (Henze and Harremoes, 1983) and they tend to accumulate until the hydrogen partial pressure is lowered.

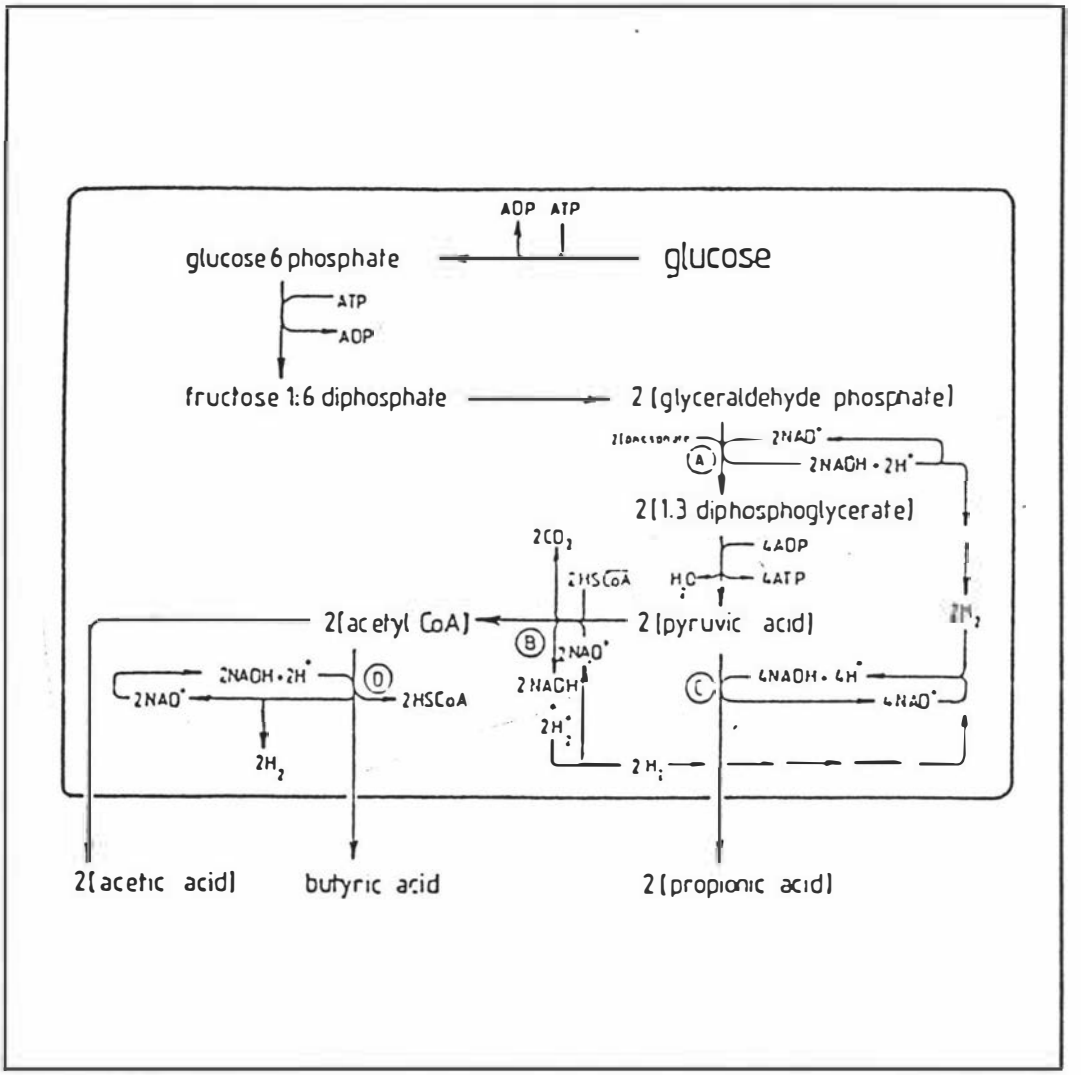

Figure 2.3: Hydrogen-regulated catabolic pathways possible for the conversion of glucose in anaerobic wastewater treatment systems (after Mosey, 1983)

The hydrogen-producing acetogens require a very low partial pressure of hydrogen to grow and can only be cultured in association with a hydrogen-

utilising bacteria. An explanation for this arises from consideration of the proposed catabolic reactions shown in Table 2.1. For both propionic and butyric 8acids the catabolic reactions are endogenic and only become exogonic when combined with hydrogen removal by the methanogens or other reactions which are sufficiently exogonic such as those performed by the hydrogen utilising SRB or NRB (under standard conditions). Figure 2.4 shows the niche occupied by the methanogenic bacteria.(Harper and Pohland,1986).

Table 2.1: Stoichiometry and change in free energy values for propionate and butyrate degradation reactions (after Harper and Pohland, 1986).

Oxidations (electron donating reactions)

1) Propionate → Acetate: $\Delta G'_o = +76.1$ kJ

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$$

2) Butyrate → Acetate: $\Delta G'_o = +48.1$ kJ

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$$

3) Acetate → Methane: $\Delta G'_o = -31.0$ kJ

$$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$$

Respirative (electron accepting reactions)

4) Hydrogen → Methane: $\Delta G'_o = -135.6$ kJ

$$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + H_2O$$

5) Hydrogen → Acetate: $\Delta G'_o = -104.6$ kJ

$$HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + H_2O$$

6) 1 + 4: $\Delta G'_o = -102.4$ kJ

$$4CH_3CH_2COO^- + 3H_2O \rightarrow 4CH_3COO^- + HCO_3^- + H^+ + 3CH_4$$

7) 2 + 4: $\Delta G'_o = -39.4$ kJ

$$2CH_3CH_2CH_2COO^- + HCO_3^- + 3H_2O \rightarrow 4CH_3COO^- + CH_4 + H^+$$

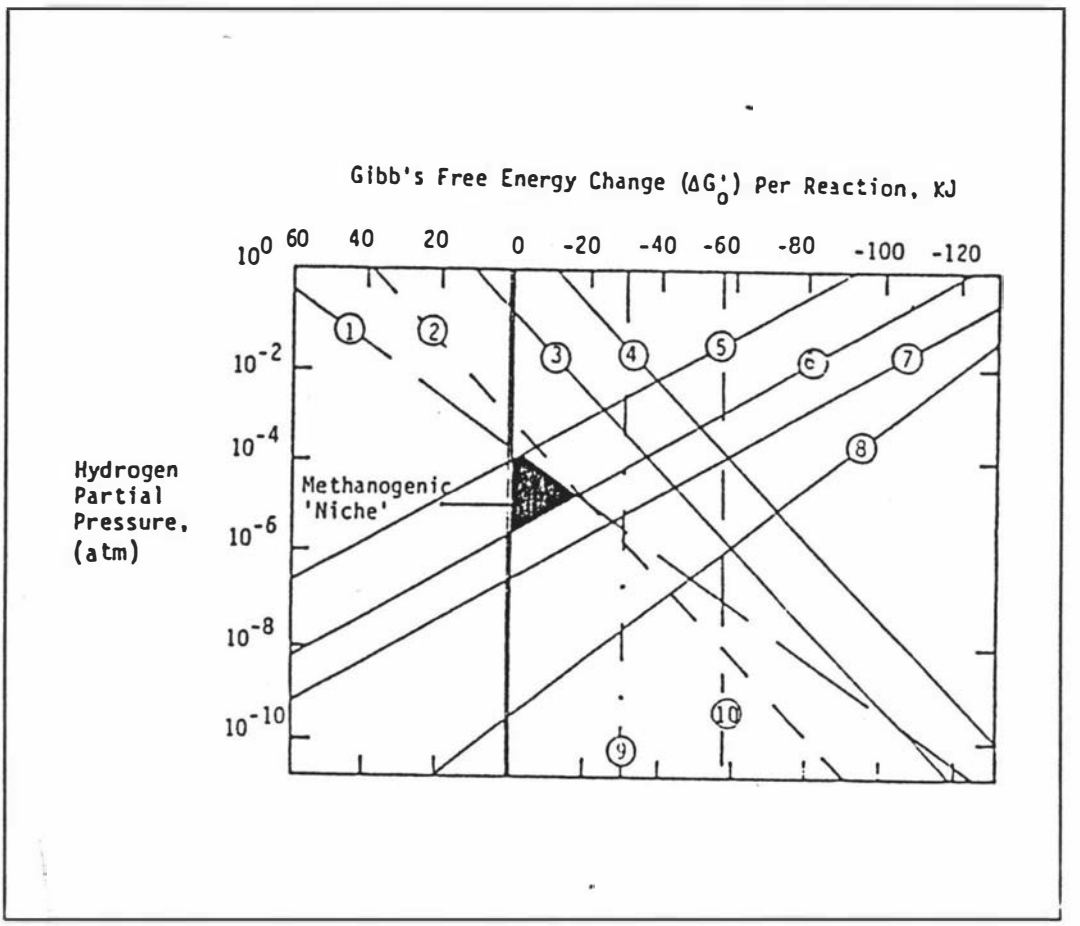

Figure 2.4 Graphical representation of the hydrogen-dependent thermodynamic favorability of acetogenic oxidations and inorganic respirations associated with the anaerobic degradation of waste organics. (1)Propionic acid oxidation to acetic acid. (2) Butyric acid oxidation to acetic acid. (3) Ethanol to acetic acid. (4) Lactic acid to acetic acid. (5) Acetogenic respiration of bicarbonate (CO_2). (6) Methanogenic respiration of bicarbonate. (7) Respiration of sulphate to sulphide. (8) Respiration of sulphite to sulphide. (9) Methanogenic cleavage of acetic acid. (10) SRB-mediated cleavage of acetic acid. Acetic acid, 25 mM; propionic, butyric, lactic acids, and ethanol, 10 mM; sulphate and sulphite, 5 mM; bicarbonate, 20 mM; methane, 0.7 atm. (Harper and Pohland 1986).

The methanogenic bacteria occupy a vital role in nature allowing the degradation and recycle of organic matter that would otherwise accumulate (Zeikus, 1977; Bryant, 1979). This is due to their ability to degrade some substances in the absence of light and exogenous electron acceptors such as oxygen, nitrate and sulphate (Bryant, 1979). The substrates utilised by the methanogens are very limited. Acetate, hydrogen, carbon dioxide, methanol, various methylamines and carbon monoxide have all been reported to be degraded under certain conditions (Mah, 1981; Bryant, 1979). Most of the identified species can reduce carbon dioxide, which is vital in maintaining the low hydrogen partial pressures required by the acidogenic bacteria (Mah, 1981) but very few are capable of utilising acetate as the sole source of carbon and energy. *Methanothrix soehngenii* has been reported as only utilising acetate (Huser *et al*, 1982). It is commonly found as a major component of granular sludge and it has been suggested that a low Ks value gives an advantage in situations of low acetate concentrations, as is found under diffusional limitation (Morvai *et al*, 1992).

2.3.4 Environmental factors

The anaerobic digestion process involves the interaction of many species of bacteria in a complex ecosystem. Bacterial growth is slow for many of the species and optimal conditions for each are often different. It is therefore important that environmental conditions providing overall optimum performance are established and maintained. The slow growth rate means that recovery from upset is often a slow process.

Two temperature optima have been observed for anaerobic digestion, the mesophilic range of 30-40 ^{o}C and the thermophilic of 55-65 ^{o}C. Within these ranges the typical mixed methanogenic consortia populations will vary biochemically via reaction rate and growth rate effecting the species mix present. Comparisons of mesophilic and thermophilic operation have been reported with mixed conclusions. Treatment of cellulosic wastewaters, especially those of the forestry industry, seems generally to be more effective at mesohilic temperatures

(Ghosh *et al*, 1985b; Liao and Lo, 1985a; Puhakka *et al*, 1988; Stephenson *et al*, 1993).

pH and alkalinity have proved important in the anaerobic digestion process. Sufficient alkalinity is needed to buffer accumulations of acids from the influent or from VFA generation. pH optima have for *Methanothrix* been reported as 7.4 - 7.8 with an operational range of 6.8 to 8.2 (Huser *et al*, 1982). This organism is thought to be predominant in many reactors and this may explain the observed pH behaviour of some mixed cultures. The pH inside granules has been reported as higher than that of the bulk liquid. This may contribute to the predominance of the bacteria inside granules. The alkalinity of wastewaters needs to be assessed to establish whether addition of alkalinity is necessary. Generally wastewaters low in nitrogenous material, such as forestry wastewaters require alkalinity addition which may become a significant cost.

Nutritional requirement of many elements have been demonstrated for the methanogenic consortia (Goodwin *et al*, 1990a; Kida *et al*, 1991a). Many of these elements, especially the various metal cations also exhibit toxic effects at higher concentrations. This is further complicated by antagonistic and synergistic interactions between the various cations (Kugelman and Chin, 1971). Availability of these elements is also altered by complexing and chelation interactions (Kragten, 1978; Speece, 1983).

Toxicity of a wide variety of compounds originating from the feed or as a result of degradation is a common problem. However acclimation of the anaerobic sludges is often possible (McFarlane and Tan, 1985; Speece, 1983; Parker *et al*, 1992). Thus, providing allowance for the acclimation period is made, successful treatment of toxic wastewater is possible.

The toxicity of VFAs has been the topic of some debate but successful two-phase systems prove that high feed VFA concentrations (up to at least 8000 $mg.l^{-1}$)

need not be inhibitory provided sufficient alkalinity is present to maintain optimal pH levels.

2.4 CONCLUDING REMARKS

The anaerobic digestion literature in recent times shows a vastly increased knowledge over the great range of interrelated aspects of this subject. At least in part this is due to the synergistic effect of combining basic microbiology, biochemistry, lab, pilot plant and full-scale installation studies. Anaerobic digestion in the forestry industry is showing an even more rapid growth as the controllability of the process is proved and improved. The economic benefits of its use ensure its continued application in this high water use industry.

The forestry industry wastewaters exhibit an extremely high variability both as a result of varying operating conditions from mill to mill and from the wide variety of wood (and other materials) pulped. In New Zealand *Pinus radiata* represents greater than 90% of the pulp wood stocks. Elsewhere this species is of no economic importance and pulp wood stocks are a much greater mix of wood types and species. Personal experience at the Pan Pacific Forest Industries TMP mill strongly suggests that the characteristics of the pulp are directly related to the species mix and tree age of wood being pulped. It seems likely that the composition of the wastewater may also be effected by these variables. In that case overseas data on the anaerobic digestion of TMP wastewaters may not be particularly applicable to the New Zealand situation. This project was initiated (in 1986) to examine the anaerobic degradation of *Pinus radiata* based TMP wastewater and to investigate the potential for removal of the fine suspended solids prevalent in this wastewater within the reactor.

CHAPTER THREE MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 General chemicals

Industrial grade urea and sodium bicarbonate were obtained from the National Dairy Association (Palmerston North, New Zealand). Food grade acid calcium mono-phosphate was obtained from the Soil Science Department, Massey University, (Palmerston North, New Zealand). All other chemicals were obtained from B.D.H. Chemicals N.Z. Ltd (Palmerston North, New Zealand) or May and Baker N.Z. Ltd (Lower Hutt, New Zealand) and were of analytical reagent (AR) grade unless otherwise specified.

3.1.2 Gases

Bottles of methane, dry nitrogen and dry air of 99% purity and helium of 99.995% purity were obtained from New Zealand Industrial Gases Ltd.(Palmerston North, New Zealand).

3.1.3 Chromatography materials

10% w/v Free Fatty Acid Phase (FFAP) on acid washed Chromasorb W (80 - 100 mesh) was obtained from Alltech Associates Inc. (Deerfield, Illinois, U.S.A.). Poropak S (80-100 mesh) was obtained from Waters Associate Inc. (Milford, Massachusetts, U.S.A.). A 30 m, 0.25 mm ID SP2330 fused silica capillary column was obtained from Supelco Inc. (Bellefonte, Pennsylvania, U.S.A.).

3.2

3.1.4 Glassware

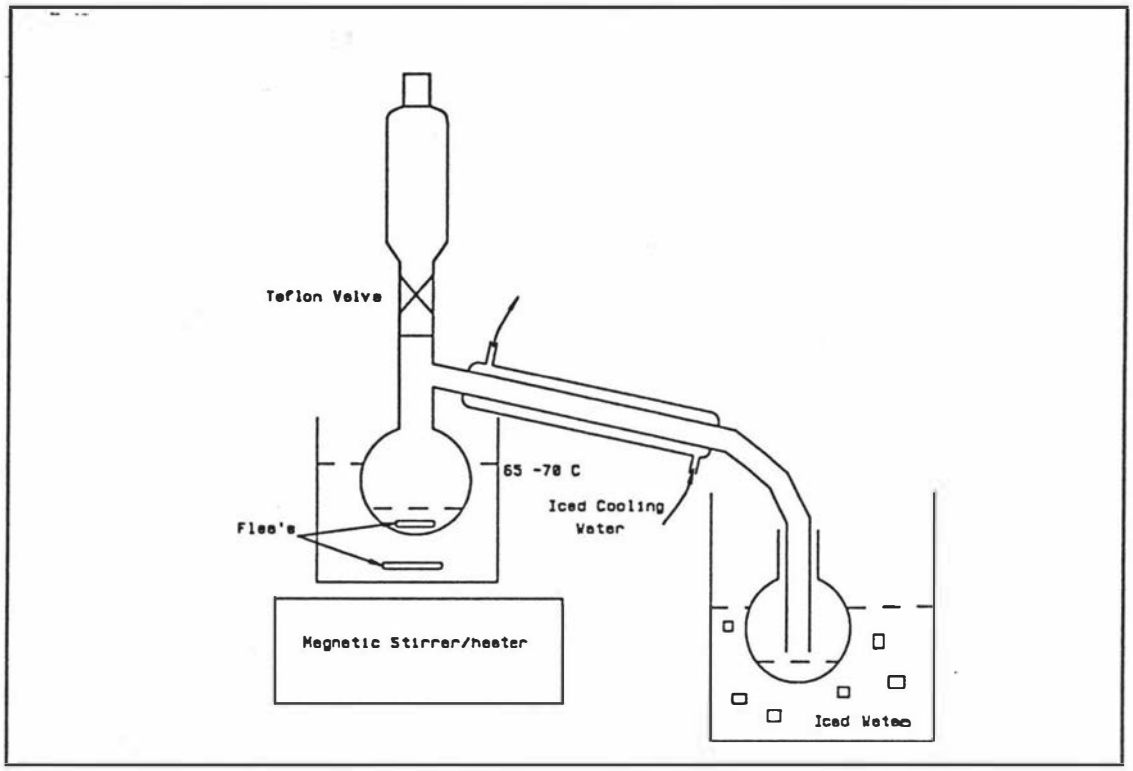

Figure 3.1 Diazomethane Generator.

A diazomethane generator, adapted from a design from the Forest Research Institute (F.R.I.) (Rotorua, N.Z.), was constructed by the Massey University Glassblower (Palmerston North, New Zealand). It is shown in Figure 3.1. All other glassware used was standard laboratory equipment. Glassware was soaked in hot 10% aqueous caustic soda to remove pine resins, if necessary, then washed in hot water containing "Pyroneg" (Diversey-Wallace Ltd, Auckland, N.Z.), or "Dri-decon" (Decon Laboratories Ltd, Hove, Sussex, U.K.), then rinsed three times in distilled or reverse osmosis water and air dried.

3.1.5 Feed

Three types of feed were used during the course of this study. All the feed stocks were industrial effluents and as such of variable composition.

3.1.5.1 Lactic acid casein whey ultrafiltration permeate

This was a high-strength completely soluble waste, here after referred to as lactic whey permeate, modified with the addition of nitrogen in the form of urea, phosphate in the form of acid calcium mono-phosphate and sodium bicarbonate for buffering capability. Two batches were used of averaged strength 44,000 mg.ℓ^{-1} COD and 50,000 mg.ℓ^{-1} COD, respectively. The lactic whey permeate was collected from the New Zealand Dairy Research Institute (NZDRI) in 20 ℓ containers and frozen to -18 °C for storage. Batches of three containers were thawed overnight, poured together into a stirred vessel and mixed until freeze-precipitated compounds had dissolved. The lactic whey permeate was then stored at 4 °C until required and was always used within one week of thawing. For each days use, sufficient lactic whey permeate was diluted to the required concentration level and urea, calcium phosphate and sodium bicarbonate added to the desired quantities. A typical composition of lactic casein whey permeate can be seen in Table 3.1 (anonymous, 1985).

3.1.5.2 Whole mill effluent

Whole mill effluent was collected from a thermo-mechanical pulp mill (Pan-Pacific Forest Industries Pulp Mill, Hawkes Bay, New Zealand). It consisted of all mill wastewaters post 1 mm contra-shear sieves and prior to ocean outfall discharge. The inclusion of wash waters resulted in a dilute wastewater compared to the screw press effluent. Table 3.2 gives a typical analysis.

3.4

Table 3.1 A typical composition of lactic casein whey permeate

Parameter	Concentration (mg/ℓ^{-1})
total COD	48,000
dissolved COD	47,500
BOD_5	34,000
total solids	50,000
lactose	40,000
ash	7,000
phosphate	1,800
nitrogen	500
calcium	1,200
pH	4.6

Table 3.2 A typical analysis of the whole mill effluent from the Pan-Pacific Forest Industries thermo-mechanical pulp mill.

parameter	Concentration ($mg.\ell^{-1}$)
COD	5,800
dCOD	3,200
Total solids	5,800
Dissolved solids	2,300
Suspended solids	3,400
Volatile suspended solids	3,200
pH	6.0

Whole mill effluent was only used for about one month and consisted of one batch. It was then replaced with screw press effluent.

3.1.5.3 Screw press effluent

This effluent is more typical of that which would be treated should a secondary treatment system be installed. It was collected from the screw press feeders of the thermo-mechanical pulping refiners at the Pan-Pacific Forest Industries pulp mill. This waste stream was diverted into a shallow concrete drain that had been cleaned. It was then pumped with a submersible pump to 205 ℓ plastic lined steel drums, driven to Palmerston North and cooled over night. Twelve batches of two to three cubic metres each were collected.

It was found that the composition of the screw press effluent varied from drum to drum by up to $\mp$ 40 %. This variation was largely associated with the suspended solids concentration and linked parameters such as total COD and resin acid composition. Variation in dissolved solids parameters was less than $\mp$ 10 %. To reduce the observed variation between drums progressive blending of the screw press effluent in an 800 ℓ tank was utilised. This reduced the observed variation between drums to less than $\mp$ 10 %. A settling period of 40 to 50 minutes before each drum was refilled from the blending tank allowed the settling out of larger wood fragments. The drums of screw press effluent were stored under refrigeration at 2 $\mp$ 1 °C. Approximately 10 % of each batch was discarded as the settled solids fraction. Removal of the larger, more readily settling solids made obtaining a uniform feed composition from each drum much easier. Vigorous stirring before and during removal of each days feed quantity proved sufficient to provide a relatively constant feed composition. Prior to use the effluent was filtered through a 1 mm screen.

A similar degree of variation in effluent composition was also found between the individual batches collected from the pulp mill. The range of composition of the screw press effluent is shown in Table 3.3.

Table 3.3 The composition of thermo-mechanical pulp mill screw press effluent used for this work and that of one specific batch.

parameter	Concentration range[a] ($mg.\ell^{-1}$)	Concentration of batch 10 ($mg.\ell^{-1}$)
COD	10,500 - 18,000	12,100
dCOD	6,000 - 11,000	7,400
Total solids	6,000 - 11,000	8,300
Dissolved solids	2,500 - 7,500	5,800
Suspended solids	2,000 - 4,000	2,500
Volatile suspended solids	1,800 - 3,800	2,100
Total resin acids	120 - 1,000	600
Nitrogen	50 - 150	n.d.
Phosphorus	10 - 25	10
pH	4.0 - 6.0	4.6

n.d - not determined

a - analyses performed after blending, settling of large suspended solids and filtration through a 1 mm screen.

The feed was supplemented with urea, acid calcium mono-phosphate and sodium bicarbonate at usual concentrations of 1.0 - 1.5 $g.\ell^{-1}$, 0.1 - 1.5 and 1.2 - 2.0 $g.\ell^{-1}$ respectively. The concentration added was varied with respect to the COD of the feed to give a COD:N:P ratio of approximately 100:5:1. Supplementation at higher levels showed no evidence of improved methane production or volatile fatty acid (VFA) removal.

3.2 ANALYTICAL PROCEDURES

3.2.1 pH value

The pH of digester samples was measured using an E.I.L. model 7055 pH Meter (Electronic Instruments Ltd, Chertsey, Surrey, England) equipped with manual temperature compensation and an E.I.L. series 1160 combination pH electrode. The electrode was calibrated twice weekly with standard commercial buffer solutions of pH 4.0 and pH 7.0 and pH values were recorded to the nearest 0.05 unit.

3.2.2 Alkalinity

Alkalinity of 10 ml feed or digester effluent samples was determined by Potentiometric titration to pH 5.75 and pH 4.3 with 0.05 N hydrochloric acid. The two end points allowed the calculation of the ratio of intermediate alkalinity to partial alkalinity (IA/PA) following the method of Ripley *et al.* (1985). This ratio represents the ratio of volatile fatty acid alkalinity to bicarbonate alkalinity.

3.2.3 Volatile fatty acids (VFA)

Volatile fatty acids were determined following the chromatographic procedure of Banfield *et al.* (1978). A Shimadzu GC-8A gas chromatograph (Shimadzu Seisakusho Ltd, Kyoto, Japan) fitted with a flame ionisation detector was used with a 3 m x 4 mm i.d. glass column packed with 10% w/v FFAP on Chromasorb W AW. The column temperature was 135 °C and the injector port temperature was 162 °C. The carrier gas was nitrogen at 1.8 $kg.cm^2$ head pressure. The air and hydrogen were supplied at 0.5 $kg.cm^2$ and 0.6 $kg.cm^2$ respectively. Digester samples were acidified to pH 1.5 with concentrated hydrochloric acid and centrifuged at 2700 x g for at least 10 minutes. The supernatant liquor was decanted off. The sample volume of 1 µl was drawn from this solution and delivered using a Hamilton model 1801rn syringe (Hamilton Company, Reno, Nevada, U.S.A.).

3.8

A Shimadzu C-R6A chromatopac integrator (Shimadzu Corporation, Tokyo, Japan) was used to evaluate the peak areas. A calibration curve was established from replicate determinations of a blank standard containing acidified distilled or reverse osmosis water and two standard acid solutions the composition of which is shown in Table 3.4. The stock solutions of these standards were prepared by accurately weighing and dispensing appropriate volumes of the individual AR grade acids. The stock solutions were stored at 4 °C. The calibration samples were drawn from these stock solutions and stored at 4 °C when not in use.

Table 3.4 Composition of volatile fatty acid standard solutions.

Acid	Concentration	
	Low standard ($mg.\ell^{-1}$)	High standard ($mg.\ell^{-1}$)
Acetic	250	1250
Propionic	325	1600
i-Butyric	70	400
n-Butyric	233	400
i-Valeric	70	400
n-Valeric	130	400

3.2.4 Chemical oxygen demand (COD)

The chemical oxygen demand of samples was determined using a micro-technique adapted from that of Jirka and Carter, (1975). Total COD of influent and effluent samples were measured. The dissolved COD of samples was estimated by determination of the COD of supernatant liquor from samples centrifuged at 2700 g for 10 minutes and filtered through Whatman glass fibre filter paper grade GF/C (Whatman Ltd, Maidstone, Kent, England) The COD of suspended solids (SS) was determined from the difference of the total and soluble COD measurements.

The method follows the Standard Methods procedure (APHA-AWWA-WPCF, 1975; pg. 550); reagents and reagent concentrations remain the same but sample and reagent volumes are decreased by a factor of twenty five. One ml of potassium dichromate solution and three ml of sulphuric acid reagent were added to two ml of sample in a 16 mm x 100 mm screw-capped culture vial (Hach Chemical Co., Ames, Iowa, U.S.A.). The solution was refluxed at 150 ± 10 °C for two hours. Then the absorbance of the mixture was determined colorimetrically at 600 nm using a Cecil model Ce 272 spectrophotometer (Cecil Instruments Ltd, Milton, Cambridge, England). Silver sulphate was added to the acid to ensure effective oxidation of straight-chain acids and excess mercuric sulphate was also added to the samples to precipitate any chlorine present (APHA-AWWA-WPCF, 1975; pg. 551).

Duplicate or greater analyses were performed on all samples. Distilled water blanks and 500 mg.ℓ^{-1} COD standards were included in each batch of analyses. The average blank reading was subtracted from the sample values and the COD determined by calculation in relation to the 0 and 500 mg.ℓ^{-1} COD standards. A calibration curve using five potassium hydrogen phthalate standard solutions also following the method of Jirka and Carter (1975) established that the curve was linear in the range 0 - 750 mg.ℓ^{-1} COD. Samples were diluted to bring the reading within this range.

3.2.5 Solids composition

Samples were analyzed for the following:

(1) total solids at 104 ± 1 °C,
(2) volatile and fixed residue of total solids at 550 °C,
(3) filterable solids at 104 ± 1 °C
(4) volatile and fixed residue of filterable solids at 550 °C.

The first two tests were performed following Standard Methods procedure (APHA-AWWA-WPCF, 1975; pp. 91, 95 -97) using 10 to 30 g sample weights. The nature of many of the samples meant that volumetric transfer was unreliable and so all samples are given as weight/weight rather than weight/volume. Modified Standard

Methods tests were performed for the other two tests as the samples could not be filtered easily. Samples were centrifuged at 2700 g for at least 10 minutes. The supernatant liquor was filtered through glass-fibre filter paper (GF/C, Whatman Ltd, Maidstone, Kent, England.). The treated samples were dried at 104 ± 1 °C for at least 16 hours to determine filterable solids and then ignited at 560 ± 10 °C for two hours to determine volatile and fixed residue solids composition. Suspended solids (non-filterable residue) and volatile suspended solids were determined by the difference of total and filterable solids.

3.2.6 Gas composition

Gas produced by the digesters was routinely analyzed by gas chromatography for methane content. Hydrogen could also be determined but was only found in a few batch assay tests.

A Varian Aerograph model 920 gas chromatograph was used. This was fitted with a thermal conductivity detector and connected to a Sekonic flat bed recorder. A 1.5 m x 6 mm ID. stainless steel column, packed with Porapak type S, was operated at 110 °C. The injector port temperature was 138 °C, the detector body temperature was 125 °C and the katharometer bridge current 130 mA. The carrier gas flowrate was 45 $m\ell.min^{-1}$ with a reference gas flowrate of 6 $m\ell.min^{-1}$. A sample volume of 100 $\mu\ell$ was delivered by a gas-tight syringe (Hamilton Company, Reno, Nevada, U.S.A.).

Calibration curves for methane and hydrogen were established using peak heights obtained by injecting varying amounts of the pure gases. Sample injections were alternated with pure gas injections until matching heights were achieved usually in triplicate.

3.2.7 Resin acids

Resin acids were determined following the chromatographic method of the Forestry Research Institute (Rotorua, New Zealand). A Carlo Erba GC 6130 Vega series 2 (Carlo Erba Strumentazione, Milano, Italy.) fitted with a flame ionisation detector operated at 250 °C was used with a 30 m x 0.25 mm ID. fused silica SP-2330 capillary column (Supelco Inc, Bellefonte, Pennsylvania, U.S.A.). A Carlo Erba AS 550 auto-sampler was used to inject 1 µℓ into a split/splitless (ssl) injector port operated at 250 °C. The column oven was temperature programmed as follows: Start at 35 °C rising to 220 °C at 4 °C/min., hold at 220 °C for 16 minutes then cool to 35 °C.

The carrier gas was helium at 80 kPa, hydrogen and air at 95 kPa and 80 kPa respectively and nitrogen as make up gas at 100 kPa. Samples were acidified to pH 1.5, freeze-dried on a "VirTis" laboratory freeze drier (The "VirTis" Company, Gardiner, New York, U.S.A.) and an internal standard of arachidic acid added. Samples were then extracted with distilled dichloromethane, methylated with diazomethane and made up to an appropriate concentration with dichloromethane ready for injection.

The computer based integration package DAPA version 4.64 (Dapa Scientific Pty. Ltd, Kalamunda, Western Australia) was used to evaluate the peak areas. Peak identification and relative response factors were by mass spectrometry at Forest Research Institute (Rotorua, N.Z.) and Hort Research (Palmerston North, N.Z.).

3.3 SERUM BOTTLE ASSAYS

3.3.1 Equipment and instrumentation

Serum bottles of 70 mℓ nominal volume (actual volume 69.0 ± 0.5 mℓ) stoppered with butyl rubber closures were used in a modification of the assay technique of Owen *et al.* (1979). The liquid volume was 40 ml and inoculum volume

approximately 1 ml. Experiment one was incubated in a temperature controlled room and all others in an incubator. Incubation was at 35 ± 1 °C in the dark and stationary. All bottles were thoroughly mixed by hand before and after gas sampling and occasionally between sampling. Gas production was measured by displacement of a 25 ml glass syringe. The barrel was lubricated with distilled water and the needle bent to 90° to allow the gas volume to be read at atmospheric pressure (Owen *et al.* 1979). The measured gas was discarded rather than re-injected to avoid losses in the dead space of the syringe. Tests with injected methane and nitrogen gases showed recoveries of at least 95 % of the injected gases at STP using this method. Methane content of the gas was measured following the method described in Section 3.2.6. The gas was sampled from the bottle immediately after the gas was discarded so that the gas pressure was near atmospheric.

3.3.2 Operating conditions

Granules or sludge from reactors C and D were used as the inocula for these experiments. Details of the experimental program are described in Chapters Five and Six.

3.4 SCANNING ELECTRON MICROSCOPE SAMPLE PREPARATION

Granules were removed from reactor D and kept under reactor liquor and refrigerated until processing was commenced. Processing was as follows: The granules were fractured fresh from the reactor liquor to provide the surfaces and particle size desired for the scanning electron microscope (SEM) work. The primary fix was 3% glutaraldehyde, 2% formaldehyde in 0.1M phosphate buffer at pH 7.2 left overnight. Samples were washed three times in the same buffer as above. An acetone dehydration series consisting of 25%, 50%, 75% and 95% v/v acetone in water for 15 to 20 minutes each and 2 x 100% for 90 minutes each was used. Samples were then critical point dried using liquid carbon dioxide. The dried samples were glued using conductive silver paint to an aluminium SEM stub and sputter coated with gold. Layer thickness was 20 - 40 nm. A Cambridge 250 Mk III scanning electron

microscope was used to study and photograph the samples. The protocol was recommended and carried out by the staff of the Electron Microscope Unit of the Batchelar Research Centre (Hort Research, Palmerston North, New Zealand). Some of the funding for this unit was provided by the New Zealand Lottery Grants Board.

3.5 CONTINUOUS DIGESTION EXPERIMENTS

3.5.1 Reactor B. An upflow anaerobic sludge blanket reactor

This reactor was constructed from "QVF" glassware (Quickfit Visible Flow process plant and pipeline, Corning Process Systems, Corning Ltd, Stone, Staffordshire, England). The apparatus is illustrated in Figure 3.2. The working volume, including sidearm and tubing was 20.4 ℓ. No gas solids separator was installed because it was thought that a high degree of solids retention would increase the wood /micro-organism ratio in the bed to a detrimental level. The expanded diameter at the top of the reactor allowed a decrease in superficial velocity to aid solids settling. Rising gas bubbles from the column generated a liquor circulation in this chamber such that the flow at the walls and overflow point was downwards. This enhanced solids separation to some extent. It was expected that this design would provide sufficient retention of methanogenic biomass for effective reactor operation. Initial reactor operation confirmed that the biomass retention was sufficient. Reactor liquor overflowed into a side arm from which the reactor recycle was withdrawn. A solids wasting point was provided at the bottom of the side arm. The reactor effluent was withdrawn from the side arm by overflowing through an inverted siphon to provide a gas seal.

Gas was removed from the top of the reactor through thick walled butyl rubber tubing to a condensate trap and a wet work experimental gas meter (Brand catalogue number 2505.62, Rudolf Brand GMBH + CO, Wertheim-Glashüte, West Germany). Bubbling the gas through the condensate trap gave a visual confirmation of gas evolution and provided a small positive pressure in the reactor.

Samples for pH and volatile fatty acid analysis were removed via a junction in the recycle line. This was to avoid changes in pH due to carbon dioxide evolution and changes in volatile fatty acids with time in the effluent bucket. Feed and effluent samples were taken as representative samples of the mixed feed or accumulated effluent. These were usually 24 hour samples. Sludge samples were taken by insertion of a semi-rigid tube through the top of the reactor.

Reactor B was installed in a constant temperature room at 35 ± 2 °C. Any particular point in the room varied by ± 1 °C but there was some temperature stratification. Thus the air temperature next to the reactor from bottom to top increased by about 2 °C. The condition of the cork and plaster ceiling prevented the use of a ceiling fan to give a more uniform room temperature. Smaller fans about the room did reduce the amount of stratification. Recycling of the reactor liquor ameliorated the temperature range experienced within the reactor.

Some evidence that the sludge bed of reactor B was poorly mixed was observed. A triangular wire frame stirrer as shown in place in Figure 3.2 was installed. This was driven at one r.p.m. by a Masterflex fixed speed pump drive (1 r.p.m., model 7544-01) for 15 minutes every 2 hours. The stirring action resulted in increased reactor inlet blockages and no improvement in reactor performance was noted. Use of the stirrer was discontinued but it was left in place.

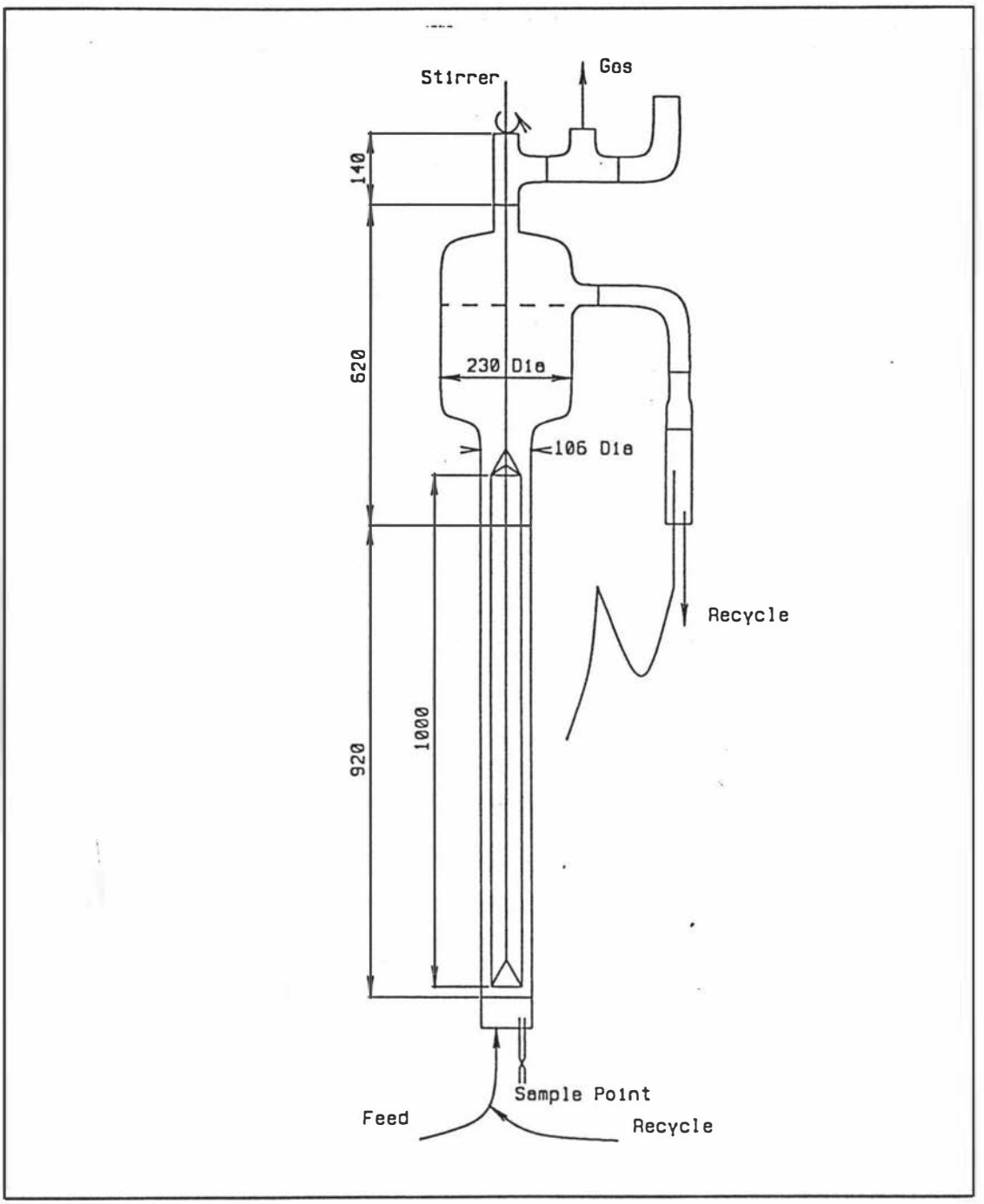

Figure 3.2 Reactor B

3.5.1.1 Feed and recycle provision.

Feed and recycle were pumped to an inlet at the bottom of the reactor using Masterflex peristaltic pumps incorporating variable-speed drives (model 7546-00) fitted with interchangeable pump heads and silicone rubber tubing (Cole-Palmer International, Chicago, Illinois, U.S.A.). To reduce blockage problems pump tubing of 16 series (3.1 mm nominal diameter) was used. To maintain the desired feed flow rate the pump drive was controlled with a cam-timer to give a pump run time of approximately 15 seconds every minute. The recycle pump used 15 series (4.8 mm nominal diameter) tubing. Solids in the feed and effluent caused abrasion in the pump tubes, especially at the folding line, which shortened the tubing life markedly. Typically failure occurred after about three weeks. Replacement with Norprene (Reg. T.M. Norton Company, Masterflex catalogue number 6404-) eliminated this abrasion problem.

Flow tubing for the reactor was originally flexible PVC joined with 'Serkit Systems' fittings (Auckland Tool and Gauge Co. Ltd, Takapuna, Auckland, New Zealand) and hose clips. Unfortunately, even when drilled out, the constriction in flow diameter at the fittings caused blockages due to accumulation of resinous solids and mineral scale. A change to semi-rigid nylon tubing joined externally with offcuts of Masterflex silicone greatly extended the time between blockages.

Feed was from stirred glass or polyethylene vessels from within the controlled temperature room. This was done to provide the shortest possible feed line between feed vessel and feed pump. If these lines were too long settling of the wood fibres caused blockage problems in the pump tube. After an infection at the pulp mill from which feed was sourced, presumed to be *Klebsiella* species, volatile fatty acid production in the feed vessel became a problem. To reduce this the feed vessel was moved to a nearby cold room and the feed was circulated to the feed pump. The modified feed system is shown in Figure 3.3. The use of a Shacklock dishwasher pump (part number 388344) gave sufficient mixing on return to keep feed solids suspended.

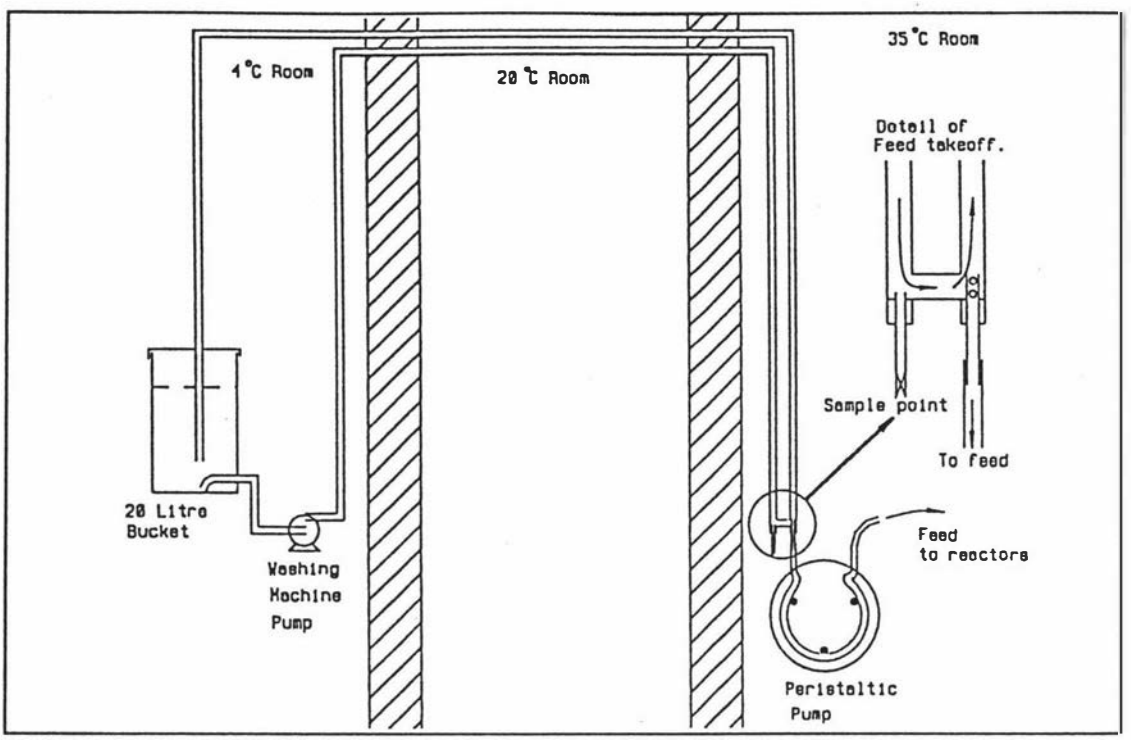

Figure 3.3 Final feed system.

3.5.2 Reactor A: A modified upflow anaerobic sludge blanket reactor

Reactor A was also constructed from "QVF" glassware with a working volume of 12 ℓ. It was similar in concept to reactor B but had certain significant differences which are shown in Figure 3.4 as compared to Figure 3.2. The settling zone or "head" of reactor A was considerably smaller than that of reactor B so that suspended solids settling was reduced compared to reactor B. No mechanical stirring was provided for reactor A.

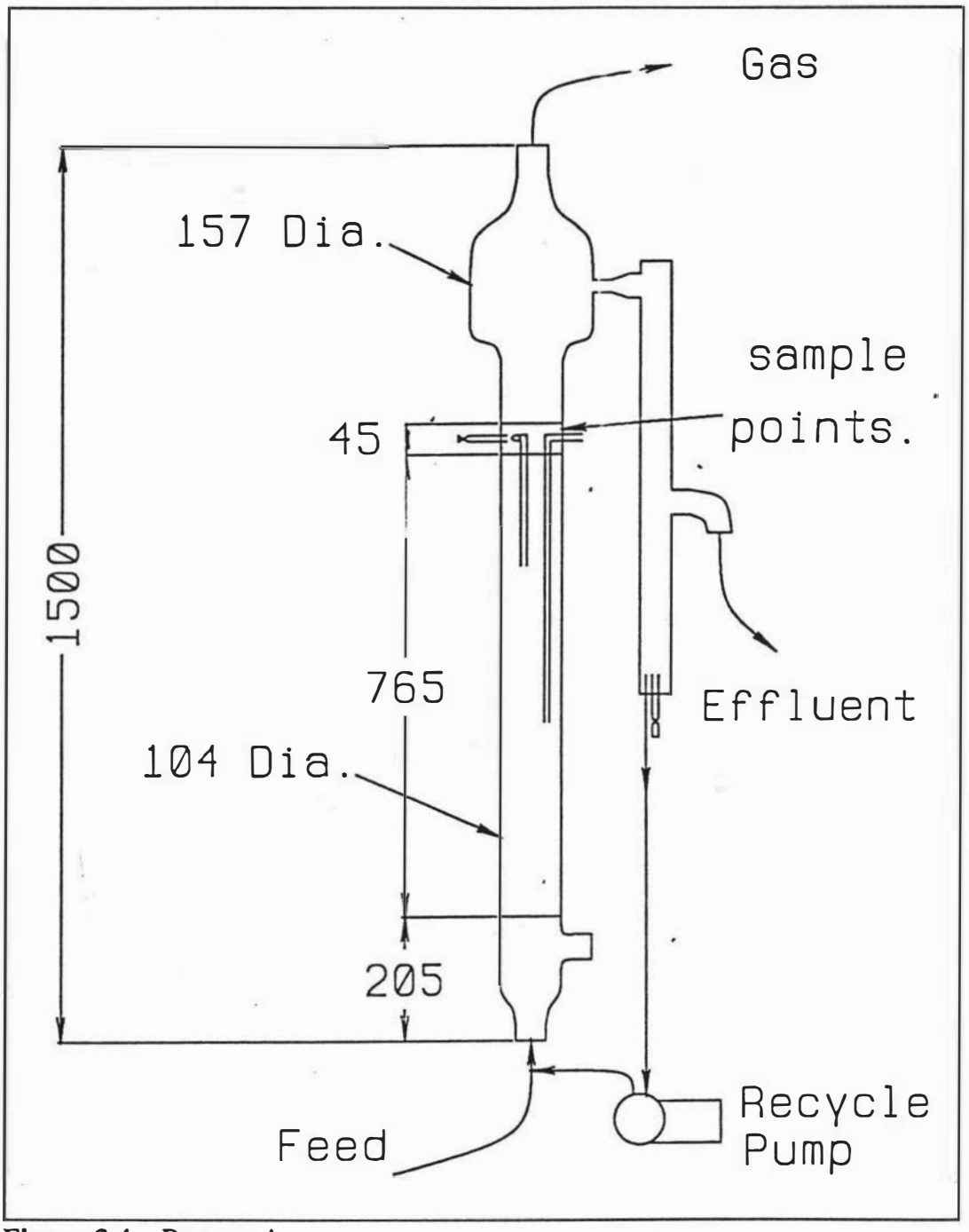

Figure 3.4 Reactor A

The feed and recycle inlet area went through a number of configurations to try to reduce the many blockage problems that resulted from the feed suspended solids once 50 % feed concentration was reached. Reactor B went through a similar evolution in inlet design. The provision of feed to the reactor followed that described in Section 3.5.1.1.

3.5.3 Reactor C: A modified upflow anaerobic sludge blanket reactor

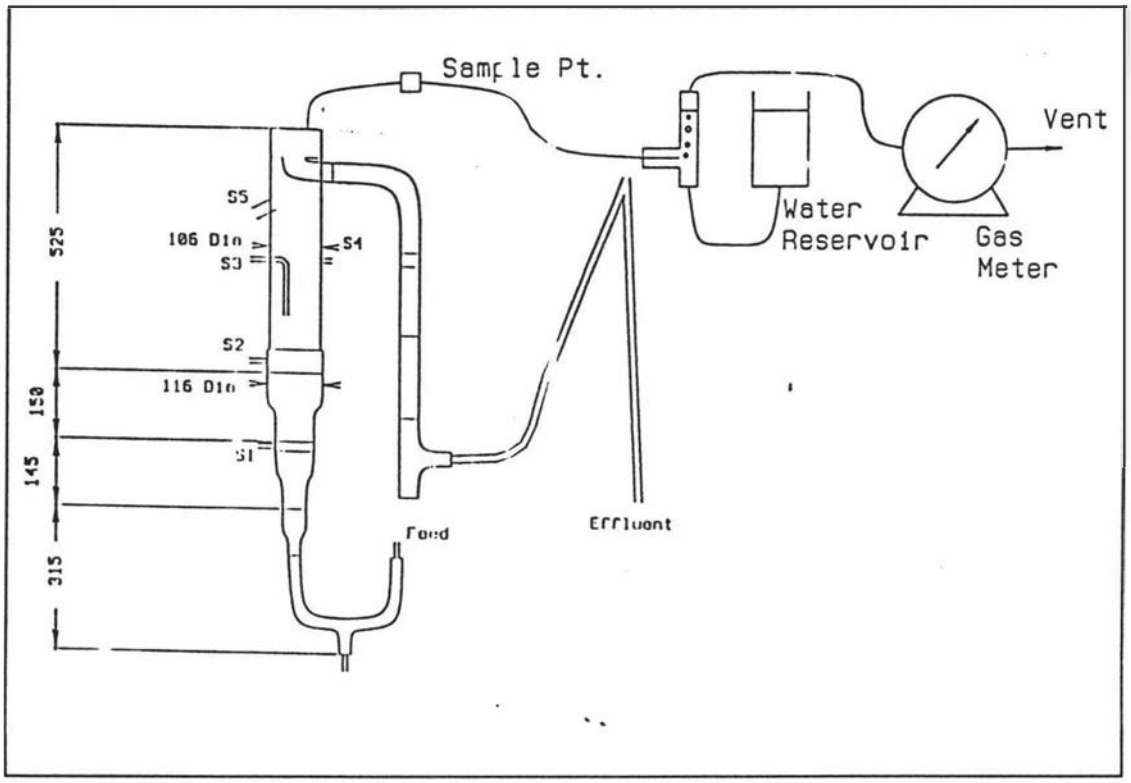

Figure 3.5 Reactor C.

This reactor was constructed from "QVF" glassware, perspex and stainless steel and was similar in concept to reactors A and B, but did not have the larger diameter settling chamber and feed was dripped downwards through a gas space to prevent blockages from granular sludge entering the inlet. There was no recycle and effluent flowed over a weir in the centre of the main reactor tube and into a side arm of the reactor. From here effluent was removed via an inverted siphon to a collection bucket. The final version of this reactor is shown in Figure 3.5. The final total reactor volume was 6.75 ℓ and had five sample points as indicated. Samples for volatile fatty acid and alkalinity analysis were usually removed from the side arm. Gas was removed from the top of the reactor through thick walled butyl rubber tubing to a condensate trap and a "wet work" experimental gas meter (Brand catalogue number 2505.62, Rudolf Brand GMBH + CO, Wertheim-Glashüte, West Germany). At a later date a second gas meter was installed in series with the first meter to check on variations in meter response.

The feed pumping system was similar to that of reactors A and B but used 15 series Norprene with intermittent pump operation via a cam timer for feed entry into the reactor.

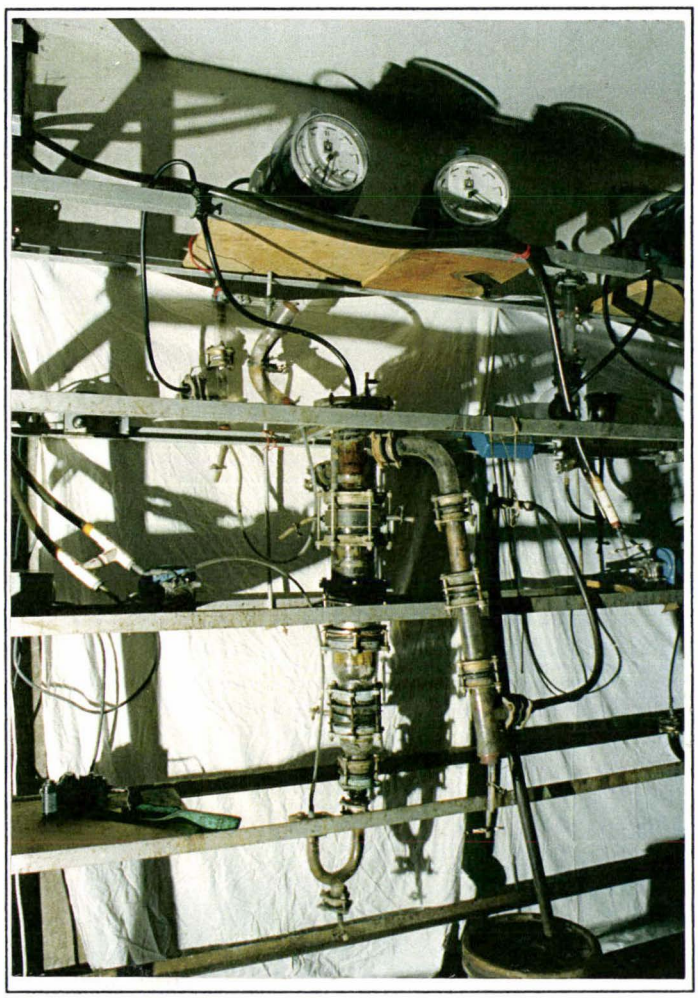

Figure 3.6 Reactor C

Reactor C was slightly modified with the addition of two "QVF" spacer rings to give a small increase in liquid reactor volume to match that of reactor D. The modified reactor can be seen in Figure 3.6. Reactor C had a reactor liquid volume of 6.25 ℓ and a liquid height of 1.06 m. Total liquid volume was 7.0 ℓ on average (side-arm liquid height varied to some small extent).

3.5.4 Reactor D

Reactor D was constructed from 110 mm internal diameter perspex tube with a bottom section of "QVF" glassware. It had a perspex side-arm. Reactor liquid volume was 6.29 ℓ and liquid height was 0.90 m. Reactor D can be seen in Figures 3.7 and 3.8. The volume of the side-arm on reactor D was less than that of reactor C resulting in a total liquid volume of 6.96 ℓ. Liquid depth from the weir down to the 0.75 ℓ mark was 0.653 m for D and 0.693 m for reactor C. Thus, although not identical, the reactors were regarded as being geometrically similar. All reactor peripherals for reactor D were of the same design and equipment as described for the final version of reactor C. All the reactors described were operated in the same temperature controlled room under the same conditions.

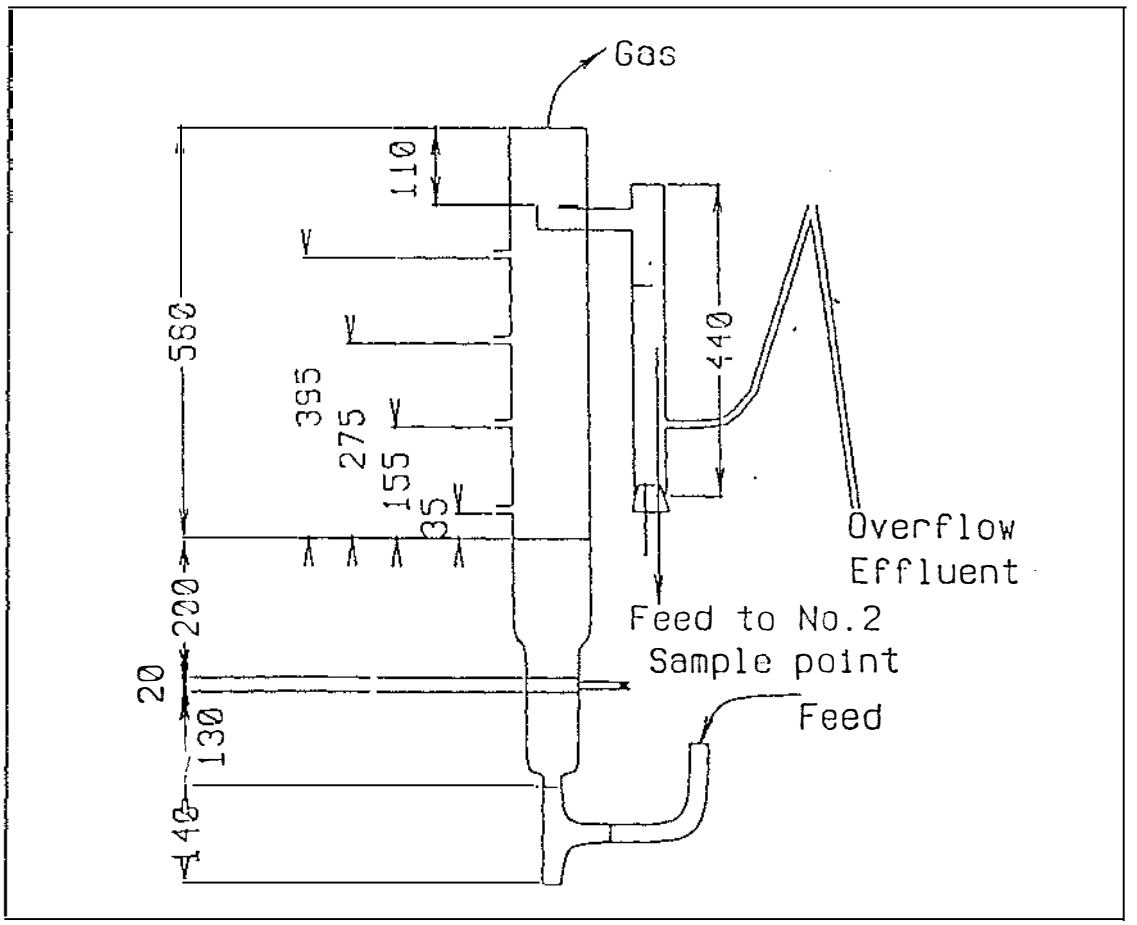

Figure 3.7 Reactor D

Reactors C and D used the same feed system described for reactor B and shown in Figure 3.3.

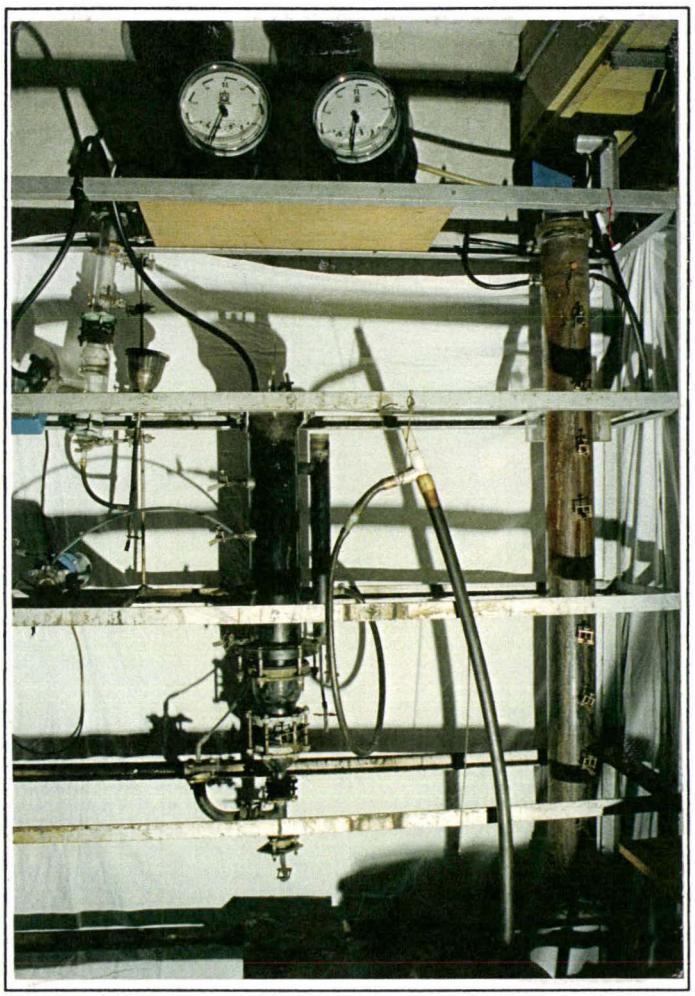

Figure 3.8 Reactor D.

3.5.5 Reactor summary

Table 3.5 A summary of the characteristics of the reactors used in the project.

Reactor	Type	Start-up	Operation
A	-12 ℓ UASB[a] -No GSS[b] -Small settling zone -Recycle	-Granular dairy effluent sludge -Whey/SPE[c] feed -Slow SPE % ramp	-Slow loss of granulation -Tightly flocculated biomass -Prone to blockage
B	-20 ℓ UASB -No GSS -Large settling zone -Recycle	-Granular dairy effluent sludge -Whey/SPE feed -Very slow SPE % ramp	-Rapid loss of granulation -Loosely flocculated biomass -Prone to blockage
C	-7 ℓ UASB -No GSS -No settling zone -No recycle	-Non granular forestry industry and reactor B sludges -100 % SPE feed -HRT ~ 1 day -Restarted[d] as pair with reactor D	-Rapid granulation -Bed matrix formed -Few blockages -High rate operation
D	-7 ℓ UASB -No GSS -No settling zone -No recycle -Twin to reactor C	-Split sludge of reactor C -Highly granular seed with ~1.1kg granules	-Continued granulation -Very high rate operation

a the reactor design is most similar to that of the UASB

b gas solids separator

c dilute lactic acid casein whey permeate and screw press effluent

d reactor C sludge was split into granule and liquor fractions, equal amounts of the two fractions were used to seed reactor D and reseed reactor C so that both reactors started from the same base.

3.5.6 Inoculum sources and preparation

The initial granular sludge for reactors A and B was obtained from the New Zealand Dairy Research Institute (N.Z.D.R.I.) one cubic metre pilot scale UASB reactor. This reactor had been treating cheese whey and lactic acid casein whey at 14 to 18.5 kg $COD.m^{-3}d^{-1}$ loading rate. The sludge consisted of uniform 1 mm diameter creamy coloured granules with little floc based or single cell growth. Temperature of this sludge was 33-35 °C.

Effluent from an 18 l UASB reactor treating lactic acid casein whey ultrafiltration permeate at about 10 $kgCOD.m^{-3}d^{-1}$, was used as a supplement to the above seed both at initial inoculation and at later dates to provide active anaerobic biomass to reactors A and B after severe upsets. The effluent from the whey permeate reactor contained mainly single cell growth with a little floc based growth. It was collected from the effluent collection vessel and so had been exposed to the atmosphere for up to 24 hours. The temperature of this effluent was about 35 °C .

For the start up of reactor C, forestry industry wastewater sludges were used. A wide variety of sludges were used to try to provide as great a variety of forestry industry acclimated anaerobic bacteria as possible to the reactor. Sludges were obtained from the central North Island (New Zealand) pulp mills using a stainless steel bucket and rope to dredge the following pond bottoms;

Number one pond. Tasman Pulp and Paper Company Ltd, Kawerua, New Zealand. (Tasman No 1 pond).

Leachate stream, solids dumping valley. Tasman Pulp and Paper Company Ltd, Kawerua, New Zealand. (Tasman leachate).

Primary settling pond. Winstone Samsung Pulp Mill, Ohakune, New Zealand. (Winstone No 1).

Secondary settling pond. Winstone Samsung Pulp Mill, Ohakune, New Zealand. (Winstone No 2).

Pond 2, clarifier underflow. New Zealand Forest Products Pulp and Paper Mill, Tokoroa, New Zealand. (NZFP No 2).

Pond 3, clarifier overflow and intercept basin. New Zealand Forest Products Pulp and Paper Mill, Tokoroa, New Zealand. (NZFP No 3).

These sludges were poured and scraped into 20 l plastic containers leaving about one litre head space and sealed to prevent further oxygen entry. Facilities for oxygen-free nitrogen flushing were not available and some oxygen contact was inevitable. The containers were vented frequently to allow for gas evolution. Once back in Palmerston North the sludges were mixed in the ratios shown in Table 3.5, and allowed to settle. A greater quantity of the Tasman No 1 sludge was used as a result of the extensive gas evolution of this sludge during transport. After twenty minutes settling time the liquor was decanted off and the settled sludge was screened through a 4 mm sieve to remove leaves and other debris. None of the above industry sludges exhibited any form of granulation. Two litres of this sieved sludge was then mixed with three litres of fresh Reactor B sludge. This sludge was removed from the bottom sample point, shown in Figure 3.2, minutes before use. At the time of this sludge removal all granulation had been lost from the reactor B sludge.

Table 3.6 Ratio and source of pulp mill sludges.

Quantity (ℓ)	Sludge source
3	Tasman No 1
1	Tasman leachate
1	Winstone No 1
1	Winstone No 2
1	NZFP No 2
1	NZFP No 3

The rate of gas evolution from the container of Tasman No 1 sludge was 5 - 10 times that of any of the other sludges. Vast upwellings of gas observed at the pond during sludge collection also suggested that this pond was very active anaerobically.

The inoculation for reactors C and D, when reactor D was introduced, consisted of splitting the sludge and liquor of Reactor C equally and placing the resultant fractions into each reactor. Each reactor was then topped up with fresh reactor C effluent. Subsequent operation of the reactors is discussed in Chapter 4.

3.6 ANALYSIS OF DATA

The analytical procedures used for this project are described in Section 3.2. Calculation of the reactor parameters follows that of Standard Methods (APHA-AWWA-WPCF, 1975). Calculation of some removal efficiencies posed some problems because of the accumulation of feed suspended solids and resin acids in the sludge beds. This elevated the apparent removal efficiencies. The development of a granular and highly structured sludge bed in reactors C and D prevented representative sampling of the sludge bed without considerable disruption. Apparent total COD and suspended solids (SS) removal efficiencies were calculated from the feed and effluent COD and SS measurements. These "apparent" values imply that all influent COD and SS entrapped within the sludge bed have been degraded. Thus, theoretical COD and SS removals were calculated using an indirect method based upon the methane production. The theoretical removal values indicate the amount of COD or SS actually degraded to methane over the time in question. Allowance for analytical and calculation errors must be made.

3.6.1 Theoretical COD removal

To obtain the theoretical COD removal (COD to methane) the methane production at STP was divided by the theoretical methane yield of 0.351 m^3 CH_4.kg $COD_{removed}^{-1}$.

For example, for a short period of operation of reactor C 126 ℓ of methane at STP was produced from an input of 614 g COD:-

$$CH4COD_{rem} = 0.126\ m^3\ CH_4 \div 0.351\ m^3\ CH_4.kg\ COD_{removed}^{-1}$$

$$CH4COD_{rem} = 0.359\ kg\ COD_{rem}$$

This figure was then divided by the input COD to give the "theoretical" COD removal efficiency

3.28

$$E_{CH4} = 0.359 \text{ kg } COD_{removed} \div 0.614 \text{ kg } COD_{in}$$

$$= 0.585 \text{ kg } COD_{removed}.\text{kg } COD_{in}^{-1}$$

$$E_{CH4} = 58.5\ \%$$

3.6.2 Theoretical suspended solids removal

The theoretical SS removal was calculated, again, from the methane production using the COD_{rem} as calculated above. It was assumed that dissolved COD (dCOD) does not accumulate within the reactor. Any production of dCOD within the reactor from suspended solids results in removal of those suspended solids from the reactor. The measured dCOD removal was subtracted from the theoretical COD removal figure. The remaining methane production and thus COD removal must have come from degraded suspended solids COD (SSCOD). Continuing the example above 296 g dCOD was removed over the period.

$$SSCOD_{rem} = 0.359 \text{ kg } COD_{rem} - 0.296 \text{ kg } dCOD_{rem}$$

$$SSCOD_{rem} = 0.062 \text{ kg } COD_{rem}$$

The SSCOD removal efficiency was then calculated by dividing the $SSCOD_{rem}$ by the measured quantity of SSCOD entering the reactor.

$$E_{SSCOD} = 0.062 \text{ kg } SSCOD_{rem} \div 0.240 \text{ kg } SSCOD_{in}$$

$$= 0.258 \text{ kg } SSCOD_{rem}.\text{kg } SSCOD_{in}$$

$$E_{SSCOD} \approx 26\ \%$$

To calculate the accumulation of SSCOD in the reactor the "theoretical" COD removed ($CH4COD_{rem}$) was subtracted from the change in COD measured between the feed and effluent (ΔCOD).

$$SCOD_{ACCUM} = \Delta COD - CH4COD_{rem}$$

$$= 0.438 \text{ kg COD} \div 0.359 \text{ kg } COD_{rem}$$

$$= 0.79 \text{ kg SSCOD}$$

To calculate the theoretical SS removal and accumulation it was assumed that the COD of the feed SS was uniform at least over the use of a batch of feed and that for this purpose that E_{SSCOD} will approximately equal the efficiency of SS removal (E_{SS}). Thus the incoming feed SS (SS_{in}) was multiplied by the SS removal efficiency to give the "theoretical" SS removal (SS_{rem}). The COD/SS ratio may vary from batch to batch depending upon the resin acid concentration of the feed SS.

$$SS_{rem} = SS_{in} \times E_{SSCOD}$$

$$= 0.127 \text{ kg SS} \times .258$$

$$SS_{rem} = 0.033 \text{ kg SS}$$

This figure can be subtracted from the change in SS measured between the feed and effluent (ΔSS) to give the accumulation of SS in the reactor SS_{accum}.

$$SS_{accum} = \Delta SS - SS_{rem}$$

$$= 0.067 - 0.033$$

$$SS_{accum} = 0.034 \text{ kg SS}$$

These calculations were performed for all the "pseudo steady states" discussed in this chapter.

3.6.3 Wood fibre and bacterial cell volume

The volume of wood SS was calculated by multiplying the mass of SS by the specific bulk index of 2.5 ml.g SS^{-1}. This index was estimated from that for wet radiata (1.9 - 2.1) and TMP fibre paper (3.0 - 3.2) (Kininmonth and Whitehouse, 1993; Ducker, 1980).

The mass of bacteria produced was calculated from the cell yield (g VSS.g COD_{rem}^{-1}) and the theoretical COD removal. The volume of bacterial cells was calculated from the mass of bacteria produced by multiplying the mass of VSS by the specific bulk index of 3.8 ml.gVSS^{-1}. This index was calculated from the density of bacterial cells (1050 $kg.m^{-3}$) and a ratio of VSS to wet weight for bacterial cells of 0.25.

CHAPTER FOUR UASB REACTOR PERFORMANCE

4.1 INTRODUCTION

The first reactors built were intended to produce data, but were in fact used largely to solve mechanical, acclimatization and analytical problems. Because of the long time constants involved, solving these problems took a great deal of time. They have produced useful observations and eventually a good supply of acclimatised sludge, as well as an understanding of how to cope with the problems caused by using real mill effluent as the substrate rather than the much more easily handled defined medium. The main problem was that the effluent contained a lot of fine solids and fibre particles in the stream. The ability to handle this was regarded as being the essence of the project and the penalty has being a long drawn out preliminary work with numerous operational difficulties having had to be overcome by a series of equipment modifications.

Once the UASB reactor design had been finalised and operational problems overcome, the performance of the reactor system needed to be established. Information on the relationship between the degree of treatment of the wastewater, the organic loading rate and the hydraulic retention time applied to the reactor are needed for large scale reactor design and comparative analysis of different reactor systems. This information is also useful for operational control of the reactor and to gain a better understanding of the reactor system. The analysis of this data forms the main subject of this chapter.

The dynamic nature of the anaerobic digestion process in combination with the deviation of these reactors from the ideal completely mixed reactor precludes the establishment of a true steady state operation. An approximation of steady state for these processes is defined as a period of operation where inlet and outlet conditions are nearly constant. This approximation is acknowledged by the use of the term "pseudo-steady state".

4.2 EXPERIMENTAL PROGRAMME

One period of "pseudo steady state" operation was analysed for reactor B. The reactor sludge had lost all granulation and was in a floc based form.

Reactor C was initially operated for 1357 days and it was with this reactor that the final reactor configuration was attained. A significant portion of this time was taken up with the recovery from a severe inhibition of methanogenic biomass activity attributed to the leaching of zinc from a galvanised storage drum into the TMP wastewater. Two "pseudo steady state" periods of operation were analyzed for this reactor after recovery was complete.

To gather further "pseudo steady state" data reactor D was established from reactor C as described in Chapter Three. A series of "pseudo-steady state" runs at different organic loading rates were performed using these two reactors. Reactors C and D were started at organic loading rates of 7.6 and 7.2 kg $COD.m^{-3}.d^{-1}$ respectively. Reactor C, the "parent" reactor for reactors C and D had been operating at an average OLR of 11.5 kg $COD.m^{-3}.d^{-1}$ for 90 days prior to the division of its sludge as inocula for these two reactors. The lowered OLR was to partially allow for the reduction in each sludge mass to approximately 40% of that in reactor C. Once "pseudo-steady state" operation was established at these OLRs and sufficient data collected, the feed rate was increased in a 3-fold step change in C and a 4-fold step change in D. "Pseudo-steady state" was deemed to be in existence once reactor parameters had held steady for three hydraulic retention times (HRT). Then this state was monitored for at least ten HRTs without significant changes in reactor parameters. After approximately seven weeks operation a new feed batch was introduced. This batch had an average COD one and a half times that of the previous batch resulting in 50% increases of OLR in the reactors. A rapid increase in volatile fatty acids (VFAs) in reactor C necessitated a drop in feed rate. The major features of the programme are reviewed in Table 4.1.

Table 4.1: An overview of the experimental programme for reactors C and D.

Reactor C			Reactor D		
Duration (days)	OLR[a] ($kg.m^{-3}.d^{-1}$)	HRT[a] (days)	Duration (days)	OLR ($kg.m^{-3}.d^{-1}$)	HRT (days)
0-21	7.6	1.55	0-29	7.2	1.62
22-74	18.9	0.62	30-75	28.0	0.44
94-186	16.6	1.06	76-186	38.4	0.46

a - OLR and HRT values are averages over the entire period.

4.3 FEED VARIATION

Up until early 1991 the SPE collected showed volatile fatty acid (VFA) concentrations of about the detectability limits of the analysis (10 - 20 $mg.\ell^{-1}$). After this time an infection at the pulp mill, presumed to be *Klebsiella* by mill staff, produced total VFA concentrations of 900 - 1200 $mg.\ell^{-1}$ in the effluent when collected. Changes in the feed VFA concentrations were minimal provided the feed was keep under quiescent conditions. However even short periods of agitation caused changes in VFA concentrations over the next day. The various strategies adopted to try to provide a relatively uniform SS concentration thus caused increased VFA concentrations to 1800 - 2400 $mg.\ell^{-1}$ in the drums of feed. Storage under refrigeration of up to 4 months showed minimal change (< 5 %). VFA concentration changed by about 10 % during the use of each drum (5 -10 days). The feed entering the feed system showed a variation of approximately 15 % over the usage of each batch of feed. In the feed system VFA concentration changed more rapidly presumably because of the constant agitation of the circulation pump. The feed system became a batch fed, continuously emptied acidification reactor. For the first of the two feed batches that all but one

of the "pseudo steady states" (that from reactor B) were performed with, VFA concentration varied from 2,000 - 4,200 mg.ℓ^{-1} over the period between additions of feed to the circulation system. The full extent of the range was not necessarily attained in each period. For the second batch, in which the "pseudo steady states" Cc, Cd and Dc were performed the VFA concentration ranged from 1,000 - 5,000 mg.ℓ^{-1}. Again in this case most of this range was not covered in any one day. The only effect evident of this VFA concentration variation was a variation in the methane composition of the reactor gas. The increase in methane percentage followed the increase in VFA concentration closely. Analysis of gas composition a number of times over a period between feed additions, repeated on different days, showed that this variation was actually in the carbon dioxide composition of the digester gas and that the methane production rate remained as constant as may be expected from this type of reactor

4.4 REACTOR D: OPERATION

Day to day monitoring of reactor D was primarily by gas evolution and VFA concentration. As each step change in OLR was made, an almost immediate increase in VFA concentrations and decrease in methane yield occurred, as shown in Figures 4.1, 4.2 and 4.3.

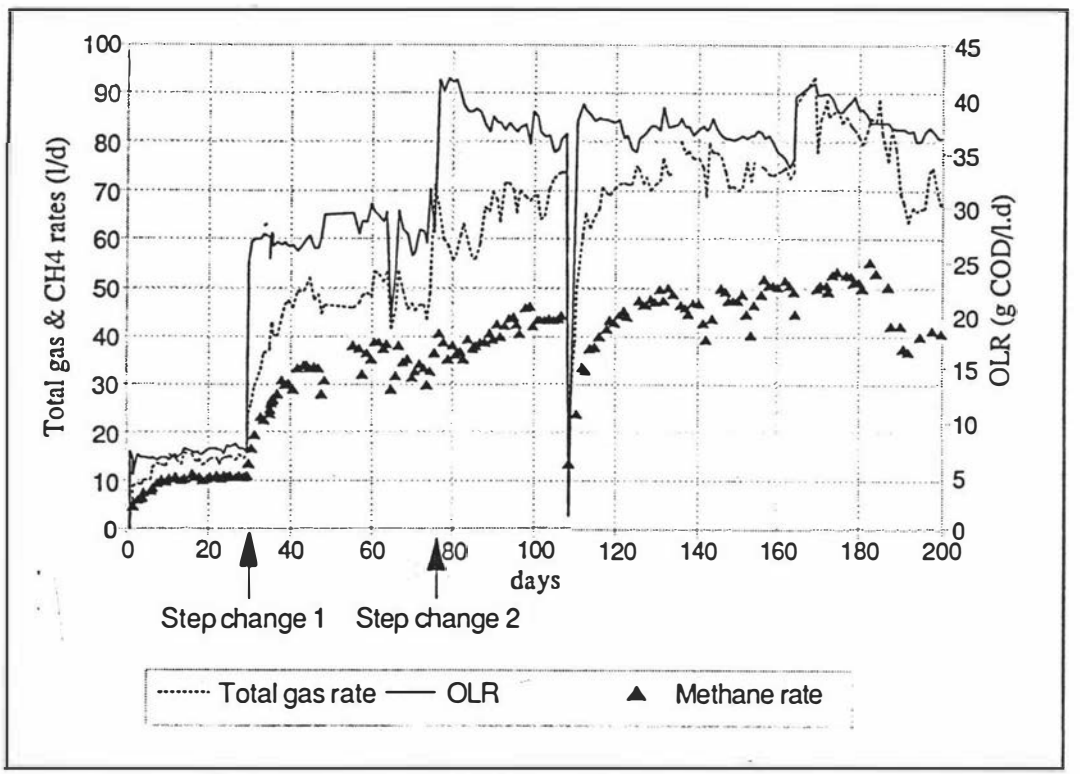

Figure 4.1 The total gas production rate, methane production rate and organic loading rate for reactor D.

4.4.1 Volitile fatty acids

At the start up OLR of, 7.2 kg $COD.m^{-3}.d^{-1}$, the elevated VFA concentrations were quickly reduced to below 200 $mg.\ell^{-1}$ total VFAs. The first step change to 28 kg $COD.m^{-3}.d^{-1}$, saw the acetic acid level rise immediately to 950 $mg.\ell^{-1}$ and then quickly reduced to approximately 300 $mg.\ell^{-1}$, although this was not as low as that of the previous "pseudo-steady state". Propionic acid however rose from 20 $mg.\ell^{-1}$ to a concentration of over 1000 $mg.\ell^{-1}$ within one day and remained at about this level for the duration of this phase. The second step change to

38 kg COD.m^{-3}.d^{-1}, again increased acetic acid to approximately 1000 mg.ℓ^{-1}. This was rapidly halved and then slowly reduced subsequently. Another increase occurred at about day 110 as a result of sludge inhibition after a feed interruption. This effect was noted in the earlier work discussed in Section 4.3.1 and has been reported in other anaerobic treatment of TMP wastewaters (Habets and de Vegt,1991). After this the acetic acid concentration held steady at approximately 250 mg.ℓ^{-1}. Over the same time period, propionic acid which had reached 2400 mg.ℓ^{-1} the day after the step change, slowly began a steady decline in concentration. After 100 days of operation a propionic acid concentration of 300-400 mg.ℓ^{-1} was obtained. Clearly the reactor was under some stress at this high OLR. No explanation was established as to why the propionic acid concentration failed to drop significantly during the stage of operation at 28 kg COD.m^{-3}.d^{-1} OLR.

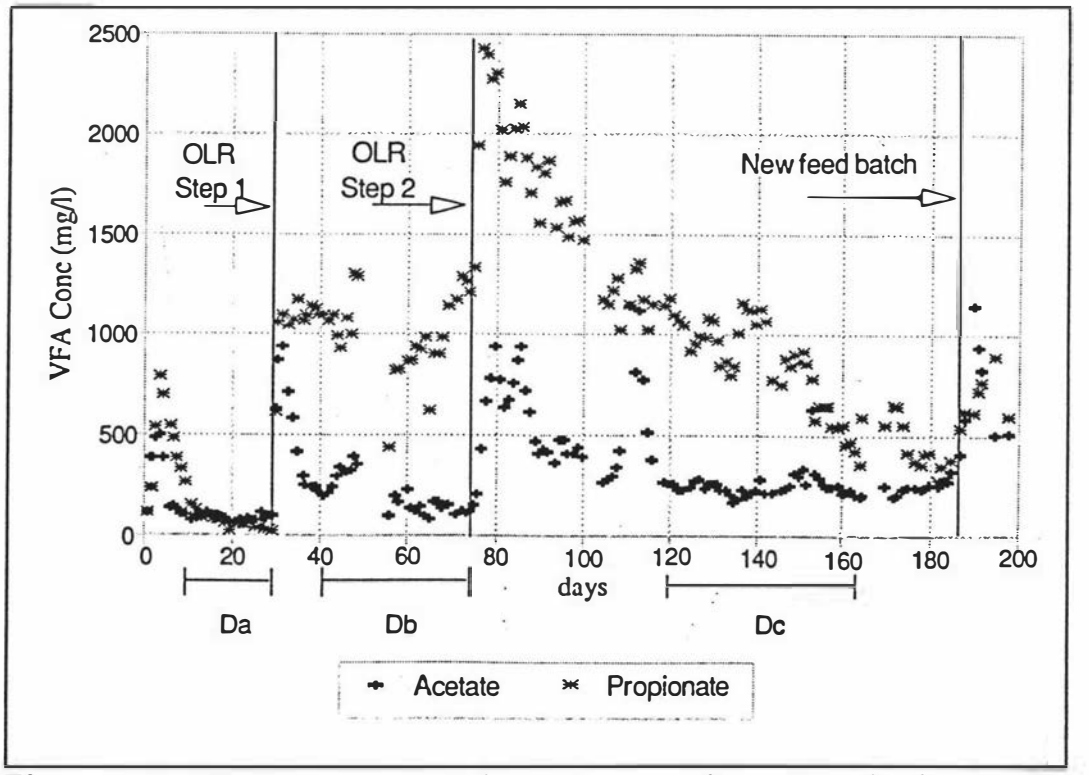

Figure 4.2 The acetate and propionate concentration at the exit of reactor D. The three "pseudo steady state" time periods for this reactor are indicated.

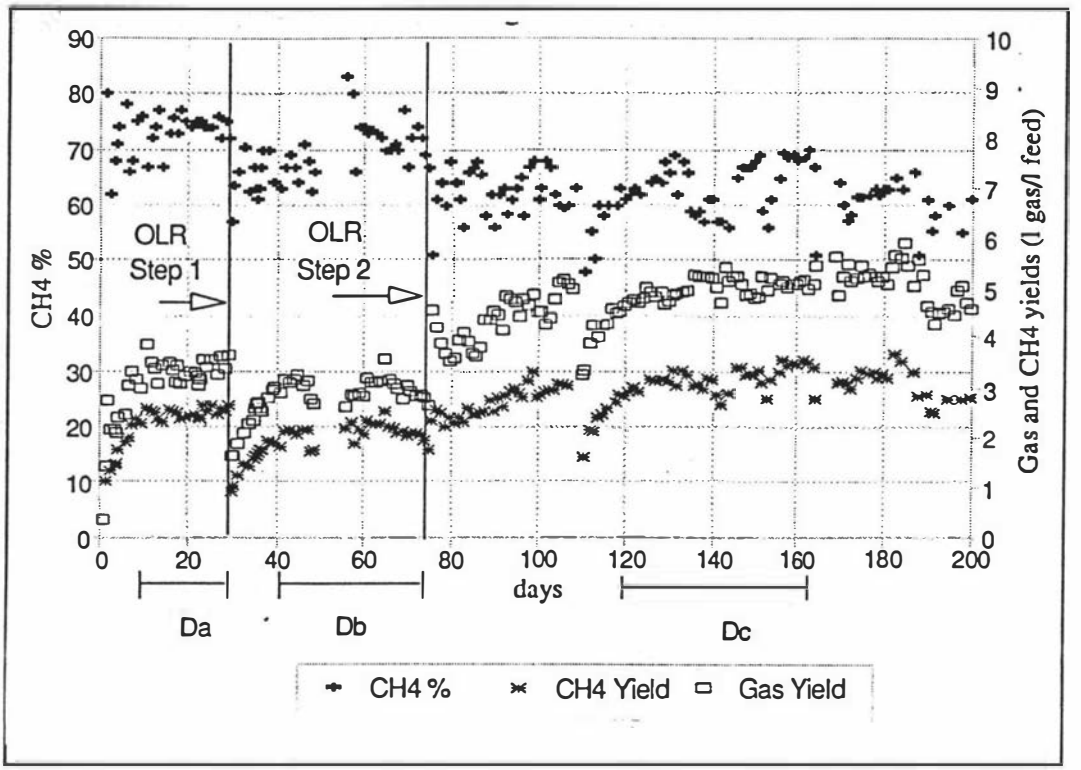

Figure 4.3 The methane composition of the digester gas, methane yield and gas yield for reactor D.

4.4.2 Methane percentage

The methane percentage of the gas is some what variable as discussed in Section 4.3 but the average methane composition drops with each step in OLR. The drop in methane percentage after the second step change shown in both Figure 4.3 (Reactor D) and Figure 4.9 (Reactor C) appears to be an artifact of the varying carbon dioxide (CO_2)production relative to it's removal as dissolved CO_2 in the effluent. The gas production rate:feed rate ratio (gas yeild) varies from feed batch to feed batch as a function of feed strength. Calculation of the solubility of unreacted CO_2 in a carbonate:bicarbonate aqueous systems following the method of van Krevelen and Hoftizer (Danckwerts, 1970) shows that variation in the ratio of CO_2 in the gas to that dissolved in the effluent alone is sufficient to explain the observed changes in methane concentration. The second feed batch, started on day 74 is more concentrated than the previous batch and the methane yield is higher. The removal of CO_2 remains constant, assuming saturation of the effluent, higher production of CO_2 results in proportionally

higher CO_2 in the digester gas. This is illustrated in Figure 4.9 for reactor C where methane percentage drops on the advent of the new feed batch on day 74, however the OLR is reduced not increased. Thus methane percentage of the gas is not as OLR linked as may first appear. The average methane percentage varies between 60 and 75 %.

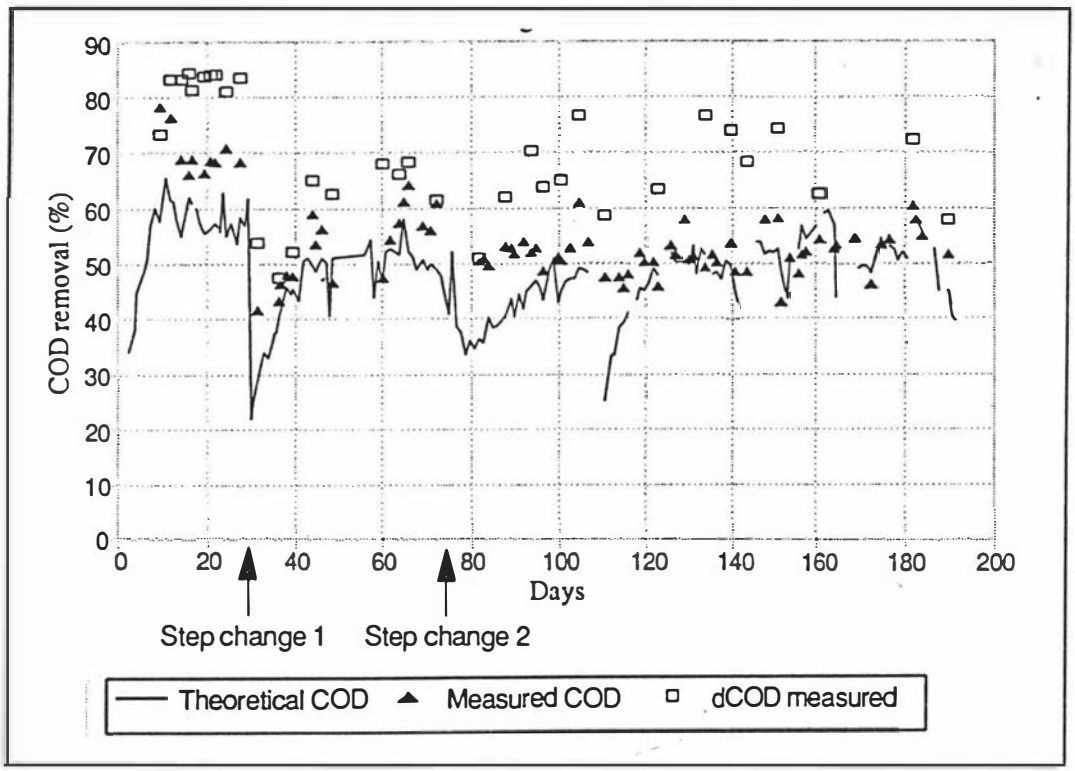

Figure 4.4 Theoretical minimum COD removal efficiency (COD to CH4), apparent COD removal efficiency (COD measured) and actual dissolved COD removal efficiency (dCOD measured) for reactor D.

4.4.3 Feed suspended solids entrapment in the sludge bed

Examination of Figure 4.4 shows that the COD removal averaged about 50% for the highest OLR, once somewhat recovered from the step change. This was not particularly different from that seen at 28 kg $COD.m^{-3}.d^{-1}$. The theoretical COD removal, calculated from methane production, deviates from the measured values at the start of this run and from approximately day 75 to day 95. The bed level values shown in Figure 4.5 suggest that high amounts of feed suspended solids

(SS) were entrapped in the bed over these time periods, as indicated by the increased slope of the line, which would have had the effect of elevating apparent COD removal. While the increase in slope could be due to bed expansion from increased gas evolution and entrapment, the liquid superficial velocity in the reactor dropped slightly. Thus it is likely that some of the increase in the sludge bed level was due to accumulated feed SS and accounts for the apparent greater than theoretical COD removal. The three groups of three straight lines shown on Figure 4.5 are calculated from the "pseudo steady state" data described in Section 4.7. Calculations are detailed in section 3.6. The lower line, " wood accum", represents the volume of the calculated wood SS accumulation in the reactor over the relevant "pseudo steady state". The upper line, "biomass accum", represents the volume occupied by the accumulated wood fibre plus that occupied by the calculated increase in bacterial cells, assuming that all the bacterial cells are retained with in the reactor bed. The middle line, "biomass lost", represents the volume occupied by the accumulated wood fibre plus that occupied by the calculated increase in bacterial cells, assuming that all the bacterial cells are lost from the reactor bed and an additional and equal mass of wood SS is retained. The actual accumulation is likely to lie between the two upper lines for each "pseudo steady state" because neither extreme, of complete retention or complete loss of bacterial cells is likely. Overall the trends of the two upper lines follow the rate of bed accumulation. That agreement is not as good for parts of the line is likely due to bed volume changes due to liquid superficial velocity changes on day 29 and changes in gas production rate and degree of gas retention in the sludge bed. The sudden bed volume changes on days 84 - 86 and 90 - 94 of approximately 0.5 ℓ magnitude can only be due to gas entrapment changes. Figure 4.1 shows no sudden changes in OLR that might suggest sudden changes in liquid superficial velocity. The good overall agreement of the observed bed level and that calculated from the "pseudo steady state" data gave increased confidence in the calculated suspended solids retention values and the theoretical COD removal efficiency calculations.

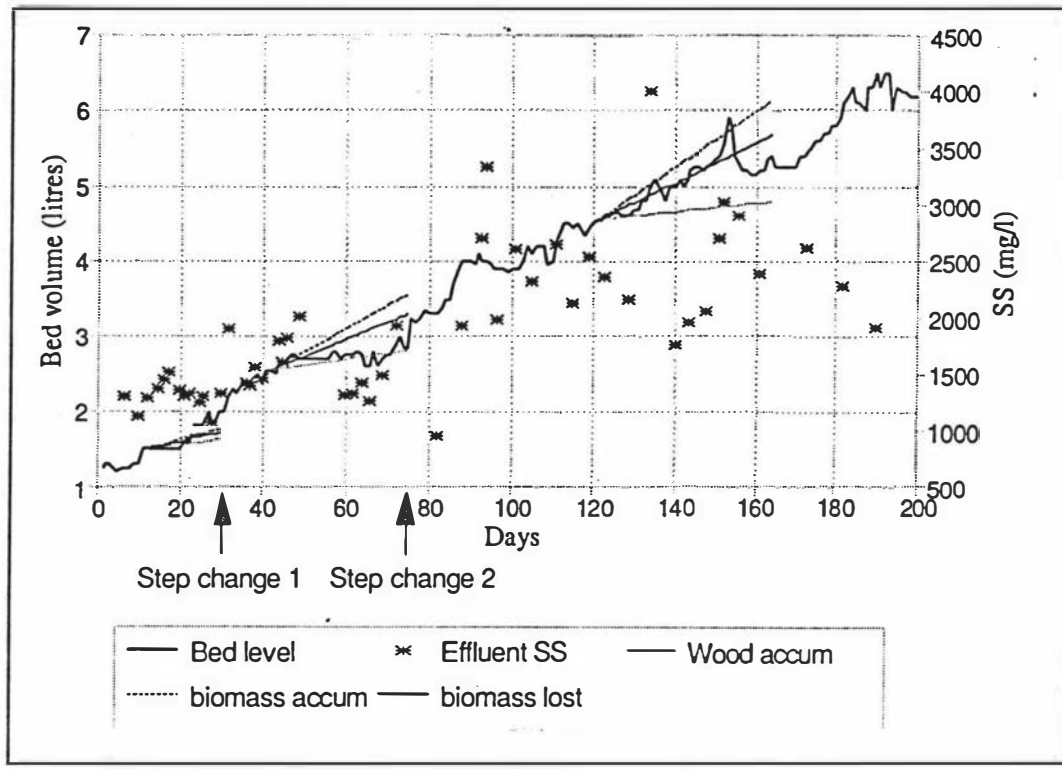

Figure 4.5 Bed volume and effluent suspended solids concentration for reactor D. The calculated volumetric wood suspended solids accumulation (Wood accum), wood plus bacterial suspended solids accumulation (biomass accum) and wood plus bacterial suspended solids accumulation where wood replaces the bacterial cells all of which are assumed to be lost from the reactor (biomass lost) are plotted for each "pseudo steady state".

4.4.4 Problems arising from high suspended solids accumulation

In the later stages of operation of reactor D, from approximately day 180, the bed had filled the reactor and carry-over of granular sludge created severe effluent blockage problems. This period is shown in Figure 4.6. The bed rose to 25-30 mm above the reactor overflow weir shown in Figure 3.6. On day 225 approximately half the sludge bed volume was removed by inverting the reactor and shaking vigorously. Structural integrity of the bed was such that it moved down the reactor as a single plug, only breaking up around the protruding overflow weir. The removed sludge was washed through a 2.5 mm sieve to remove fines and 0.75 l of larger granules were replaced in the reactor. OLR

and HRT where maintained at the same levels as those prior to this event. Within three days of this fines wasting gas production was back to prior levels. This is evidence that the fines in the top part of the bed were not performing a significant role in the methanogenic consortium.

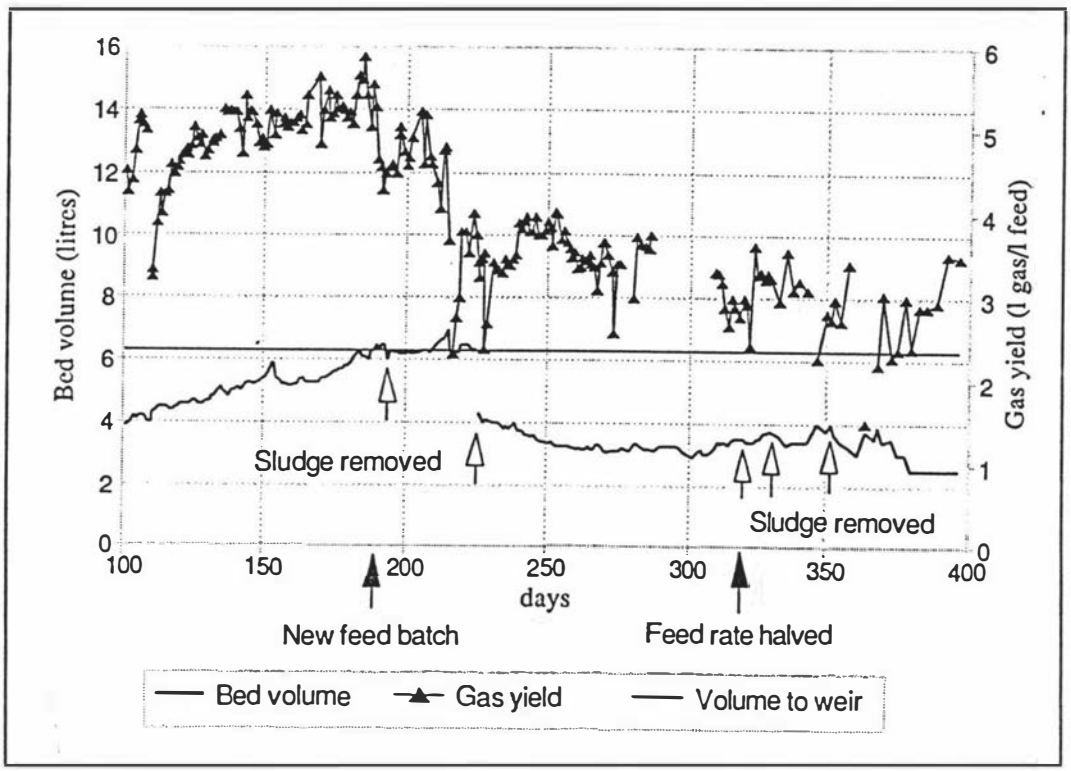

Figure 4.6 Bed volume and gas yield (ℓ gas/ ℓ $feed_{in}$) for reactor D. The volume of the main body of the reactor is also plotted.

From approximately day 190 the gas yield (litres gas/litre feed input) was declining as shown in Figure 4.6. This coincides with the start of a new feed batch. Increases in acetate and propionate concentrations, shown in Figures 4.2 (D) and 4.8 (C), indicates that some change has occurred in feed composition. Thus though reactor C's gas yield was reasonably constant, it is likely that the change in feed batch at about day 187 was, at least, partially responsible. On day 193 0.75 ℓ of sludge was removed from the top of the bed of reactor D with little effect upon the gas or methane yield suggesting that loss of bed solids was not significant. The further removal of 35% of the bed volume and the replacement of the larger granules from this volume, temporarily reversed the decline in gas yield. Subsequent decline was at a much lower rate. The bed

volume was also declining to some degree after this large sludge wasting event probably as a result of the reduction in influent SS on about day 235 and reduced gas production. Other analytical commitments prevented detailed analysis of this period thus no rigorous conclusions can be drawn.

It appears that the bed volume began to increase again from day 300 but three significant sludge removal events, indicated on Figure 4.6, greatly reduced this effect. The total volume of removed sludge was approximately 3 ℓ.

Tracer studies were conducted over a period following day 275 and are evident in Figure 4.6 by the lack of gas yield data. NaCl was used as the indicator. This appeared to cause inhibition of gas production in the reactor and chloride ions have been identified as a cause of problems in serum bottle assays described in Chapters 5 and 6. The tracer plots were not successful because of tailing of a very long duration. This invalidated any statistical analysis of the data but the shape of the initial response curve suggested the sludge bed was predominantly plug-flow and the liquor above close to completely mixed. Similar indications were found for reactor C.

4.5 REACTOR C: OPERATION

Reactor C was geometrically similar to reactor D and was operated in much the same mode as reactor D.

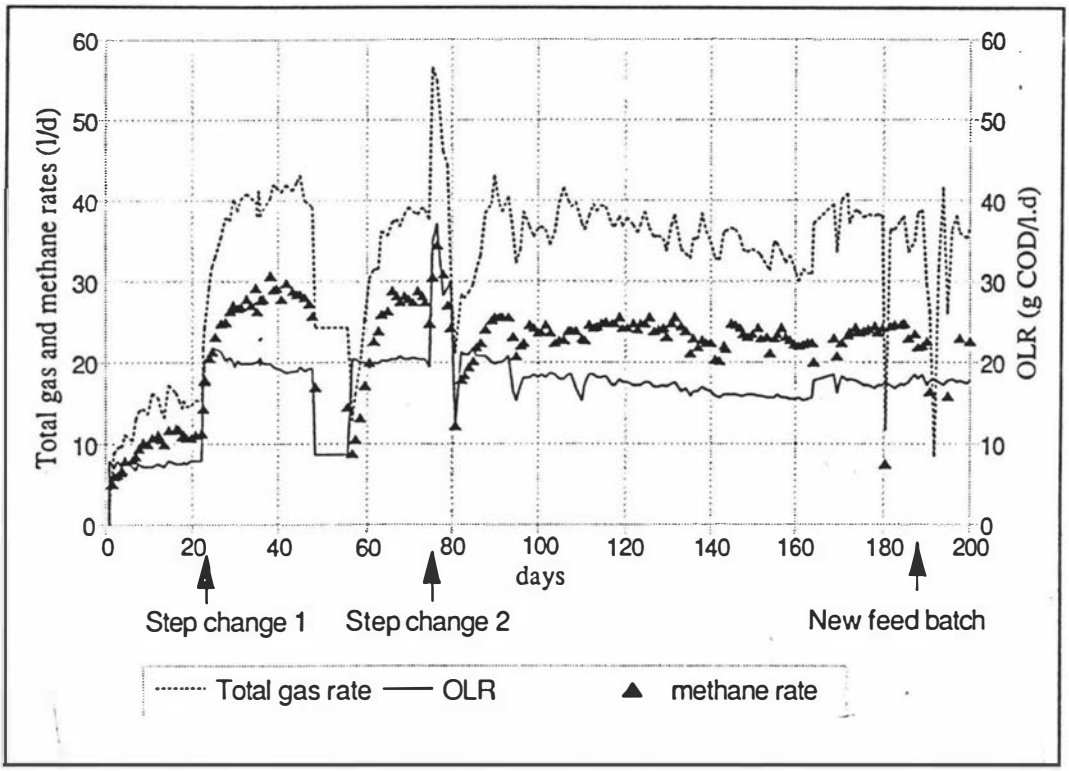

Figure 4.7 The total gas production rate, applied organic loading rate and the methane production rate for reactor C.

4.5.1 Volatile fatty acids

The first stage of operation of reactor C was very similar to that of reactor D as was expected. For the first 30 days influent acetic and propionic acid concentrations were on average 25% lower for reactor C because of a quirk of the batch feed- continuous withdrawal of the feed circulation systems. No appreciable difference in performance was noted other than a 5% greater dCOD removal for reactor D shown by comparison of Figures 4.4 and 4.10. At the step change to an average OLR of 18.9 kg $COD.m^{-3}.d^{-1}$, on day 21, VFA concentrations only reached half that seen in reactor D, as shown in Figures 4.8 and 4.2 respectively. Propionic acid was removed at a similar rate to acetic acid

(calculated from the slope of the point in Figure 4.8). This indicates that reactor C was not as stressed by this three-fold step change as reactor D was by a four-fold step change, as might be expected.

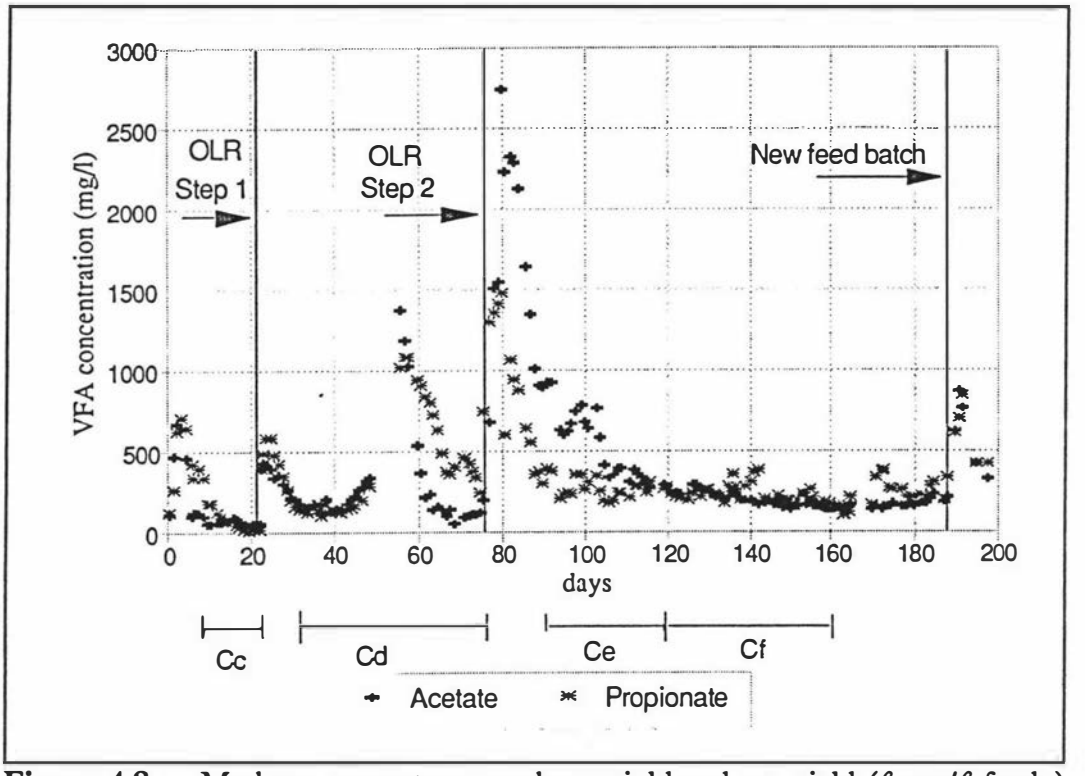

Figure 4.8 Methane percentage, methane yield and gas yield (ℓ gas/ℓ $feed_{in}$) for reactor C.

When the new, more concentrated feed batch was introduced to reactor C, on day 74, the first lot added to the feed system had not been settled properly and had a very high solids content and a greater proportion of fibrous solids. This resulted in acetic acid concentrations of 2750 mg.ℓ^{-1}, 2.5 times those in reactor D shown in Figure 4.8. However, propionic acid peaked at approximately 1400 mg.ℓ^{-1} compared to 2500 mg.ℓ^{-1} for reactor D. This difference approximates the difference in propionic acid concentration between the two reactors before the feed change. Because of the high acetic acid accumulation the feed rate was reduced, on day 83, to approximate the OLR before the feed change. Removal of propionic was then rapid. Acetic acid concentration also dropped, with approximately the same rate, after five days at approximately 2500 mg.ℓ^{-1}. No further change was observed in propionic acid concentration once it was just

below 1000 mg.ℓ^{-1} for four days, Feed rate was dropped by 10 %, on day 90, to aid recovery. Reactor C then had an extended period of steady methane production with low VFA concentrations but variable effluent SS.

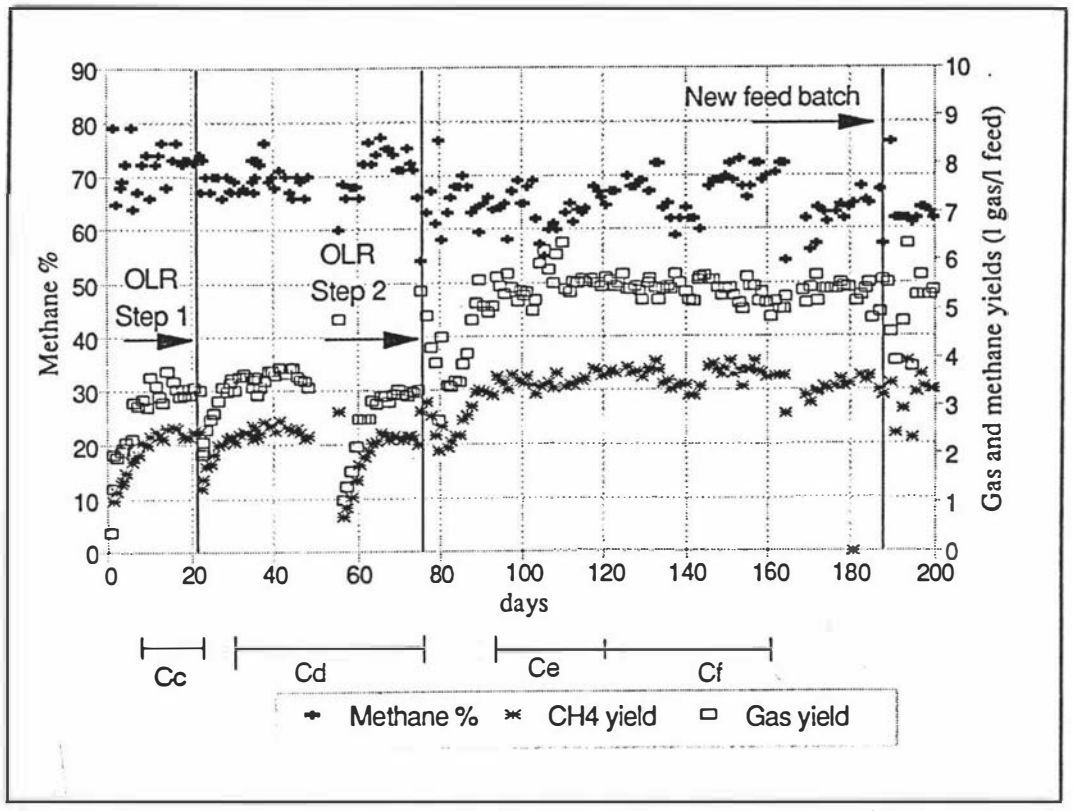

Figure 4.9 Methane percentage, methane yeild and gas yeild (ℓ gas/ℓ $feed_{in}$) for reactor C

4.5.2 The effects of feed suspended solids entrapment

COD removals, when the reactor was running well were remarkably similar no matter what the OLR applied, as is shown in Figure 4.10. Reactor D showed a slightly larger range of average values as expected from the larger range of applied OLR. The decline and then scattering of the measured COD reduction values, after day 74, are due to SS entrapment and shedding from the sludge bed. Examination of the bed volume data shown in Figure 4.11 and the COD removals in Figure 4.10,, shows that while the bed volume is increasing the measured COD reduction is generally larger than that calculated from methane

generation. This is because solids entrapped in the bed but not yet degraded are missing from the implied mass balance of the measured COD removal values. Once the bed volume begins to fall the measured COD removal values become much more scattered and on average lower. This variation represents the solids shed from the bed. These solids have been observed being lost from the bed through large up-wellings of gas. Thus solids loss appears to occur, predominantly, in bursts followed by long quiescent periods. A general impression formed was that the degree of this activity varied from day to day. It is likely that this is responsible for the variation in SS measurement.

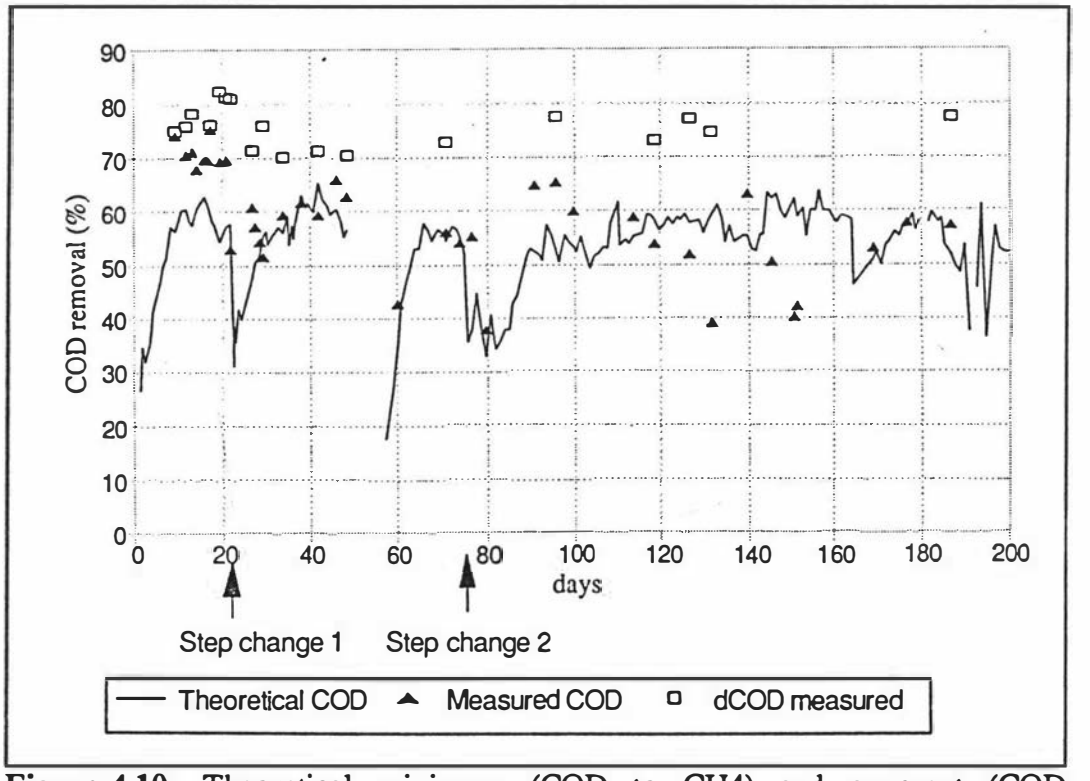

Figure 4.10 Theoretical minimum (COD to CH4) and apparent (COD measured) total COD removal efficiencies and actual dissolved COD (dCOD) efficiency for reactor C.

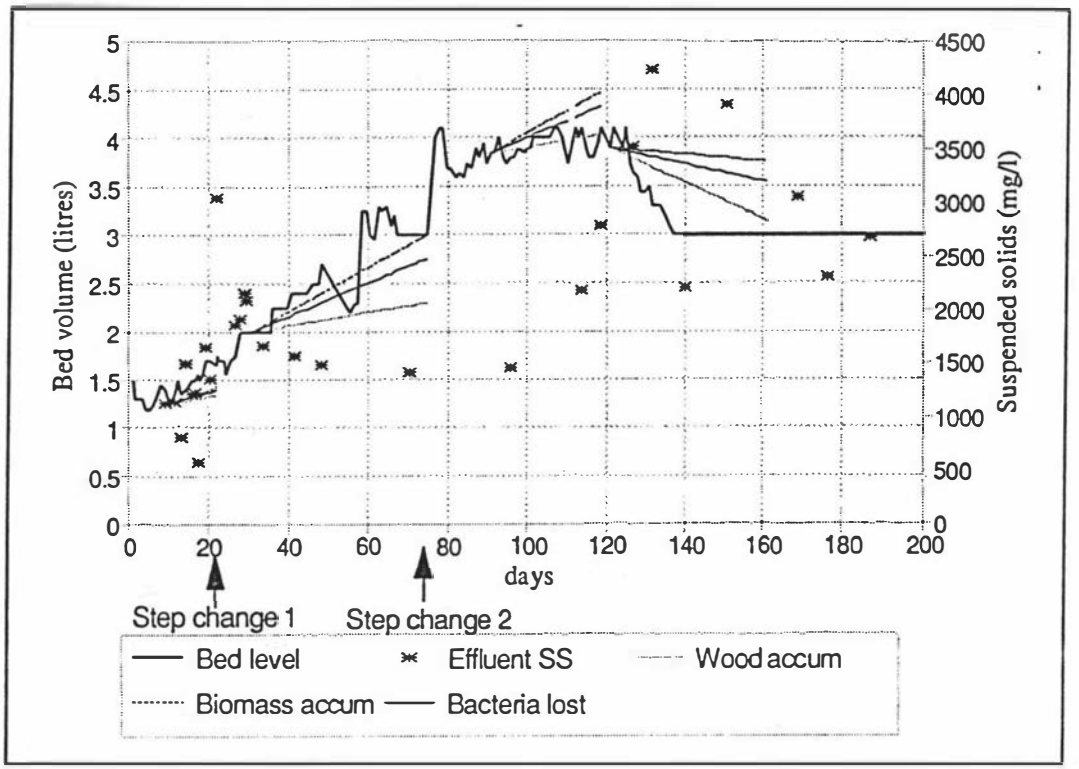

Figure 4.11 Bed volume and effluent suspended solids concentration for reactor C. The calculated volumetric wood suspended solids accumulation (Wood accum), wood plus bacterial suspended solids accumulation (biomass accum) and wood plus bacterial suspended solids accumulation where wood replaces the bacterial cells all of which are assumed to be lost from the reactor (biomass lost) are plotted for each "pseudo steady state".

In reactor C, unlike reactor D, the bed did not fill the reactor body. The bed volume rose at a similar rate to that in reactor D for the first 80 days. After the feed rate was reduced on day 80 and then again on day 95, the rate of increase in bed volume decreased, then levelled off and then the bed volume began to fall as shown in Figure 4.12. This appeared to be a simple mass balance determination. The new feed was about 50% higher in SS than the old feed batch and the feed rate reduction was from an average of 11.3 $l.d^{-1}$ down to 6.6 $l.d^{-1}$. This gave a 20% reduction in influent SS rate.

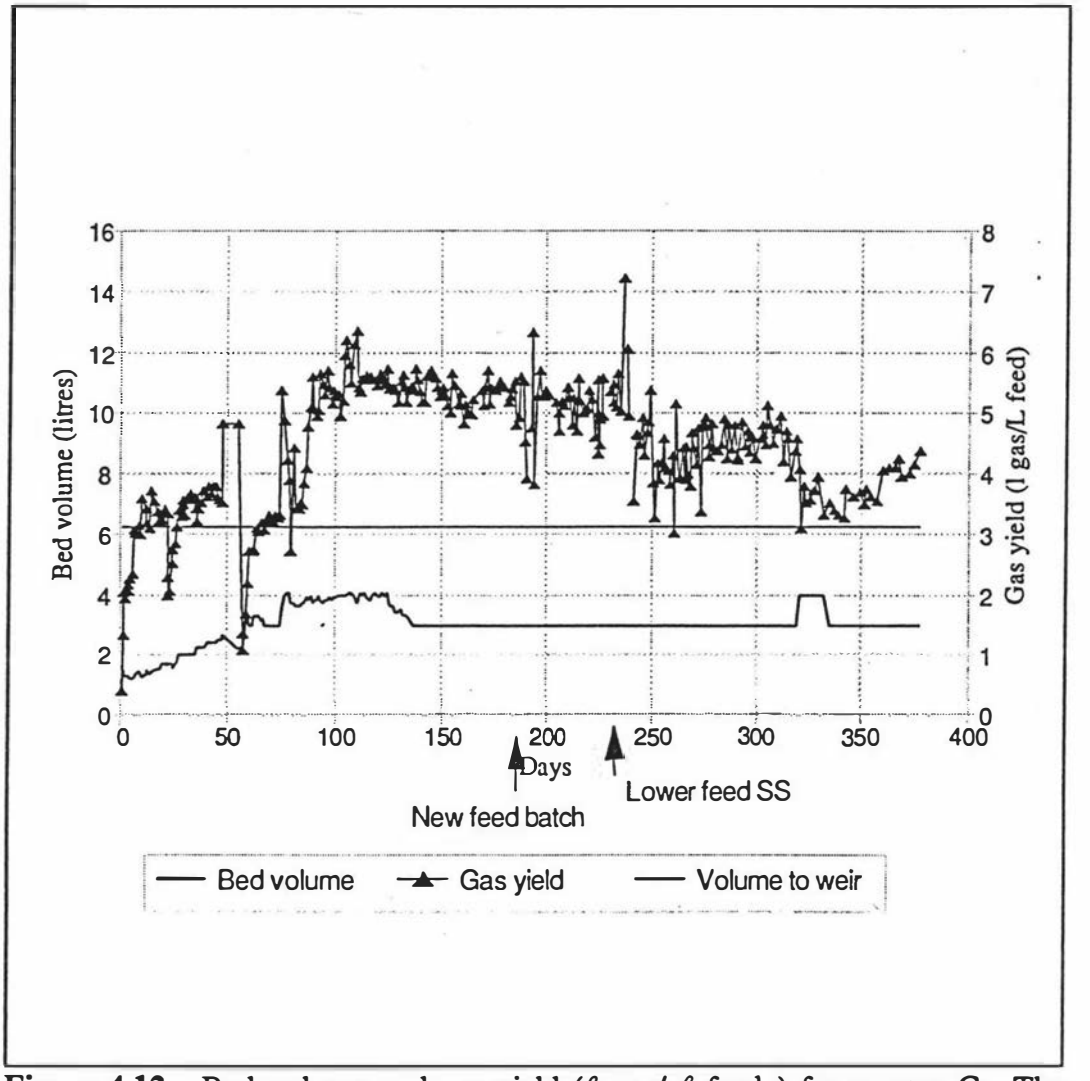

Figure 4.12 Bed volume and gas yield (ℓ gas/ ℓ $feed_{in}$) for reactor C. The volume of the main body of the reactor is also plotted.

Calculated sludge bed volume accumulation from wood and bacterial biomass are plotted in Figure 4.11, following the methods described for reactor D. As seen in Figure 4.5 (reactor D), once allowance is made for likely gas entrapment changes, the top two lines plotting the greatest and smallest combined wood/bacterial biomass accumulation have a reasonably good agreement with the recorded bed volume. The lines for "pseudo steady state" Cd do not agree particularly well but similar feed and effluent SS and COD concentrations result in larger errors for the final removal efficiencies. Once the bed level began to drop the effluent SS concentration on average doubled but with a large range of values.

The gas yield was essentially constant throughout this period from day 110 to day 240 and VFA concentrations were low and constant. No inhibitory effects were evident in that VFA levels were relatively low and constant. From day 135 sludge bed volume could not be determined but obviously bed volume decrease was greatly reduced or complete washout would have occurred. On about day 320 the bed volume was observed at the four litre mark of the reactor, as shown in Figure 4.12, indicating a possible bed increase in the latter stages of this period, although at least the top part of this bed was quite loose and flocculated in structure. This greater bed volume was reduced soon after a feed rate decrease of 50 % showing that at least some of this increase was due to bed expansion rather than solids accumulation.

4.5.2.1 Loss of sludge bed suspended solids

Observations over this time period may help to explain the mechanism of increased effluent SS. It must be considered that the very high solids inflow over days 75 and 76 followed by a lower wood SS inflow may have effected the structure and texture of the sludge matrix that built up surrounding the sludge granules and this may effect the solids and gas holding ability of the bed. Further discussion of the sludge matrix can be found in Chapter 6. The bed volume increased by approximately one litre over days 75 and 76, suggesting that this part of the bed must have a much looser structure than that formed before because the extra solids inflow of this event was approximately 60 g of wood fibre. The bed volume dropped by 0.4 ℓ over days 79 and 80 but significant extra amounts of gas must have remained in the lower part of the bed. This was observed to be the case on day 87. Very rapid changes in sludge bed volume must be a result of entrapment of gas in the bed. Reactor C when dismantled at the end of operation was found to contain 10 to 20% gas in the lower sections of the bed. Extreme cases of gas entrapment sometimes occurred after reactor upsets. Whole sections of the bed would be lifted by gas bubbles that filled the entire reactor cross section and as much as 400 mm vertically. This behaviour was most prevalent in the more frequently upset reactors A and B. It seemed to

occur when reactor upsets led to a collapse of the bed structure, blocking the liquid and gas bubble flow paths. This same type of bed structure collapse could also release entrapped gas bubbles causing a rapid bed volume decrease. Thus loss of large entrapped gas bubbles would add to the bed structure breakdown and possibly further reduce the suspended solids holding ability of the bed. Higher losses of SS would occur from the bed until the extra entrapped gas is released and bed structure is reformed.

Over days 104 to 110 elevated gas yields were noted as shown in Figure 4.12. The bed was noted to be very active in terms of large gas bubble evolution. This may represent the break through of the high fibre/high gas section of the bed postulated above. After this event the effluent SS levels began to climb suggesting the bed was becoming less cohesive. Shortly after this the bed level began to fall. The bed volume was found to be a dense cohesive mass when reactor C was dismantled on day 377 of operation, this was the normal type of structure for these sludges

The "normal" bed structure consisted of numerous iron rich granules surrounded by a thick matrix of solids with the consistency of wet clay. With a smaller bed volume, as seen after day 125, the SRT was expected to be lower and thus time for solids dissolution less. The solids mass balance for "pseudo steady state" Cd plotted in Figure 4.11 shows a net loss of SS would be expected for the period of day 120 to day 160. However, at this time percentage SS degradation was relatively high. This suggests that it is not just the bed volume and SRT that effects the degradation of SS in these granular reactors. It is postulated that the degree of development of the bed matrix is the determining factor for suspended solids degradation.

4.6 GENERAL OPERATIONAL CONSIDERATIONS

In summary, these reactors had a number of periods where "pseudo-steady state" data was be collected. COD removal, gas yield and VFA concentration data indicate that these periods were reasonably stable. The main deviation from a"pseudo-steady state" is the relatively rapid changes in sludge bed volume, but these changes are not due solely to large changes in active bacterial biomass but also to changes in the amount of wood biomass and gas accumulated in the sludge bed. This raises concerns for large scale, long term use of this system. The filling of the reactor with bacteria and wood biomass, at high loading rates, would result in the need for frequent requirement for solids wasting. The removal of the wood suspended solids prior to reactor treatment of the wastewater would reduce but not eliminate this requirement. However, given the very fine nature of a major fraction of these solids total removal may not be economic (Paterson *et al*, 1992; Bird, 1990) and the methane return from the partial solids degradation would be lost. In this case the "iron-rich" granules (discussed in Chapter 6) with their excellent settleability, robust structure and stability provide an alternative answer to these problems. These very dense granules were found uniformly distributed throughout the bed with the exception of the 25 mm diameter inlet area where the smaller granules were displaced by the influent suspended solids. The granules were exceptionally well retained within the reactor and easily separated from the finer solids. The bed wasting incident in reactor D and its subsequent performance suggest that this type of removal of wood solids could be performed when necessary without overly affecting reactor performance. Any fragmentation of granules during such a process only supplies more nuclei for granulation.

4.7 "PSEUDO-STEADY STATE" ANALYSIS

Data collected from various reactor "pseudo-steady state" periods of operation were analyzed to assess reactor performance as a function of loading rates and hydraulic retention time. The "pseudo-steady states" selected are summarised in

Table 4.2. Two "pseudo-steady states" from the early reactor C operations are included in this analysis. A "pseudo-steady state" from reactor B is also included to allow comparison between the granular growth of reactors C and D and the flocculant growth of reactor B.

Table 4.2: "Pseudo-steady state" ("SS") parameters

Reactor	"SS" Designation	Duration (days)	OLR (gCOD/l.d)	HRT (days)
B	B	1062-1112	2.1	5.48
C	Ca	1250-1269	6.15	1.09
D	Da	17-29	7.2	1.62
C	Cc	10-21	7.6	1.55
C	Cb	1306-1331	11.0	1.09
C	Cf	120-160	16.4	1.09
C	Ce	90-120	17.5	1.05
C	Cd	30-75[a]	19.4	0.61
D	Db	41-74	27.1	0.44
D	Dc	120-163	36.7	0.47

a - Upset period omitted between days 45 - 65

4.7.1 Dissolved COD removal

As expected, the general trends for COD removals are for a slow decrease as the OLR increases and this is shown in Figure 4.13. The low point in dCOD removal at point ✚Db is directly attributable to the high constant propionic acid concentration present in reactor D during this period. Similarly reactor C at point ✚Ca had slightly elevated VFA levels. However the low dCOD removal at point ✚Cd is not readily attributable to elevated effluent VFA concentrations. When the separate dCOD removal efficiency trends of reactors C and D are considered, as shown in Figures 4.14 and 4.15 respectively, it can be seen that more than the OLR applied is effecting this parameter.

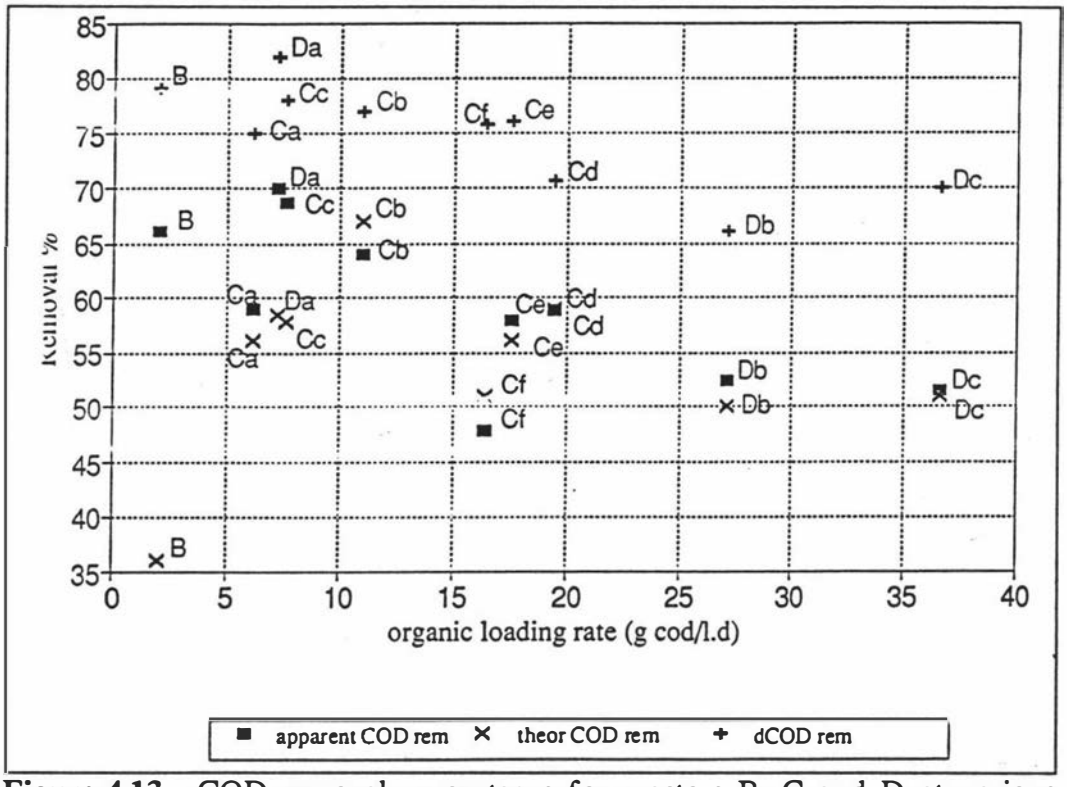

Figure 4.13 COD removal percentages for reactors B, C and D at various "pseudo steady states" versus organic loading rate.

When the hydraulic retention time is considered as shown in Figure 4.16 it can be seen that dCOD removal is directly related to the HRT and that differences in OLR (6.15 - 17.5 kg $COD.m^{-3}.d^{-1}$) at the same HRT (points ✚Ca, ✚Cb, ✚Ce

and ✚Cf) have little effect upon the dCOD removal efficiency. Point ✚Ca fits the trend relative to HRT rather than OLR. This fits the expectation that the contact time of the dissolved fraction of the feed with the reactor bacteria will be determined by the HRT because this fraction is not easily separated from the feed liquid..

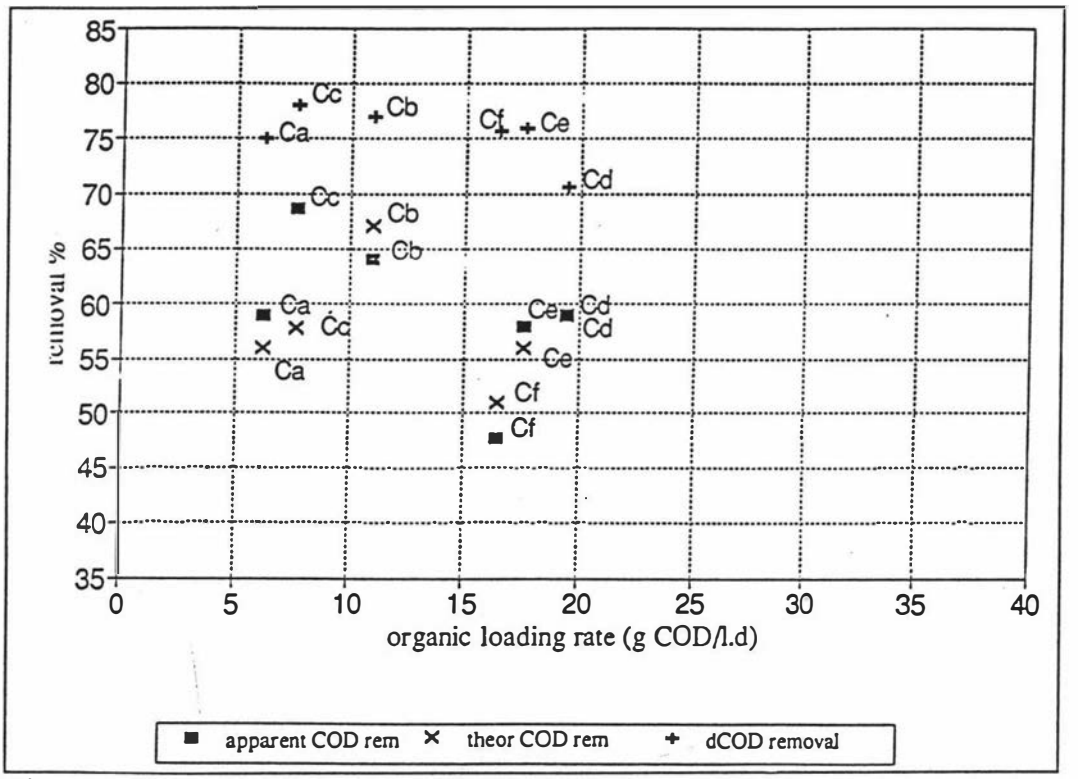

Figure 4.14 COD removal efficiencies for reactor C only.

The difference between points ✚Cc and ✚Da is attributed to the higher influent VFA concentrations for reactor D described earlier. These two "pseudo steady states" were performed soon after reactor start-up when the bed matrix had had little time to built up. Thus if the matrix fulfils an acidogenic role for the predominantly methanogenic granules better performance would be expected from the reactor receiving the highly acidified influent. That this is the case suggests that the reactors at this stage were limited by the acidogenic step of the methane fermentation.

4.7.2 Apparent and theoretical COD and SS removal

The apparent COD removal efficiency, point ■Ca, in Figure 4.13 was from a period when reactor C was operated with diluted feed. The bed volume had remained relatively constant for 800 days, suggesting little further accumulation of solids in the bed was occurring. With no gas solids separator (GSS) present effluent SS was never low unless gas production was very low. These factors in combination result in poorer apparent COD removal percentages than seen in reactors C and D at similar OLR levels (points ■Cc and ■Da respectively). In these reactors, at that time, the bed was not at equilibrium having just been re-established, considerable amounts of feed suspended solids were accumulating in the bed. Thus apparent COD removal was higher than that evidenced by methane production (theor COD_{rem}).

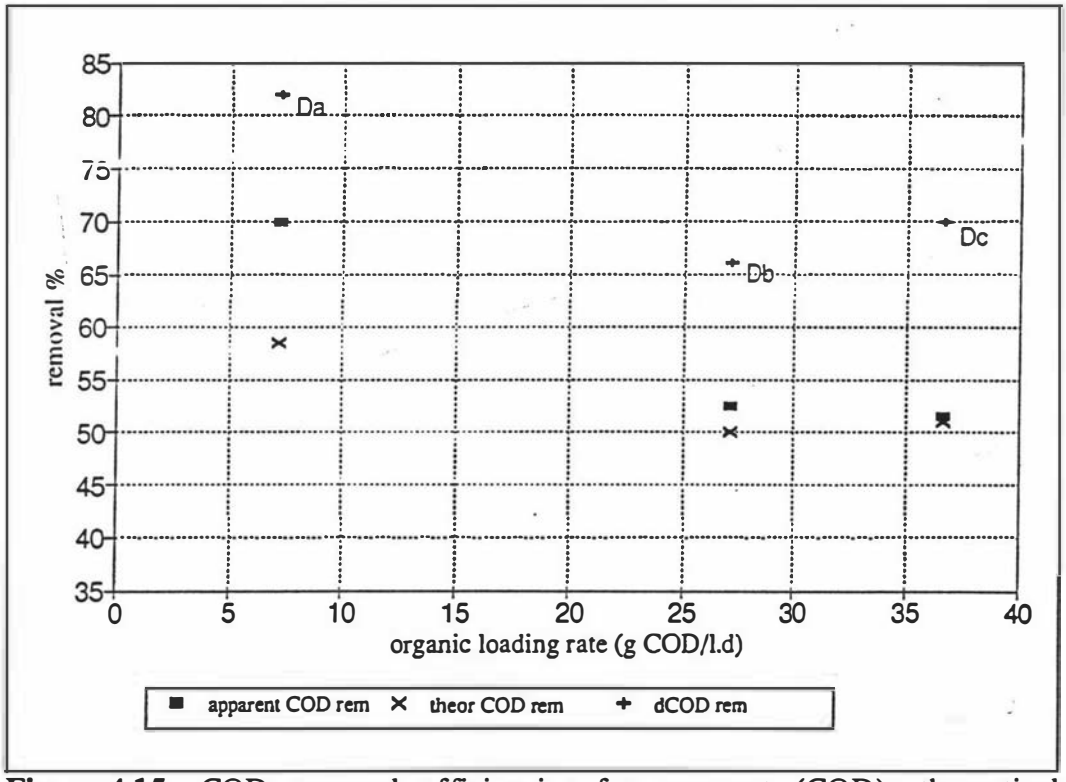

Figure 4.15 COD removal efficiencies for apparent (COD), theoretical (CH_4 COD) and dissolved COD (dCOD) for reactor D only.

The point ■Cf was from a period of operation when the bed volume began to drop as discussed in Section 4.3.4. This resulted in lower suspended solids retention and a lowered total COD removal.

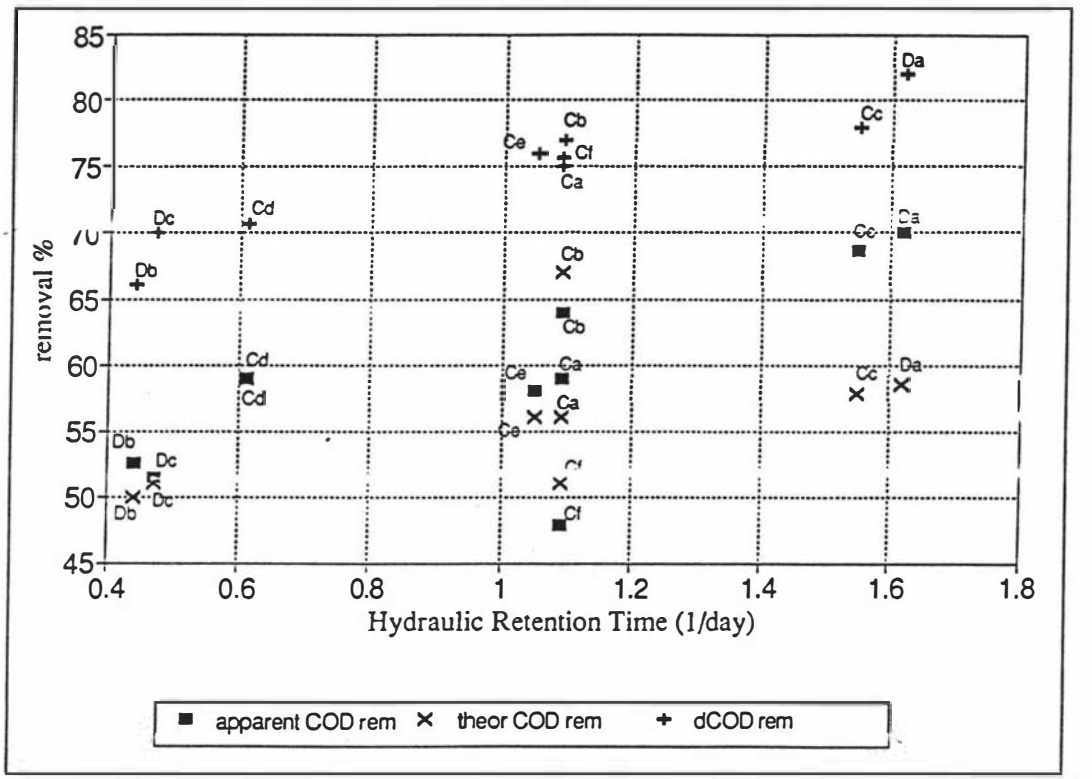

Figure 4.16 COD removal efficiencies vs the applied HRT for the "pseudo steady states".

Comparison of the measured COD removal with that calculated from the methane production shows that these two parameters generally follow each other with three notable exceptions. These are points B, Cc and Da on Figure 4.13. The relatively close agreement between measured and calculated COD removals, for most "pseudo steady state" periods, suggests that the solids degradation rate is similar to the rate of entrapment of solids within the sludge bed. This indicates that the rate of accumulation of feed SS in the reactors was low compared to the rate of incoming SS. Mass balances performed on the "pseudo steady states" using the methodology described in Section 4.2.3 show that from 6 to 10 % of the feed SS is accumulating in the bed for most of the points. For Cc and Da, where the difference between apparent and theoretical COD removal was 12 %, SS accumulation was 27 and 25 % of influent SS respectively.

Where the "pseudo steady state" Cf shows a higher theoretical COD removal efficiency (point ✖Cf) compared to the apparent COD (point ■Cf) removal, indicating a net loss of SSCOD from the reactor, the mass balance, effluent SS measurements and observations of bed volume also show a net loss of solids from the reactor. For the other "pseudo steady states" agreement between the theoretical and apparent points in Figure 4.13 and the other analysis parameters is generally good. The errors in the data analysis and the multiplying effect of the calculation methodology mean that agreement is not likely to be perfect. However the calculated, measured and observed parameters do show sufficient agreement to confirm the validity of the "pseudo steady state" data. Thus, where the theoretical removal ratio is higher than that measured, the dissolution rate exceeds the entrapment rate and *vice versa.*

Large differences in the apparent and theoretical COD removal values can be explained when the circumstances of the reactors are considered. The two "pseudo steady states" Cc and Da, at OLRs of 7.2 and 7.6 kg COD.m $^{-3}$.d^{-1}, are from soon after start-up. At this time the bed volumes were low. A rapid rise in the bed volumes showed that solids were accumulating in the beds. Thus the discrepancy between measured(points ■Cc and ■Da) and calculated COD removal (points ✖Cc and ✖Da) arises from solids held in the bed but not degraded. The other exception was reactor B, here growth was floc-based and fluidised with some degree of solids return and considerable mixing via the recycle. Calculations from the methane yield for this period shows that all the methane produced can be accounted for by the removal of the dissolved COD alone. Suspended solids were periodically shed from the bed of reactor B at effluent concentrations up to three times the influent. This shedding occured for one 4 - 5 day period over 70 days of monitoring. Thus no dissolution of the wood suspended solids is evident for this reactor.

Examination of the relationship of total COD removal with the HRT as shown in Figure 4.16 demonstrates that, unlike the dissolved COD, it is not so strongly

linked solely to the applied hydraulic retention time as shown by the four points of about HRT = 1.06 days. Clearly other factors are involved.

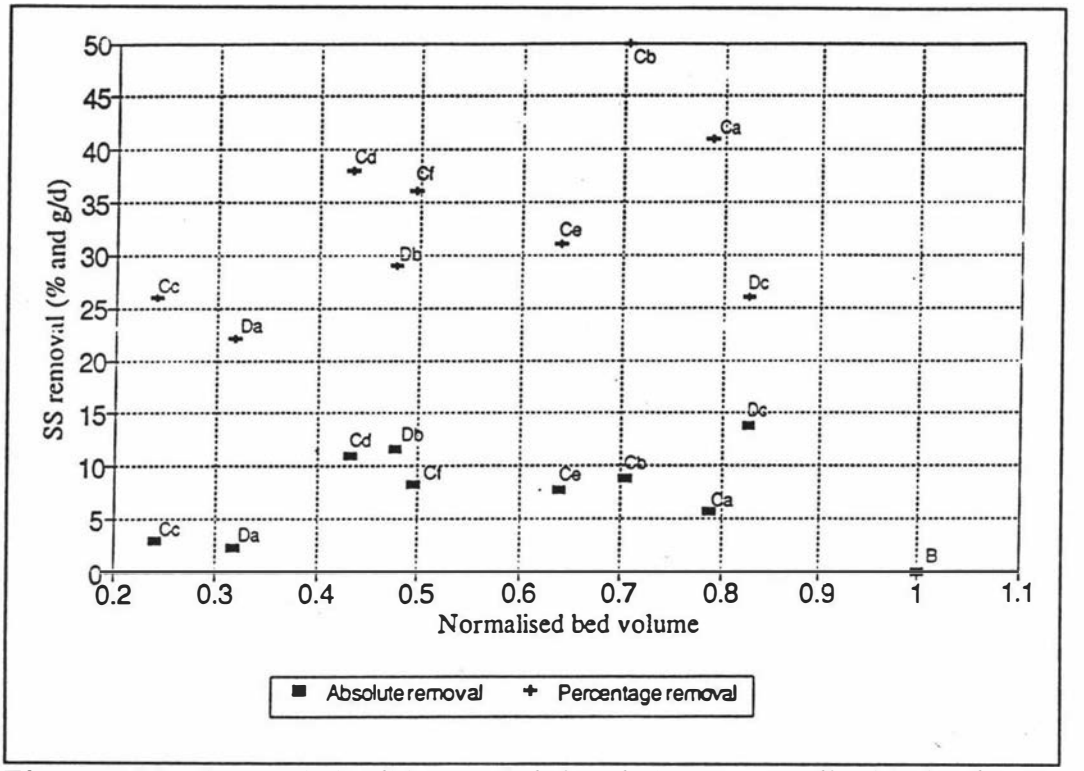

Figure 4.17 Suspended solids removals in relation to normalised bed volumes.

Calculation of suspended solids removal for the other "pseudo steady states" from the methane yield shows a strong link to the state of the sludge bed. Examination of Figure 4.17 shows that this is not simply a function of the bed volume but that an upward trend in absolute and percentage removals is evident. The SRT calculated by standard methods does not represent the retention time for the feed solids. A considerable proportion of the bed volume consists of granules which, because of their high density, have extended retention times. The actual retention time will be related to the proportion of feed SS in the bed volume and the pattern of retention of these solids. Examination of the bed of reactor C at the end of operation (day 378) showed that at least some of the feed suspended solids accumulate in the bottom of the reactor creating a filter bed for

subsequent solids. These solids will have a long residence time while those not trapped here will have a shorter residence. Thus the actual residence time distribution is related to the structure of the bed as well as the bed volume.

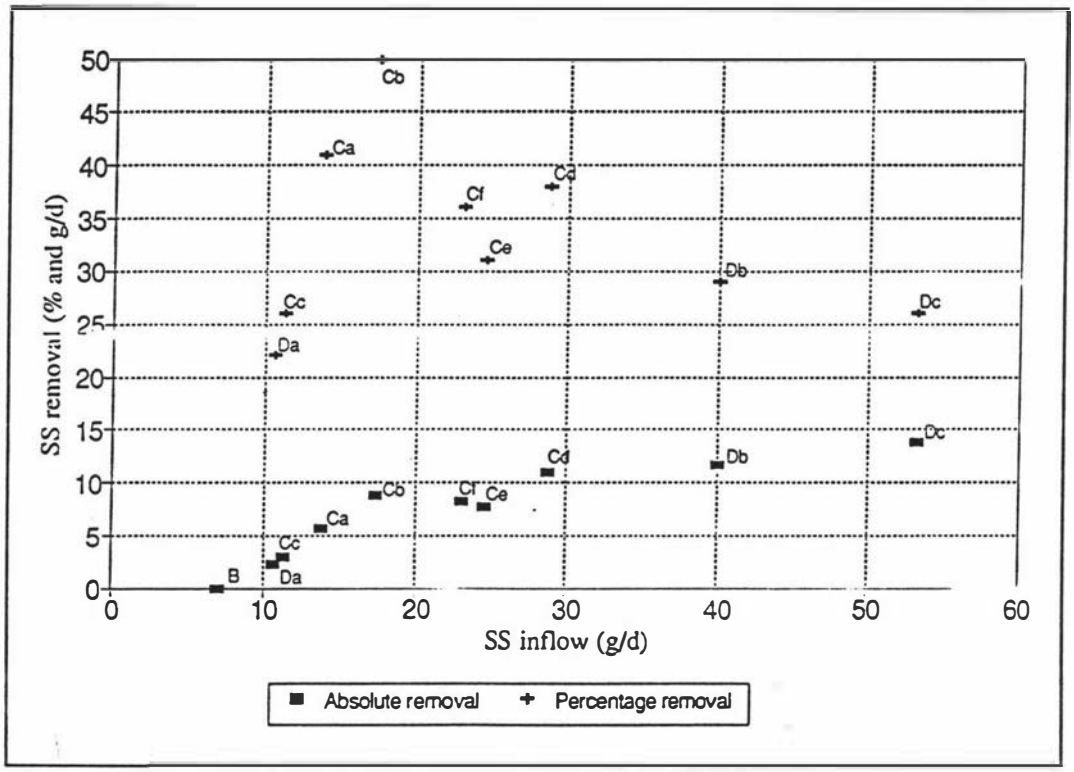

Figure 4.18 Suspended solids removals vs the SS inflow.

Reactor B, a floc-based growth reactor exhibited no SS removal as shown in Figure 4.18. This cannot be confirmed for the rest of the very long operational life of this reactor but all indications are that suspended solids removals were, at best, minimal. Calculation of total solids retention time for this period shows a similar duration to those calculated for the other reactors. Thus wood solids degradation cannot be simply a function of the residence time of the solids within the reactor. Some factor present in the dense sludge matrix of the granular reactors is missing from the open floc-based system. The major difference between the two systems is the matrix structure itself. This structure is discussed in Chapter 6 and it provides a variety of micro-environments within the reactor dependent upon depth within the matrix and height in the reactor. In reactor B, recycle and the loose nature of the flocs means that the micro-

environment experienced by the solids is essentially that of the bulk liquid throughout the reactor. Thus conditions within micro-environments of the matrix forming reactors may be more conducive to the degradation of wood solids. The VSS concentration of the granular sludge was three or more times that of reactor B. How much of this VSS was wood solids is unknown. This would be expected to give a difference in degradation rate but not determine the presence or absence of solids degradation. As has been suggested earlier reactor B did not receive as wide a variety of bacteria as did reactors C and D. Bacterial levels in the feed were not high for the life of B compared to the feed supplied to the later reactors. Bacteria capable of degrading the cellulose of the ligno-cellulosic wood solids may have been absent from the reactor. The matrix layers held the wood solids in a relatively immobile condition, this may have allowed more intimate contact between the wood solids and the degrading agent. Extra-cellular enzymes in particular would not be diluted as much in the matrix environment. Given the affinity of bacteria for surfaces, it is expected that intermit contact between wood solids and bacteria would occur as easily in the open environment of reactor B. The most likely mechanism is the provision of the necessary conditions for the degradation of wood solids by the matrix layer.

Overall the COD and SS removal rates show a shallow curve in relation to the OLR shown in Figures 4.19 and 4.13. Thus similar performance results over a wide range of loading rates. To some extent this was an artifact of the high concentration of finely dispersed wood solids in the influent. The lack of a gas-solids separator (GSS) in these reactors meant that a higher overflow rate of solids was expected. It was this factor, giving severe selection of settling solids, in combination with the flocculating and granule forming abilities of iron, that has been identified as being responsible for the formation of granules in this difficult wastewater. The disadvantage of this was that suspended solids are not settled well once they escape from the bed. Thus even at low loading rates effluent SS values remained high (typically 1000 - 2000 $mg.\ell^{-1}$), adversely effecting COD and solids removals. At higher loading rates soluble COD increased readily but at least some of the SS in the influent was retained by the

bed acting as a filter. Released SS lost from the reactor appears to have come predominantly from the top of the bed. At least some of the released SS was non-wood biomass. The increase in effluent SS, above the base level present because of the absence of a GSS, seems to be largely a function of the state of the bed rather than just the applied loading rate. Comparing the theoretical COD removal, calculated from methane production, with the measured values show that there were periods of operation where SS were accumulated in the bed and others where there was a loss of solids. Comparison of reactors C and D operational data shows that major solids losses from the sludge bed were a result of external events creating reactor upsets. Provision of a GSS would reduce the amount of suspended solids lost from the reactor and give a higher quality effluent. The increased retention of suspended solids in the reactor would result in a faster filling of the reactor increasing the demand for solids wasting. Further work is needed to provide the cost-benefit analysis of this option. Another option is the provision of floating or suspended anaerobic filter media above the sludge bed. This would return some of the suspended solids to the bed but would entrap a major proportion of the rest of these solids within the filter media. Another advantage of this option would be the further polishing of the dissolved COD component of the effluent. The solids loading on this filter zone would still be high, perhaps 30 to 50 % of the solids load to the reactor. Thus a large volume of filter media would be required. The success of this option is dependent upon achieving the necessary conditions for wood solids degradation and upon the rate of that degradation, otherwise clogging of the filter bed would be a major problem. Again further work is needed to investigate this option.

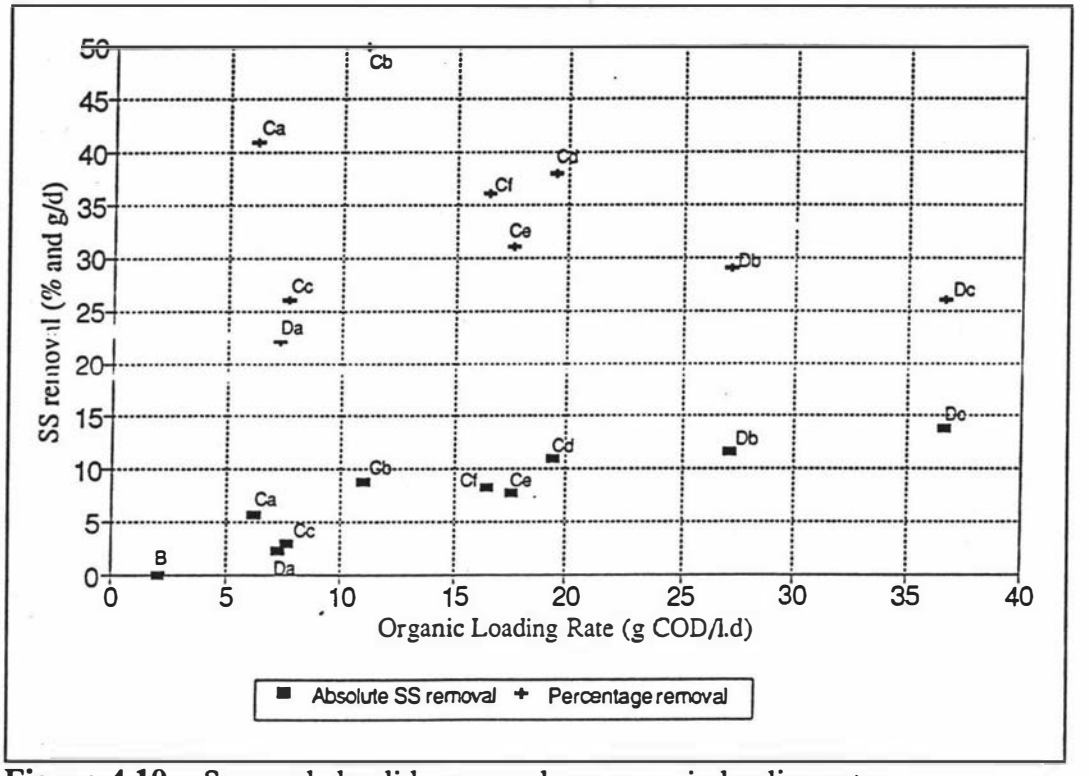

Figure 4.19 Suspended solids removals vs organic loading rates.

4.8 COMPARISON WITH OTHER TMP ANAEROBIC DIGESTION WORK

Over the past decade a number of investigations of the anaerobic treatment of TMP wastewaters have been conducted. Some of the wastewaters investigated contain other pulp mill wastewater streams to a minor extent. These streams are not reported to be the cause of any major operational problems in these instances. A wide variety of wastewater compositions have been utilised, a result of the wide variety of TMP process configurations and operating conditions used throughout the world. Table 4.3 shows the wastewater composition and operational performance reported in the literature.

Table 4.3: Wastewater composition and operational performance reported for anaerobic treatment of TMP wastewaters.

Feed COD ($mg.\ell^{-1}$)	Dissolved fraction (%)	OLR (HRT) $kg\ COD.m^{-3}.d^{-1}$ (days)	COD removal (%)	dCOD removal (%)	Ref
25200	32	5 (6)	80	76	1
25200	32	7 (4)	80	76	1
3035	75	23.1 (0.21)	29	41	2
2000-5000	95	10 - 18	62		3
7500-10400	80	6.8 (1.1)	43	54	3
7500-10400	80	13.9 (0.75)	27	54	3
7500-10400	80	13.9 (0.56)	41	57	3
7500-10400	80	15.9	34	47	3
4000	>85	9-18.5	45		3
4200	>85	8-15	30-40		3
8600	99	7 (0.92)	75		4
8600	99	7 (0.58)	65		4
8600	99	11 (0.88)	90		4
500-2400	>85	3-6 (0.16-0.25)	50		5
2500	>85 [1]	12.1 (0.2)	55		6
1800-3300	96	4.7 (0.65)	59		7
1800-3300	96	10.5 (0.27)	71		7
1800-3300	96	10.7 (0.28)	63		7
1800-3300	96	14.3 (0.16)	76		7
1800-3300	96	22 (0.14)	72		7
1800-3300	96	49.3 (0.06)	62		7
1800-3300	96	80.9 (0.04)	61		7

Feed COD ($mg.\ell^{-1}$)	Dissolved fraction (%)	OLR (HRT) kg $COD.m^{-3}.d^{-1}$ (days)	COD removal (%)	dCOD removal (%)	Ref
4000	>90	0.5-2.5 (.7-1.0)		30-55	8
4000	>90	1.4-2.1 (.9-1.7)		30-53	8
4000	>90	2.7-3.9 (.9-1.4)		28-48	8
4000	>90	2.5-11 (..3-1.6)		38-60	8

Literature for Table 4.3:

1 M^{c}Farlane and Tan, (1985)
2 Richardson *et al*, (1991)
3 Habets and de Vegt, (1991)
4 Vinas *et al*, (1993)
5 Rekunen, (1985)
6 Sierra-Alvarez *et al*, (1990)
7 Rintala and Lepisto, (1992)
8 Jurgensen *et al*, (1985)

As can be seen from Table 4.3 most of the wastewaters treated are almost completely soluble. Only M^{c}Farlane and Tan, (1985) report any SS degradation. Analysis of the data presented in this paper, using the methodology described in Section 4.2.3, demonstrates that no degradation of SS is evident. Theoretical COD removals were calculated to be 22 and 32 % for the two loading rates quoted. These recalculated results are included in Figure 4.20 a plot comparing literature COD removals for TMP-type effluents with those of this work. Where a range of values have been given the data have been plotted as the maximum point of the given range in terms of both OLR and COD reductions.

The literature values show a wide range of values obtained from a number of reactor types, These are predominantly UASB reactors but include anaerobic filters, contact reactors, expanded bed reactors and two-phase reactors. It must be considered that most of the experiments described are of relatively short duration (3 - 6 months) and further work may have improved the reactor or biomass effectiveness. However no literature is available that reports so.

In comparison, experimental dCOD removals from this project fall at the high end of the data reported but are not clearly superior. However the experimental total COD removals from this project are clearly superior to those achieved with any of the other high suspended solids feedstocks treatments reported. The dissolved fraction of the feedstock COD used for this project was 55 - 60 % and the COD 12,000 -18,000 mg.ℓ^{-1}.

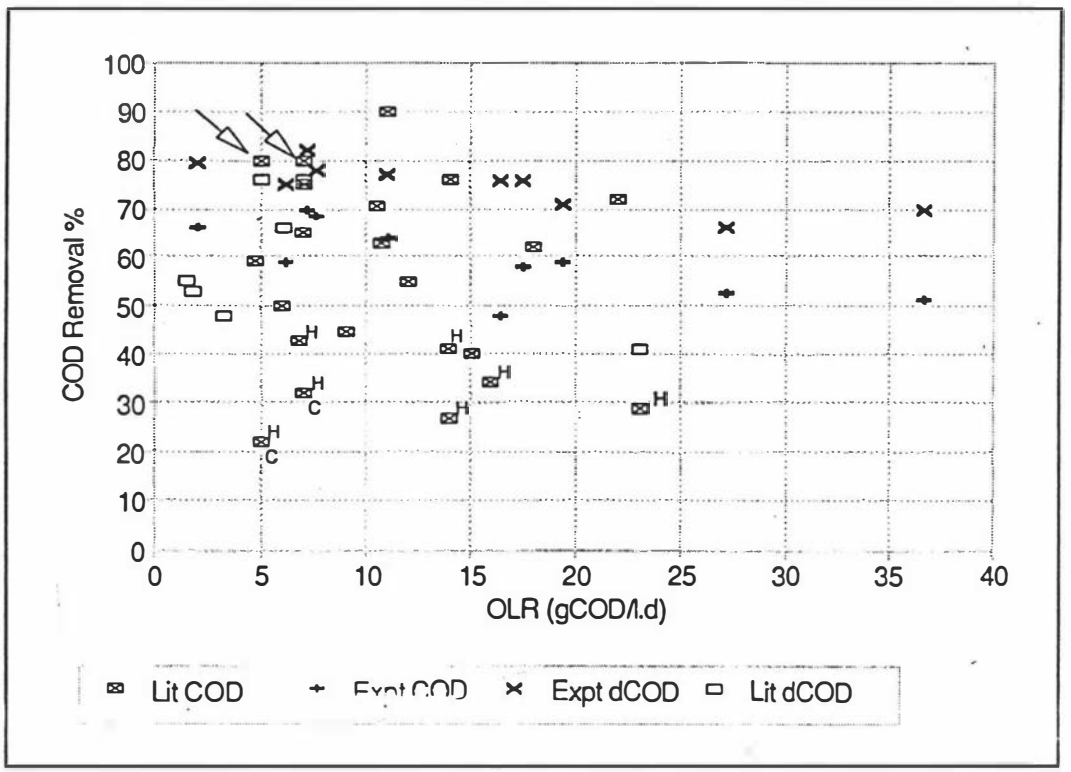

Figure 4.20 A comparison of literature COD and dissolved COD removal values with those generated in this work.

The points marked with an superscript "H" are the higher solids feed experiments.

The two points marked with arrows are those that were recalculated and the actual COD reduction points from this are marked with subscript "C".

4.9 SUMMARY

A reactor configuration has been developed that will successfully treat Thermo-mechanical pulp mill wastewater without prior solids removal. Up to 50 % degradation to methane of influent suspended solids was achieved. Effluent SS from the reactor showed a marked improvement in settling ability compared to the influent SS. The reactors did become filled with sludge but even severe solids wasting events did not seriously interrupt reactor performance. Floc based UASB systems in this work and in the literature did not exhibit any suspended solids degradation ability. The strongly granular nature of the sludge bed developed in these reactors is the only significant difference between these wood solids degrading reactors and others that showed no activity. In Chapter 6 the sludge and granules are examined to try to determine the reasons for this successful treatment of TMP effluent.

CHAPTER FIVE RESIN ACIDS

5.1 INTRODUCTION

When this work was started little was known about the toxicity of resin acids to biological treatment processes but their toxicity to fish had been demonstrated. Removal of 90-95% of resin acids from an initial concentration of 0.5 mg.ℓl^{-1} by aerobic treatment was reported but no data had been published on the anaerobic biodegradability of the resin acids (McFarlane and Tan, 1985). Recent publications have reported resin acids to be toxic to anaerobic bacteria and especially the methanogens but that the degree of solubilization in the TMP process is low. (Sierra-Alvarez and Lettinga, 1990; Habets and de Vegt, 1991). Analysis of feed and effluent samples from this work show a range from 0-18% of the total resin acids were soluble for feed and 4-25% for effluent samples, as shown in Table 5.4.

This chapter examines the changes to resin acid type and concentration resulting from treatment in UASB reactors and investigates the inhibitory effect of the resin acids upon an acclimated methanogenic consortia.

5.2 RESIN ACID NOMENCLATURE

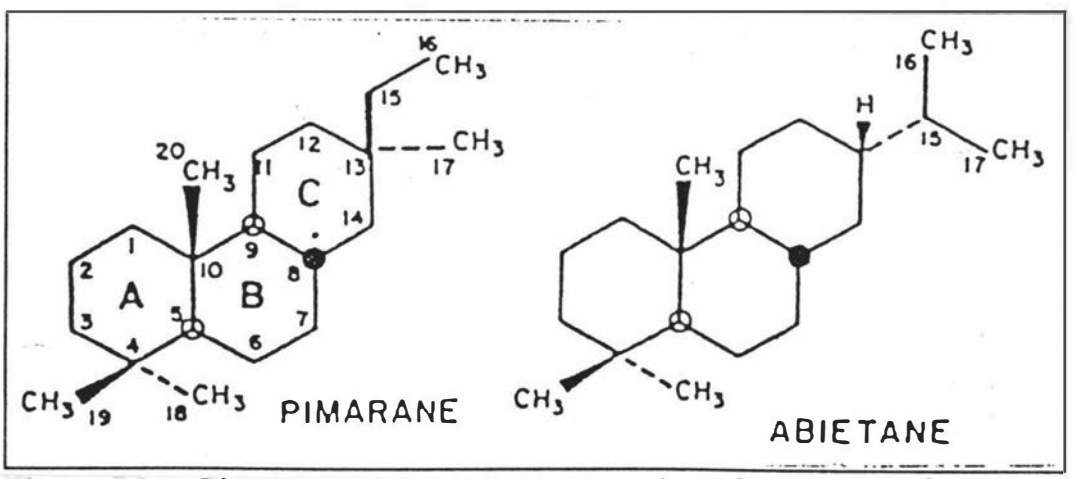

Figure 5.1 Pimarane and Abietane skeletons from Chang *et al* (1971).

Resin acid nomenclature in this document follows that of Chang *et al*, (1971). The resin acids are normally subdivided into two groups; the abietane group, which has an isopropyl side chain at position 13 and the pimarane group, which has methyl and vinyl constituents in that position. Structures and the numbering of these two parent skeletons are presented in Figure 5.1. The isopimaranes are cis-trans isomers of the pimaranes about carbon 13. Naturally occurring resin acids, defined as those observed in growing *Pinus radiata* trees have generally been given additional simple names for ease of use. These are shown in Figure 5.2 along with other resin acids significant in this work. Two other sub-groups have been named, the dehydroabietanes: the family of aromatic resin acids with an isopropyl group at position 13 and the secodehydroabietanes: the family of aromatic resin acids with an opening in ring B and also sometimes in ring A. The IUPAC nomenclature for these compounds is to say the least unwieldy. These names are often of the order of 130 characters long containing many numbers. Differences between acids are often signified by no more than a change in the accent on one of those characters. Thus the IUPAC names have not been used in this document.

5.3 RESIN ACID ANALYSIS

The resin acid sample preparation and analysis procedure was taken from that used by the New Zealand Forest Research Institute (FRI). A capillary gas chromatograph column of the same specifications and operated under the same conditions as those used by FRI was chosen to simplify the identification of eluted compounds. FRI had spent considerable time and resources on the GCMS identification of the peaks. Unfortunately by the time resin acid samples were taken to FRI for CGMS confirmation of peak identification they had changed the type of column used. Change from a very polar column to the neutral HP1

column resulted in different elution orders and co-elution. This meant that even with four very different samples analyzed by GCMS at FRI, identifications of all compounds was not possible.

To perform the identifications the specific column used in this work was installed in the Hort Research VG 70-250S double focusing magnetic sector mass spectrophotometer (VG Analytical, Manchester, England) GCMS. One sludge and one feed sample were analyzed using the normal temperature programme and carrier gas flow rate. This gave traces that were very similar to those obtained on the Carlo Erba capillary GC normally used for the analyses. The only discernable difference was in peak shape. Peaks from the GC were tailing whereas those from the GCMS were leading. This resulted in one small peak, that in the GC trace eluted on the tail of a large peak, not being resolved from the maximum of the large peak. Analysis of the mass spectra at ten points in the resulting peak allowed separation of the two overlaid spectra sufficiently to identify the two compounds.

Computer data base matching of the mass spectra was not conclusive for most of the peaks. Two reasons for this were determined. Small air leaks gave a number of low molecular weight peaks in the spectra that interfered with the matching protocol. The data base available was not a forestry industry database and did not contain all of the known resin acids. Final identifications were performed manually using mass spectra obtained from FRI (Chang *et al*, 1971 and Stuthridge, 1990). One factor noted was that, in many of the spectra from this work and those of Stuthridge, 1990 the molecular ion and other high mass peaks had a lower intensity than seen in the spectra of Chang *et al*, (1971). The reasons for this are unknown. This factor made final identification of some peaks difficult, especially where the spectra of different acids were very similar. Two peaks could only be identified as being similar to acids already identified to be the other peak of the GCMS trace. These two peaks were tentatively identified as isomers of these already assigned acids.

5.4

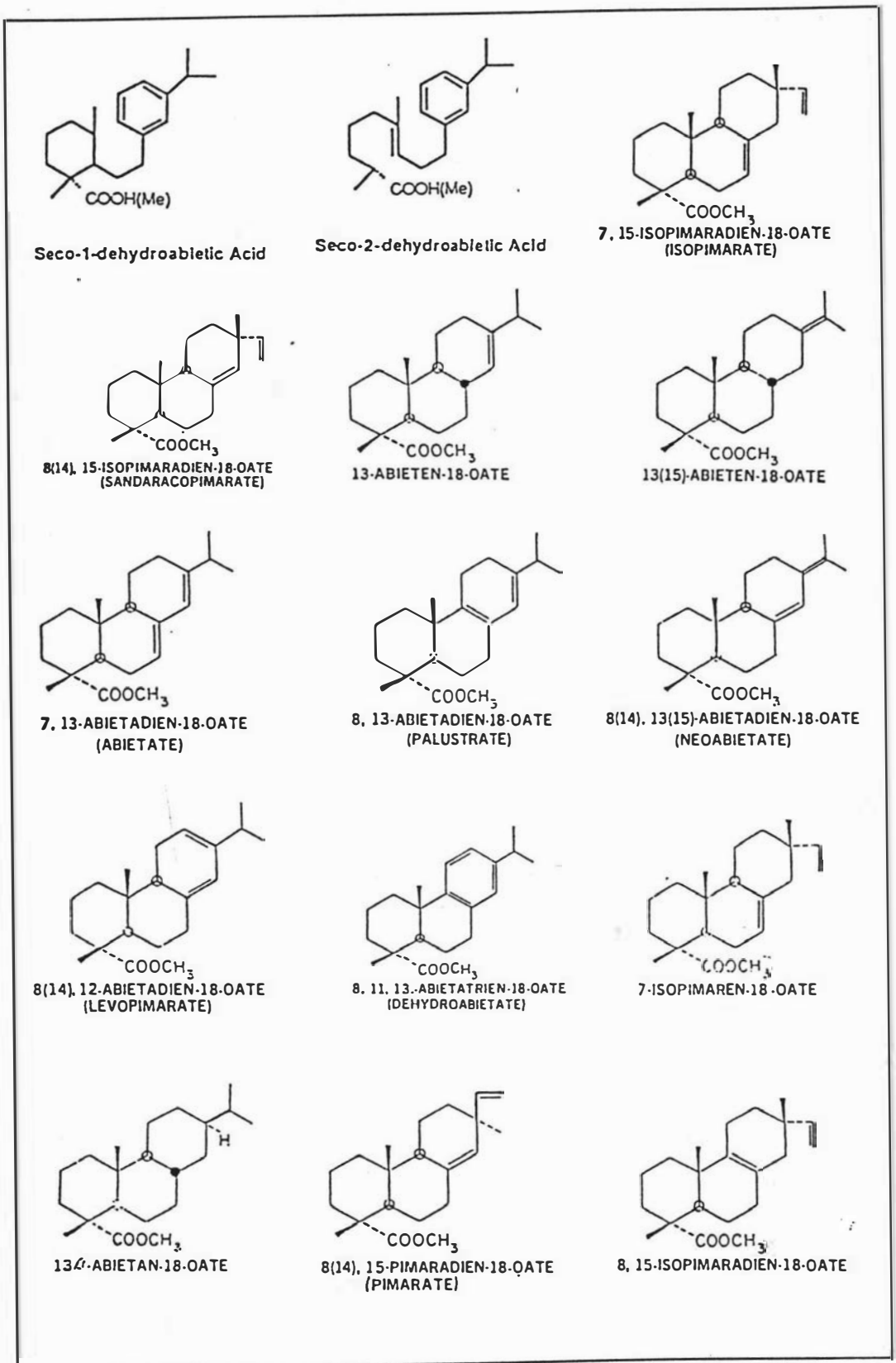

Figure 5.2 Structures, systematic and simple names of significant resin acids (Chang *et al*,1771).

There were three instances of peaks eluting at, or very nearly at, identical retention times in the two GCMS traces that were identified as being different resin acids. In either of the two samples analyzed by GCMS no spectra of the acid from the other sample could be discerned in any of the three instances. In all three cases the two acids were of different molecular weights and their mass spectra easily differentiated. While this was the case in these two samples it can not be ruled out that these acid "pairs" do not occur simultaneously in some samples. In reporting resin acid concentrations the assumption has been made that the "feed" acids of the pairs are characteristic of feed samples and the "sludge" acids are characteristic of sludge and effluent samples. However it must be considered possible that the other of the "pair" is present. The three resin acid "pairs" are shown in Table 5.1. Resin acids are named as the acid rather than as the methyl ester.

Table 5.1: Resin acid "pairs" with similar elution times

Feed	Effluent and sludge
8,15-isopimaradien-18-oic acid	8-pimaren-18-oic acid
8,15-pimaradien-18-oic acid	18-isopimaranoic acid
8,13-abietadien-18-oic acid (Palustric acid)	7-isopimaren-18-oic acid

For the first four sets of samples the dichloromethane extract weight was measured. This required the use of desiccated, pre-weighed round bottomed flasks to which the dichloromethane extract solution was added. Dichloromethane was evaporated by rotovaporator and the flask desiccated and weighed. This process was repeated until a steady weight was reached. The time required to measure the dichloromethane extract weight made the sample preparation procedure prohibitively long and equipment intensive. It was also found that feed samples had dehydroabietic acid as the predominant resin acid rather than abietic acid. Commonly, no abietic acid was present at all. In *Pinus radiata* chips subjected to pulping, abietic acid is the predominant resin acid

(McDonald and Porter, 1969; Porter, 1969). Two feed samples analyzed earlier at FRI showed the expected pattern of predominant abietic acid. When the dichloromethane extract weight part of the sample preparation procedure was omitted feed samples showed the expected resin acid pattern. This effect was not a function of different batches of feed. From this it is concluded that conditions during the extended rotovaporation and desiccation stages for the dichloromethane extract weight could lead to the isomerisation of abietic acid to dehydroabietic acid. It was not determined whether the single rotovap step in the standard extraction process was causing any isomerisation.

Resin acid samples were all extracted and methylated in batches of seven or eight. Some doubts arose about the reproducibility of results between batches. To test this replicates of one feed sample were extracted in ten subsequent extraction batches. These data, shown in Table 5.2, also serve as an estimate of precision for the procedure. No pure resin acids were available to allow the accuracy of the procedure to be estimated. Resin acid recoveries of 68% and fatty acid recoveries of 55% at pH 2 using methyl *tert.*-butyl ether have been reported (Voss and Rapsomatiotis, 1985) but that recovery is dependent upon effluent type. The recovery of isopimaric, dehydroabietic and neoabietic acids have been found to vary with acid concentration and from acid to acid (Zender *et al*, 1993). The variation is more extreme in neoabietic than the other two measured acids. Standard deviations reported for the resin acids in natural effluents suggest that most of the resin acids will show a smaller degree of variation than neoabietic. This suggests that the differences seen in resin acid recovery from different effluents may be a function of the types of resin acid present rather than other factors of that effluent and that recovery of individual acids will be relatively uniform. Examination of the standard deviations reported in Zender *et al*, (1993) suggests that the relative recovery ratios between acids will be sufficiently uniform to allow use of the resin analysis data.

The recovery of the internal standard, arachidic acid, from a pure solution was found to be 100 ± 5%, which was the limit of weighing accuracy for the quantity

normally used. However anomalous differences between some samples could be explained by incomplete recovery of the internal standard during the redissolution step. The repeat extraction and analysis of one sample as dicussed abovesuggests that this was not a problem in the later analyses.

Table 5.2: Estimation of the precision of resin acid and long chain fatty acid measurement for a typical feed sample.

Acid	Mean concentration ($mg.\ell^{-1}$)	Standard deviation (s.d.) ($mg.\ell^{-1}$)	Relative s.d. (%)
Palmitic	11.0	1.04	9.5
Oleic	15.8	0.95	6.0
Linoleic	10.0	0.77	7.7
8,15-isopimaradien-18-oic	15.2	4.44	29.2
Seco-1-dehydroabietic	5.9	2.49	42.2
Pimaric	34.8	5.86	16.8
18-isopimaranoic	12.7	3.18	25.0
13-abieten-18-oic	92.8	16.30	17.6
13β-abietan-18-oic	2.3	2.48	107.8
~7-isopimaren-18-oic	46.3	9.58	20.7
Palustric	3.3	3.14	95.0
Isopimaric	16.4	7.80	47.6
13(15)-abieten-18-oic	18.0	8.29	46.1
abietic	226.7	24.85	11.0
Dehydroabietic	66.8	5.24	7.8
Total acids	578.3	35.70	6.2
Resin acids	541.2	35.73	6.6
Long chain fatty aids	37.1	2.58	7.0

5.8

Because of problems with the capillary gas chromatograph and with the data capture software some samples had to be stored for more than a year. Where possible these were acidified to pH 1.5 and frozen. Storage temperature was below -18°C. In some cases unacidified samples that had been stored at 4 °C had to be used. To estimate the possible degradation or isomerisation of these acids a number of samples were repeat sampled after one to six months continued storage.

Table 5.3: Acid sums before and after varied storage times

Sample	First storage (months)	Second storage (months)	Sum of acids ($mg.\ell^{-1}$)	Sum of resin acids ($mg.\ell^{-1}$)	Sum of LCFAs ($mg.\ell^{-1}$)
D[a] eff	13	-	603.8	549.5	54.4
		1	565.6	518.4	47.2
D feed	7	-	559.9	520.1	39.9
		5	599.0	578.9	20.2
D eff	16	-	401.8	401.8	0
		1	375.3	375.3	0
D eff	13	-	487.8	449.8	37.9
		1	447.6	416.5	31.1
D eff	16	-	402.2	402.2	0
		1	355.4	355.4	0
D eff	12	-	198.3	197.7	0.6
		6	198	198	0
D eff	9	-	543.1	491.8	51.4
		6	556.0	527.0	29.0

a - reactor designation

Overall, the sums of total acids, resin acids and LCFAs remain approximately the same for each sample pair as shown in Table 5.3. This indicates that little degradation occurs with sample storage by whichever method. The long chain fatty acids do appear to under go some degradation but this seems to be variable from acid to acid and sample to sample. Similar types of samples processed with less storage time show similar levels of LCFAs. This suggests that the variation seen in the sample pairs is a result of the degree of reproducibility of the individual acids when using this procedure. The size of the peaks involved in most of these cases is such that signal noise would make a significant difference in peak height. Variation in the individual resin acids between the sample pairs does not follow any pattern, suggesting the variation is due to errors in measurement of the individual acids. Some show an increase in concentration with continued storage, others a decrease. It is not always the same acids changing in each set of sample pairs as can be seen in Figure 5.3. This data indicates that no significant changes in the resin acids occur during the later stages of long term storage of samples.

The sample pairs data discussed above implies that any resin acid changing process was not continuous over the length of the storage interval. However it does not determine whether changes occur during the early stages of long term storage. The use of two different storage methods provides some evidence that short term storage was not engendering changes in resin acids in samples. No significant difference was seen between similar samples stored at pH 1.5 and -18^{o}C and those stored at 4 ^{o}C at neutral pH. Any bacterial mediated changes would not occur in the acidic frozen samples. The above data suggests that these changes were not occurring in the neutral refrigerated samples. Isomerisation was more likely to occur at pH 1.5 than at neutral pH (Voss and Rapsomatiotis, 1985). Any pH and temperature mediated isomerisation was likely to occur in the period between acidification of the sample and its subsequent freezing. Again differences would be expected between similar samples stored under the two sets of conditions. Differences were not evident between samples stored by the different methods. This was circumstantial evidence that no significant

changes in the resin acid concentrations were occurring in the various samples utilised in this work.

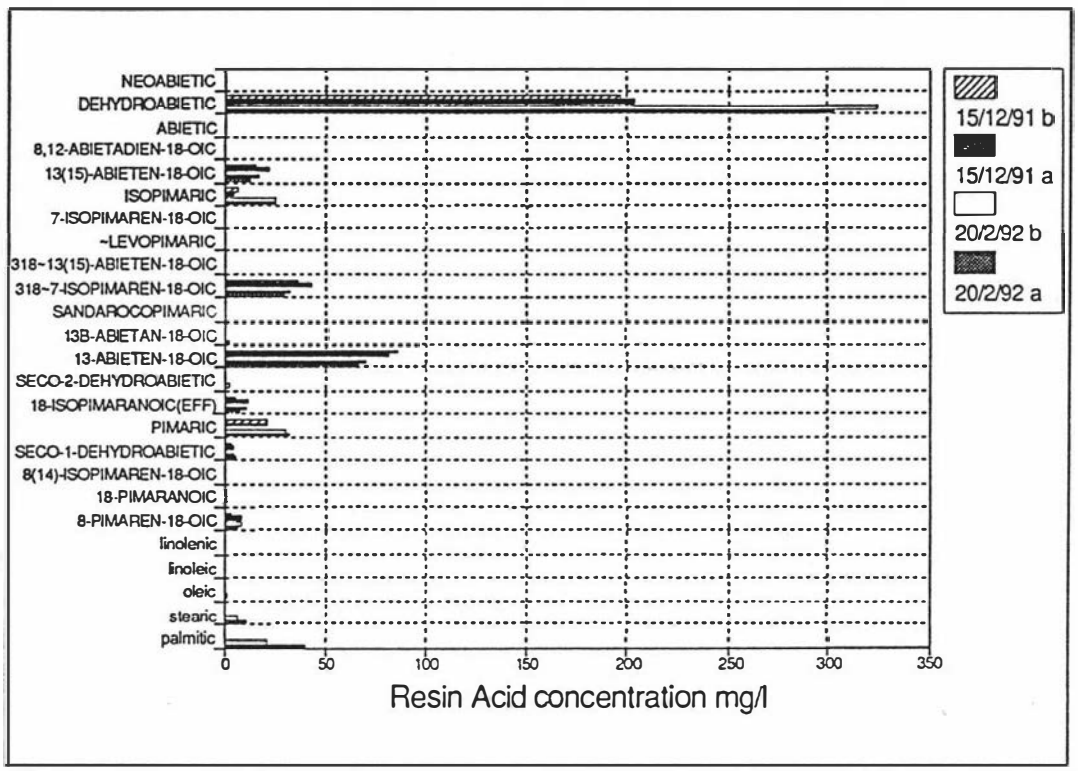

Figure 5.3 Resin Acid sample pairs. Samples are reactor D effluent. Details for the 15/12/91 and 20/2/92 samples are given in Table 5.3, the third and last sets of data respectively.
a - first analysis b - second analysis.

5.4 CHANGES IN RESIN ACIDS IN THE UASB REACTORS

5.4.1 Resin acids in the feed

Characterising the feed is difficult, as has been discussed in Chapters 3 and 4. In particular the concentration of solids is very variable both at the Pan Pacific Forest Industries(NZ.) Ltd. pulp mill and in the collected feed used for this work. In TMP wastewaters the resin acids are generally insoluble and associated with the fines (Sierra-Alvarez, 1990). This makes them predominantly inaccessible

biologically and of low toxicity. Analysis of the soluble fraction of various feed samples shows some variability but indicates low solubility as shown in Table 5.4.

Some of the dissolved resin acids may actually be part of the very fine suspended solids fraction that passed through the GF/C filter paper used for the separation of suspended solids from the samples. Thus the dissolved resin acids could be even lower in concentration than indicated in Table 5.4. This means that for this waste almost all the resin acids exist associated with filterable solids. Any problems in sampling solids accurately, as discussed in Sections 3.1.5.3 and 4.2.2 will result in similar variation in measured resin acid concentrations. The nature of the feed handling systems, from collection from the screw press feeders at the pulp mill to provision to the reactors, however modified, still resulted in a constantly varying feed stream. This is a realistic situation in that the wastewater stream at the mill also varies from a year to year down to a minute by minute basis (Paterson *et al*, 1991). While the reactors were receiving a relatively uniform feed composition averaged over a week and within each feed batch, the point in the feed addition - depletion cycle at which the feed was sampled had a great effect on the measured feed composition. Effluent composition may have varied with the feed composition, especially when hydraulic retention times were low. However effluent samples, except for VFA analysis, were effectively composite samples of each effluent collection cycle. Feed samples were grab samples.

Table 5.4: Distribution of resin and LCFAs between the dissolved and suspended solids fractions of feed and effluent samples.

Sample	Dissolved		Suspended		Resin acids dissolved fraction %
	Resin acids ($mg.\ell^{-1}$)	LCFA ($mg.\ell^{-1}$)	Resin acids ($mg.\ell^{-1}$)	LCFA ($mg.\ell^{-1}$)	
B[a] feed 8/9/89	91	28	520	98	18
D feed 14/11/91	6	0	234	24	3
C feed 14/11/91	9	0	150	15	6
C feed 10/4/92	0	0	486	41	0
B eff 8/9/89	76.32	5	1053.9	0	7
D eff 14/11/91	62.2	1.9	250.7	0	25
C eff 14/11/91	62.3	0	311.2	0	20
C eff 10/4/92	23.4	0	524.7	11	4
C start up liquor	27.5	.3	268	0	10

a - reactor designation

The resin and long chain fatty acids compositions of a typical feed sample from a well mixed storage 205 litre drum are presented in Table 5.2. This is effectively an averaged sample of feed batch 10. Naturally occurring resin acids predominate but a significant amount of the transformed resin acid 13-abieten-18-oic acid is present. This acid has not been found in feed samples acidified at the mill and only in very low quantities in "fresh from the mill" feed drums. A rapid increase in 13-abieten-18-oic acid concentration is found after the feed batch blending process. Rapid VFA production also occurs as a result of this process, believed to be a result of the mixing of bacteria adhered to the settled wood fibres with the easily digested carbohydrates dissolved in the liquid. This combination of factors strongly suggests that 13-abieten-18-oic acid is formed by microbial action. A pathway has been proposed of hydrogenation of abietic acid (Zender *et al*, 1993). Microbial activity in the feed was quite variable, as measured by VFA production mainly as a result of the inoculation effect from

the old feed when fresh feed was added to the feed vessel. This explains the variation in 13-abieten-18-oic acid concentration in feed samples. Clearly some transformations of the resin acids were occurring in the feed vessels.

5.4.2 Resin acid mass balance over a reactor.

A mass balance of resin acids has been performed for reactor D for the duration of one feed batch. Problems with the estimation of the resin acid concentration of the previous batch, as discussed above, preclude a mass balance from the startup of the reactor. A pictorial representation of the mass balance is presented in Figure 5.4.

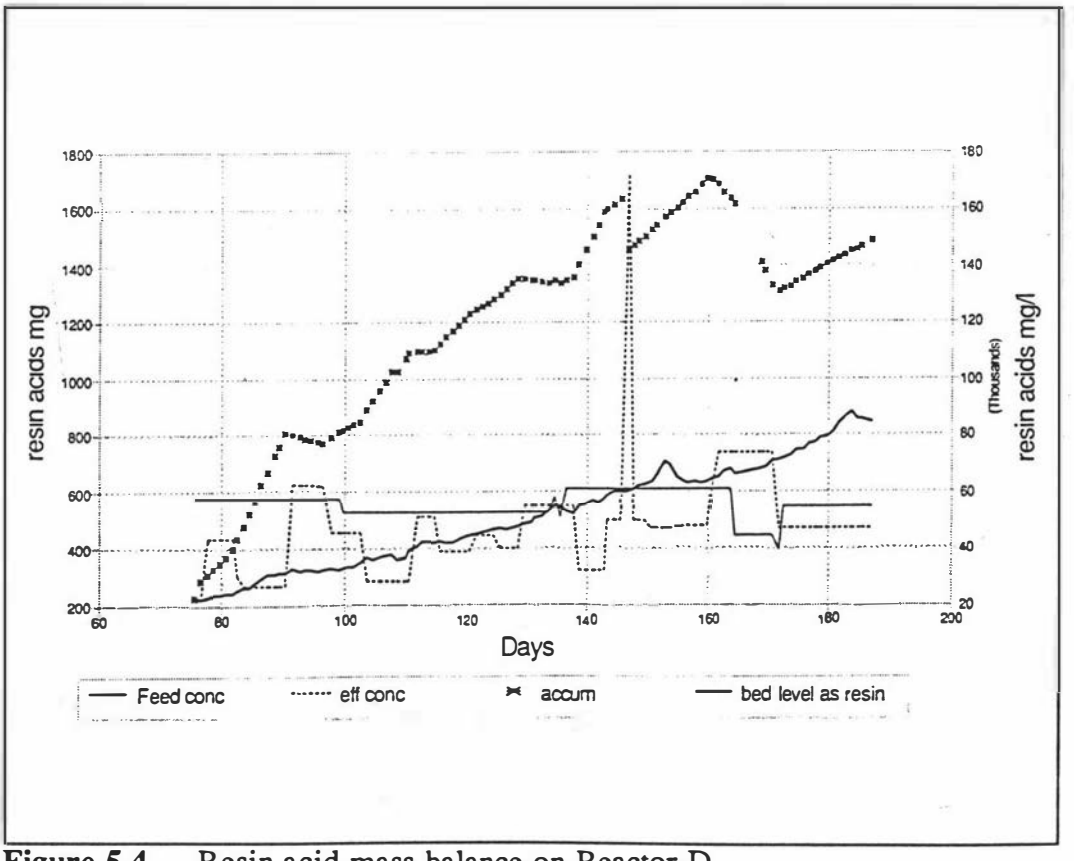

Figure 5.4 Resin acid mass balance on Reactor D.

Resin acid concentrations have been assumed to remain constant until the next measurement. The theoretical resin acid accumulation has been plotted on the same scale, assuming no degradation, as the product of bed level and the final bed resin acid concentration. This product represents the measured resin acid accumulation in the reactor. From the difference between the theoretical accumulation and measured resin acid accumulation lines it can be seen that at least 60,000 mg of resin acids was not accounted for in the mass balance and thus must have been degraded. No sludge wasting occurred from the reactor during this period. These figures represent 6 to 12% of the total resin acids fed to the reactor over the time in question. This shows that the UASB was not an effective means of removing resin acids. For the time over which the mass balance was performed the reactor, D, was operating at an average organic loading rate of 37.3 kg $COD.m^{-3}.d^{-1}$

5.4.3 The effect of organic loading rate upon resin acid degradation

The measured values of resin acids in the feed ranged from 120 to 1000 $mg.\ell^{-1}$ total resin acids. A guide to the amount of degradation taking place can be gained from examining the effluent resin acid concentrations at various organic loading rates as shown in Figures 5.5 and 5.6. For both reactors three feed batches were used. Each batch can be expected to be relatively uniform in composition on a weekly basis. The change over points occured on 74 and day 187. Generally higher RA concentrations were seen after a change in loading rate or feed batch. This concentration dropped as the "steady state", as indicated by low VFA concentrations, became established. The higher resin acid concentrations are a result of increased suspended solids concentration in the effluent brought about by changes in the bed from increased liquid and gas superficial velocity.

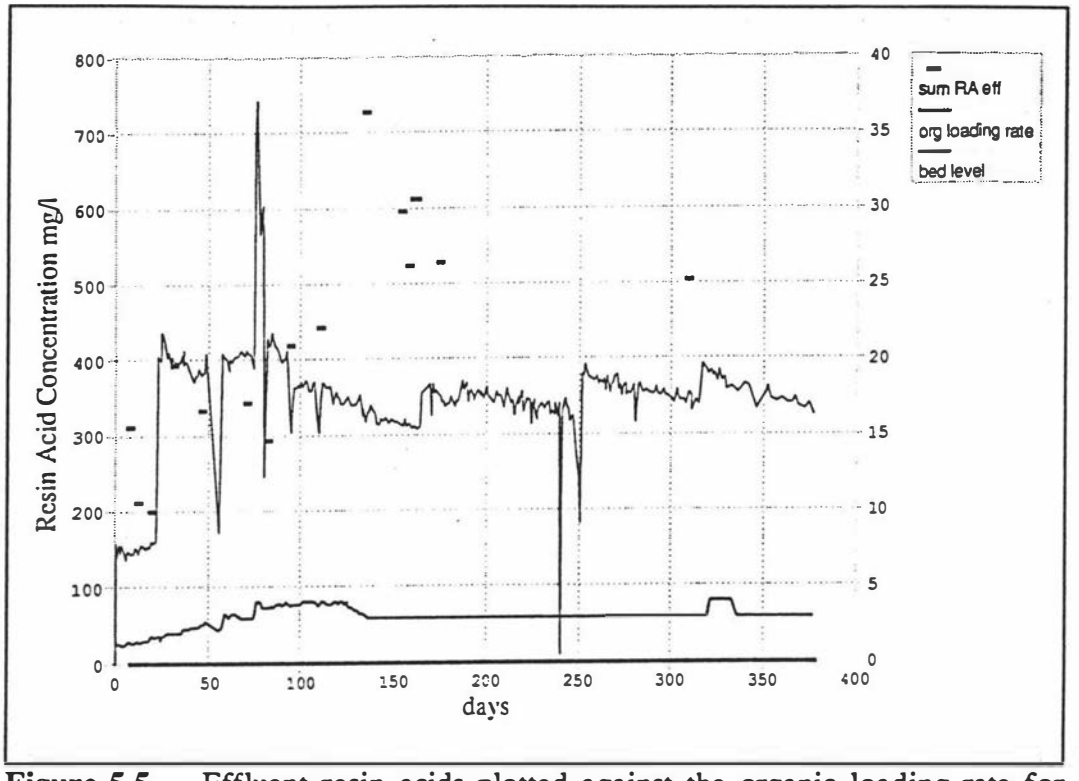

Figure 5.5 Effluent resin acids plotted against the organic loading rate for reactor C.

For the earlier loading rate steps there seems to be a link between organic loading rate and effluent resin acid concentration. However at least some of the low effluent concentration may be due to the accumulation of solids in the sludge bed as illustrated by the bed level plots. In the later stages of operation the higher concentrations seen in Reactor C may be a result of shed solids from the bed. The straight line for reactor C bed level after day 137 was a result of scale build up in the reactor and reactor flanges preventing effective sighting of the bed level. Actual bed level was less than the shown three litre level but greater than the two litre level. Thus the bed level fall probably continued for longer than is apparent in Figure 5.5. Final bed height when this reactor was shut down was the two litre level but some sludge had been removed and no flow of feed or gas was present to expand the bed. Thus, the operational bed height at the finish would have been higher. Loss of bed solids may also be the cause of high effluent concentrations for reactor D, although the bed level was not observed to fall. There was little distance (less than 15 cm) between the top of the bed and

the overflow weir and solids could have easily been carried out of the reactor by rising gas bubbles.

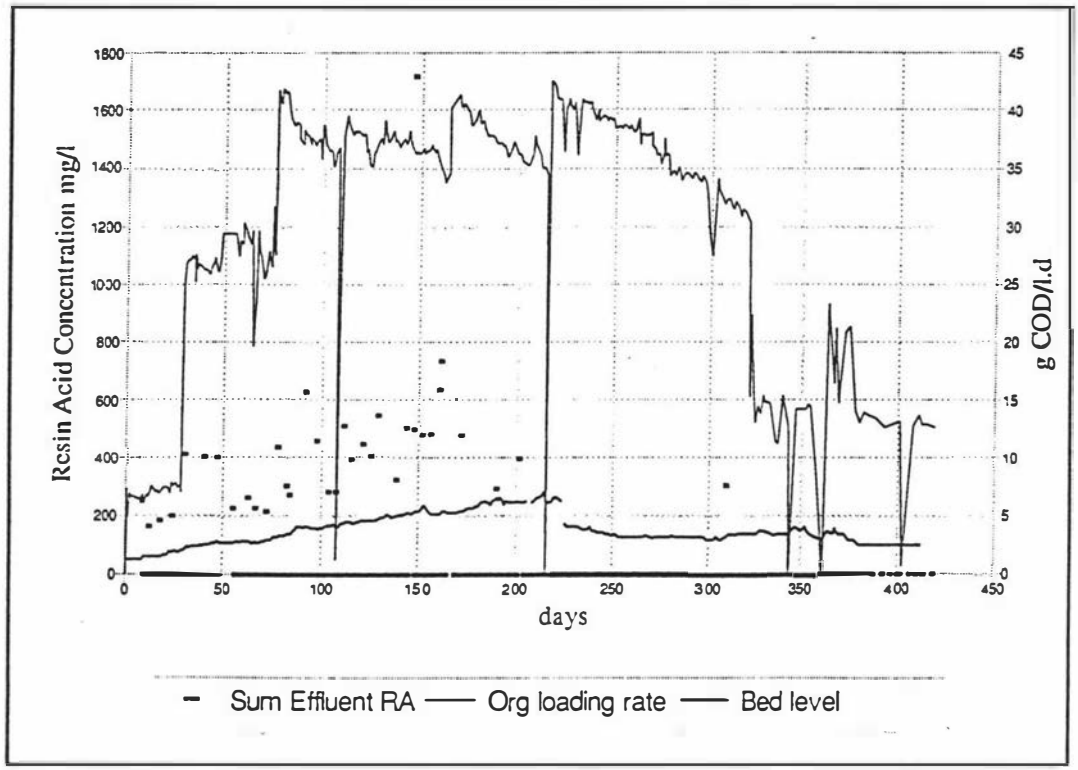

Figure 5.6 Effluent resin acids plotted against the organic loading rate for reactor D.

It must be considered that the lower early stage effluent resin acid concentration may be predominantly due to low influent concentration and accumulation of resin acids in the sludge bed. The available data is insufficient to determine if resin acid degradation was significantly better at lower organic loading rates.

5.4.4 The effect of UASB reactor treatment upon individual resin acids

The major changes in resin acids, from feed to effluent, are almost total removal of abietic acid and increases in dehydroabietic and 13-abieten-18-oic acids. These increases in dehydroabietic and 13-abieten-18-oic acids are larger as the organic loading rate is increased. Concentrations of these acids suggest that

abietic is being predominantly transformed to the other two acids. Another resin acid with a mass spectrum very similar to that of 7-isopimaren-18-oic accumulates to a lesser extent. 13(15)-abieten-18-oic acid also appears in the effluent at a low level. Isopimaric and pimaric acids appear to experience some removal at lower organic loading rates. Other resin acids are of such low concentrations that conclusions can not be drawn with any degree of certainty. The presence of three resin acid pairs with similar retention times precludes comments on changes in these acids. For the long chain fatty acids measured, all are removed completely at the organic loading rates less than 15 $kg.m^{-1}.d^{-1}$. At higher rates palmitic and stearic accumulate to some extent and oleic and linoleic are predominantly removed. Linolenic acid showed no significant presence in the feed, sludge or effluent samples. Changes from feed to effluent are shown in Figure 5.7.

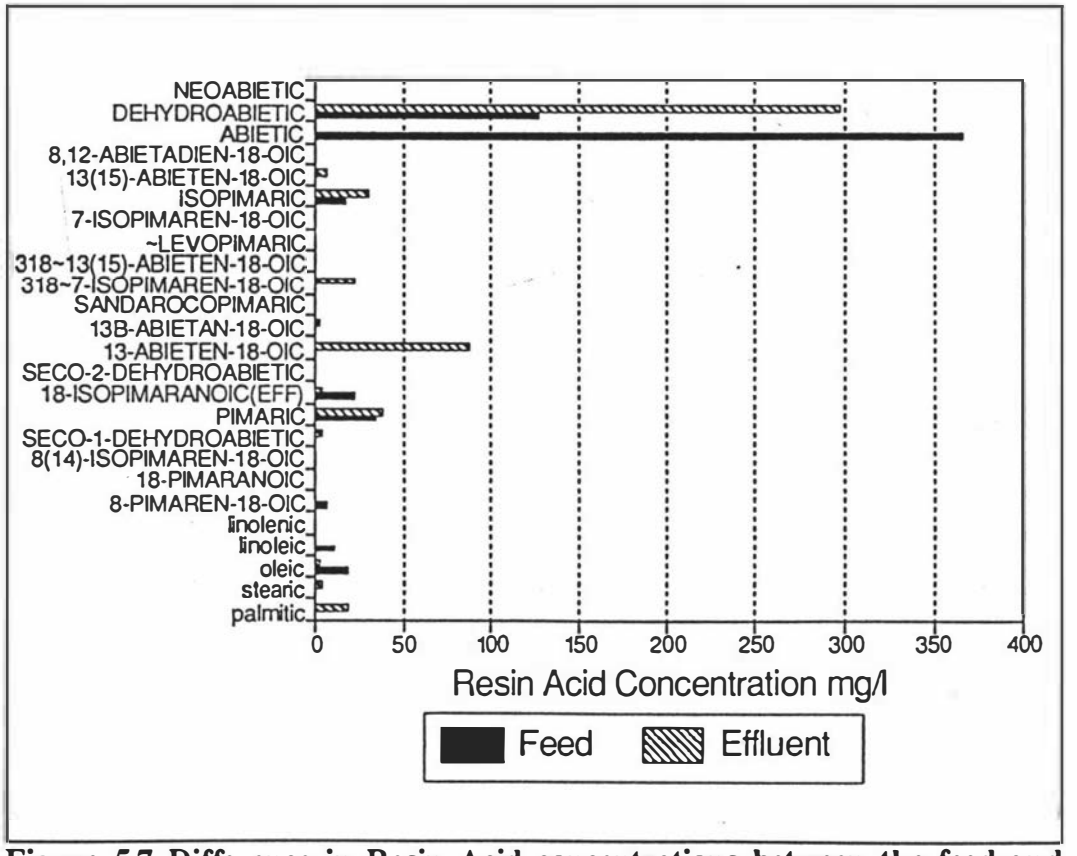

Figure 5.7 Difference in Resin Acid concentrations between the feed and effluent.

These changes in the resin acid concentrations show that abietic acid, at least, is biologically accessible. Dissolution of the resin acids must occur within the reactor, possibly when some of the suspended solids are degraded. The samples of effluent and seed sludge liquor shown in Table 5.4 have a higher average dissolved resin acid fraction than the feed samples. This again would require some dissolution of the resin acids in the reactor. The degree of removal of resin acids may be dependent upon retaining the suspended solids within the bed to allow for slow resin acid dissolution. Thus anything that disrupts the wood fibre holding ability of the bed results in higher effluent resin acid concentrations. High liquid superficial velocities may not allow wood solids sufficient retention time for resin acid dissolution.

The sludge of the reactors accumulated high resin acid concentrations. In some cases LCFAs also accumulated. Figures 5.8 and 5.9 illustrate the resin acid composition of reactor C at the start and the end of operation after the sludge was split. The seed for reactor C_{late} came from reactor C_{early} that had been operated on this wastewater for approximately four years and was already high in resin acids. In Figure 5.8 "seed granules" is the granular (>1mm) fraction of the "seed sludge". Dehydroabietic acid is more highly concentrated in the seed sludge, dehydroabietic acid must be at a much higher concentration in the sludge matrix than in the granules on a dry solids basis. 8-pimaren-18-oic and the unconfirmed isomer of 7-isopimaren-18-oic acids also show this uneven partitioning between the two types of reactor solids. This suggests that the transformation to these acids is occurring in a close association with the wood solids and diffuse reactor growth. In comparison other acids, notably 13-abieten-18-oic, appear to be accumulating within the granules. Little evidence of wood solids was found within the granules, as shown in Figures 6.20 to 6.23, thus the

transformation of this acid is not associated with the wood solids and may be occurring in the granules or associated with granular growth conditions.

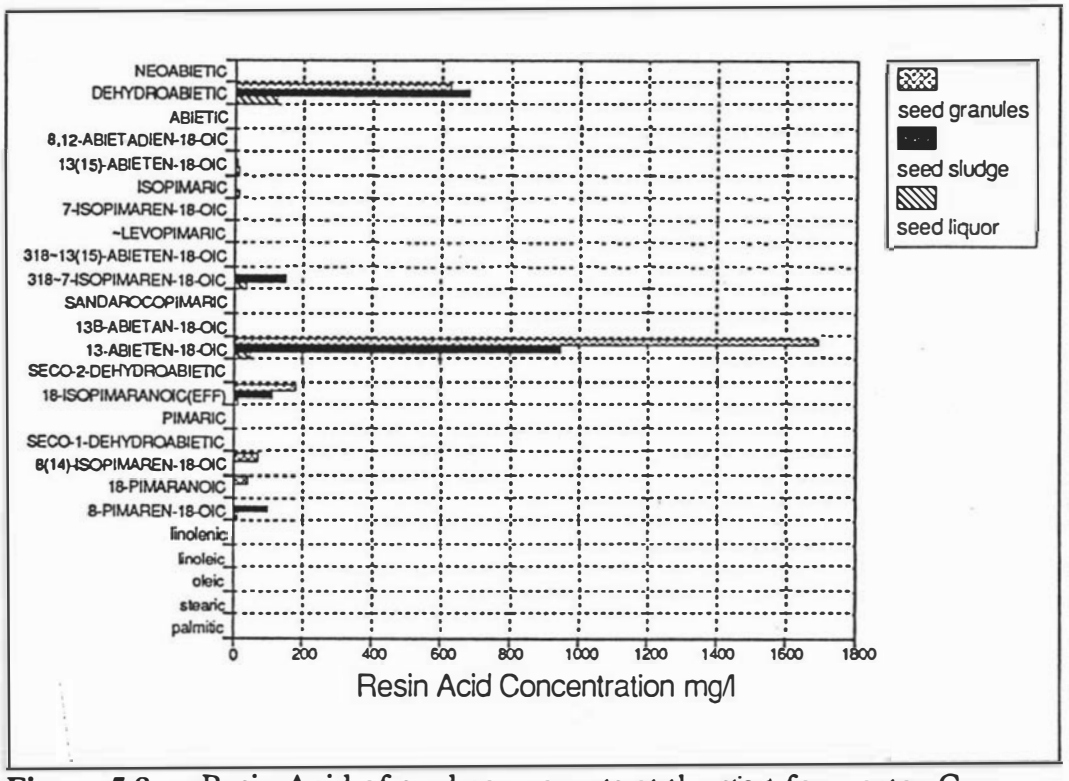

Figure 5.8 Resin Acid of seed components at the start for reactor C.

The resin acid distribution in Figure 5.9, a resin acid profile of reactor C at the end of operation, also shows evidence of this resin acid partitioning. The reactor entry was highly concentrated in feed solids as shown by the accumulated naturally occurring acids and has a very high dehydroabietic acid concentration. Further up the reactor, where granular biomass predominates lower dehyroabietic acid and higher 13-abieten-18-oic acid concentrations are seen. These accumulated concentrations were far above the reported inhibition concentrations for resin acids. For dehydroabietic acid 50% IC = 43 mg.ℓ^{-1} on the first feeding and 123 mg.ℓ^{-1} on the second feeding (Sierra-Alvarez and Lettinga, 1990). Values for 80% IC were 105 and 141 mg.ℓ^{-1} respectively (Sierra-Alvarez and Lettinga, 1990). Where 50% IC is the concentration of compound that caused

50% inhibition and 80% IC caused 80% inhibition. Sludge dehydroabietic acid (DHA) concentration in the reactor sludges reached 11,000 mg.ℓ^{-1} at the entry to reactor C and was commonly above 3,000 mg.ℓ^{-1}. Seed sludge for reactors C and D was measured at a DHA concentration of 650 mg.ℓ^{-1}. While some degree of acclimatization is to be expected, clearly most of the resin acid is not in a dissolved form.

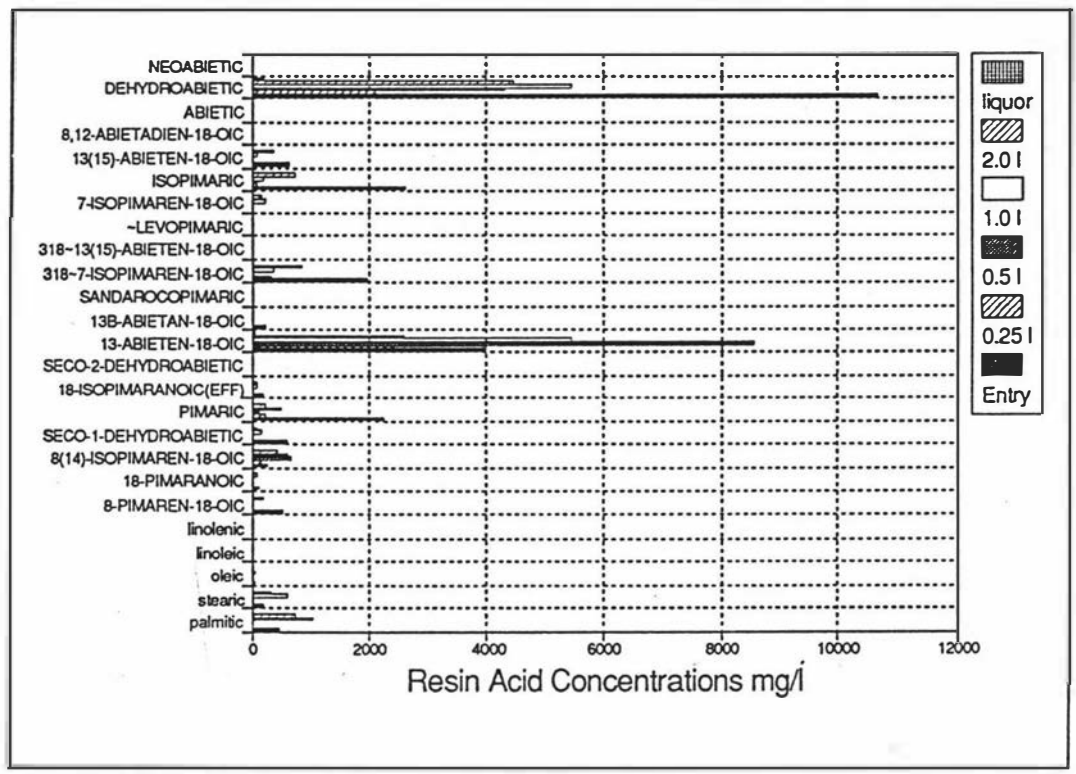

Figure 5.9 Resin Acid profile at the end for reactor C.

5.5 RESIN ACID AND FEED TOXICITY

Feed stream resin acids were clearly above the reported inhibitory concentrations especially with respect to abietic acid (Sierra-Alvarez and Lettinga, 1990). Even with most of the resin acids in solid form the variability of the feed batches collected from the pulp mill suggested that toxicity could be a problem. The interaction of resin acid accumulation in the sludges and sludge acclimatization also warranted investigation. Serum bottle assays were conducted to estimate the

toxicity of whole feed and a dichloromethane extract of the feed using an well acclimatised sludge. The methodology follows that given in Section 6.3.

5.5.1 Serum bottle experiment 6: Toxicity of a feed dilution series

This experiment was conducted after the failure of experiments 4 and 5 which were based on testing the toxicity of a dichloromethane extract of the feed. A feed dilution series was used in an attempt to duplicate the successful activity seen in the reactors. Experimental conditions are shown in Table 5.5.

Table 5.5: Experimental conditions for serum bottle experiment 6

Runs	Feed dilution	COD ($mg.\ell^{-1}$)	Resin acids ($mg.\ell^{-1}$)	VFA ($mg.\ell^{-1}$)	Ave Seed (gVSS)
1-3	0	0	0[a]	0[a]	0.33
4-5	1/20	750	20	200	0.34
6-7	1/10	1500	40	400	0.35
8-9	1/4	3750	80	1000	0.34
10-11	1/2	7500	160	2000	0.34
12-13	3/4	11250	325	3000	0.35
14-15	1/1	15000	650	4000	0.34
19-20	2/1	30000	1300	8000	0.37
21-22	3/1	45000	1850	12000	0.35

a does not include material from the seed sludge

The feed used was found to have developed high concentrations of VFAs after the experiment was started. Reactor feed total VFA concentration ranged from 1000 $mg.\ell^{-1}$ up to approximately 5000 $mg.\ell^{-1}$, as discussed in Section 4.3.2.1. Thus some degree of acclimation to high VFA concentrations can be expected but concentrations above 10000 $mg.\ell^{-1}$ will have an inhibitory effect. The pH

in the serum bottles was neutral when measured and so the VFAs will be predominantly unionised, reducing the potential inhibition. Examination of Figure 5.10 shows that inhibition is occurring in the higher feed strength runs. The top left hand curve approximates the theoretical methane yield of the runs. As the experiment progressed there is some evidence that inhibition lessened as the gas yield increased above 50% of theoretical. At this stage in the digestion VFA concentration would be dropping. This is evidence that VFAs are involved in the observed inhibition.

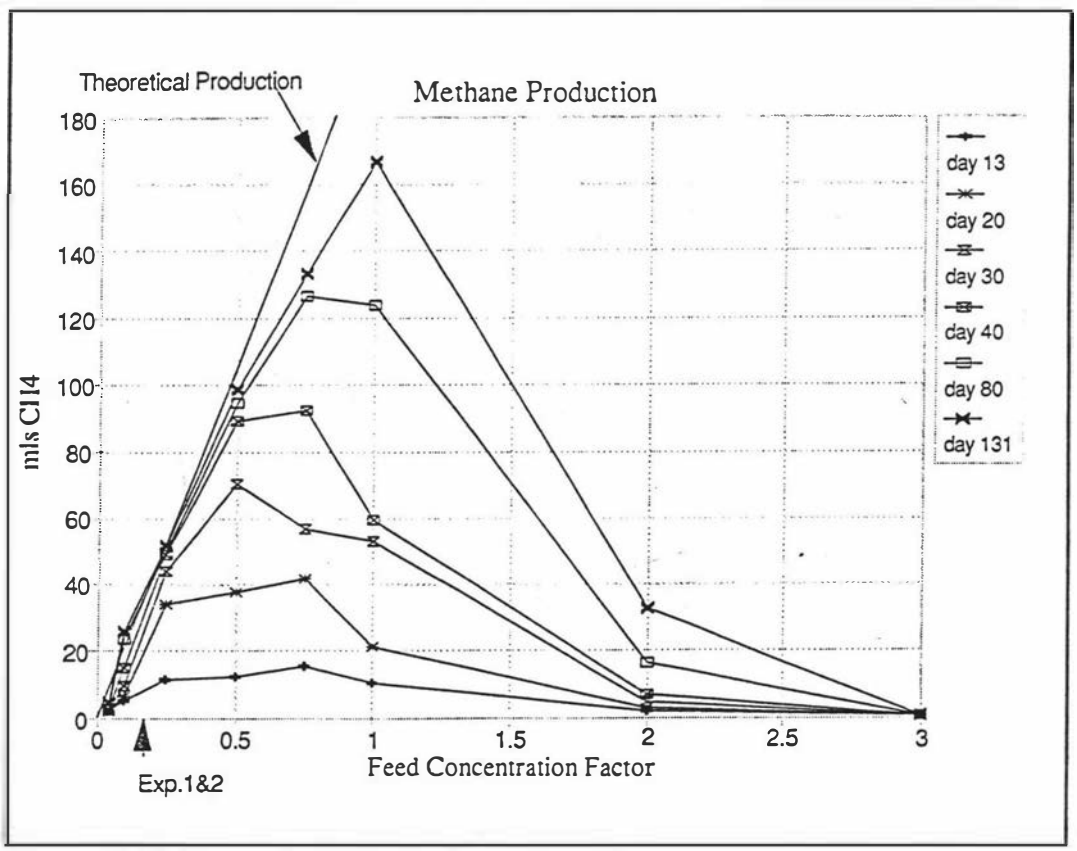

Figure 5.10 Serum bottle experiment run 6.

The low Ks values for the volatile acid users imply corresponding methane production rates for most of the digestion in these runs where no inhibition occurs (Mosey, 1983). Thus as VFA concentrations drop towards the end of each digestion similar methane production rates would occur. This is not evident in the specific methane production curves shown in Figure 5.11. Peaks in the

methane production rate at approximately day 20 result from the addition of a concentrated nutrient solution based upon the basal medium used in earlier serum bottle experiment. Thus, while the VFAs are responsible for some of the inhibition, it appears that there are other components of the feed that still exert an inhibitory effect upon the methangenic consortia.

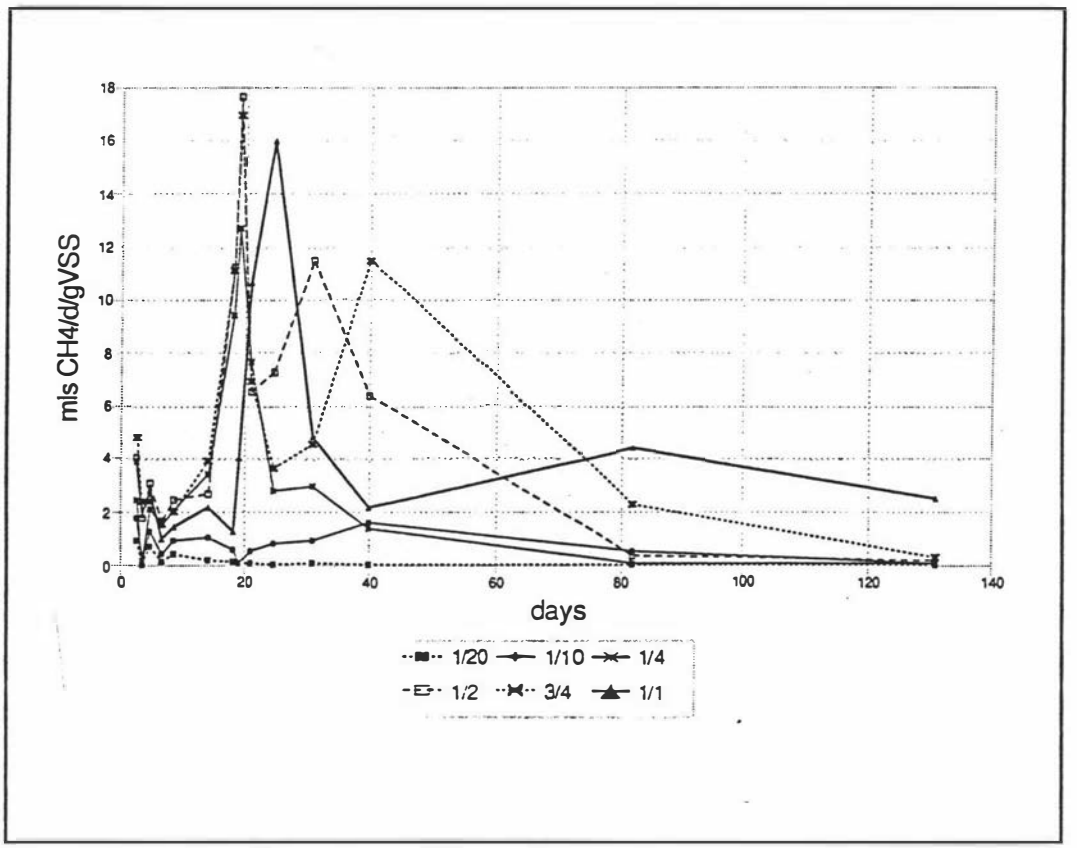

Figure 5.11 Specific methane production rate for serum bottle run 6.

5.5.2 Serum bottle experiment 8: A dichloromethane extract toxicity series

To remove the inhibitory effect of the VFAs present in the above experiment a 8dichloromethane extract of the feed was used as the inhibitory substance in a serum bottle batch assay. One litre of feed was extracted with 1.2 litres of distilled dichloromethane. The extract was then extracted with approximately 8equal volumes of distilled water ten times. This removed at least 99% of

water soluble compounds as measured by VFA concentration. The resulting dichloromethane solution was concentrated by rotovaporation at 25-30°C and redissolved in a small amount of methanol. A dilution series was created with more methanol. 0.2 ml of the required extract/methanol solution was injected into pre-prepared serum bottles containing 25% feed and urea, acid calcium mono-phosphate and sodium bicarbonate at the same concentrations used for full strength feed in the reactors. Again 0.1 ml of the concentrated modified (low chloride) basal media was injected after 4 days of incubation. Other methodology was as described in Section 6.3. Experimental conditions are described in Table 5.6.

Table 5.6: Experimental conditions for serum bottle experiment 8.

Run	Seed (gVSS)	Extract dilution	Soluble resin acids ($mg.\ell^{-1}$)	Seed+feed resin acids[b] ($mg.\ell^{-1}$)	total resin acids ($mg.\ell^{-1}$)
1-2	0.32	0[a]	0	343	343
3-5	0.32	0	0	344	344
6-7	0.31	1/1	150	340	491
8-9	0.30	1/2.67	56	334	391
10-11	0.35	1/6.5	23	353	377
12-13	0.31	1/14.6	10	335	345
14-15	0.34	1/31	5	351	356
16-17	0.31	1/62.1	2	338	340
18-19	0.33	1/248	0.6	351	352

a no methanol added, all other runs were injected with 0.2 ml methanol

b Resin acid concentration calculated from seed quantity x average resin acid concentration plus feed resin acid concentration.

Another experiment was conducted before experiment 8. Experiment 7 was similar to experiment 8 in that a similar dilution series was used for the resin acid concentrations but it showed no activity. The proposed reasons for this was

that no feed was used in the media and the resin extract concentration range of 10-580 mg/l soluble resin acids used was too highly concentrated to allow any activity.

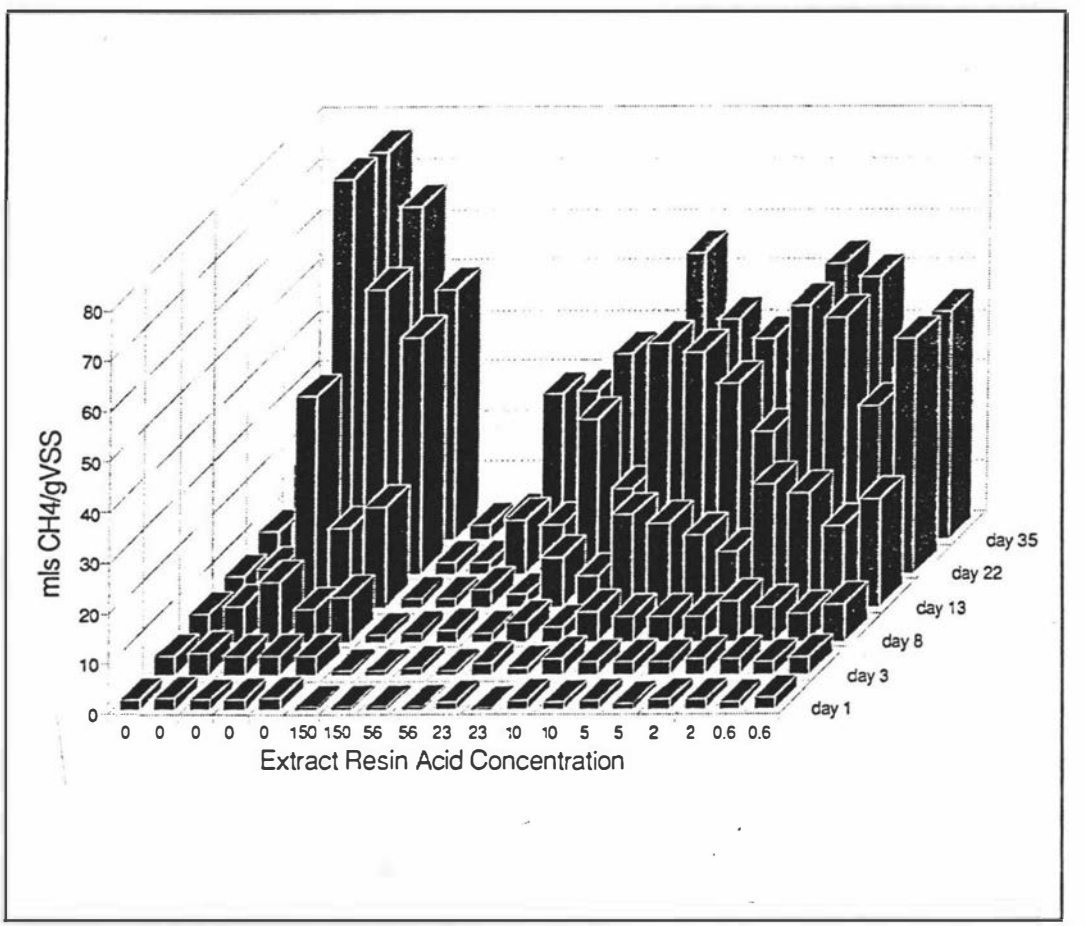

Figure 5.12 Methane production as a function of extracted resin acid concentration in Serum bottle run 8.

Methane production was definitely inhibited by the higher soluble resin acid concentrations is shown in Figure 5.12. However considerable variation is noticeable. This may reflect the differences in activity in the various seeds. Clearly the seed is not utilising the feed particularly well as evidenced by the failure of the two blank runs to produce much methane. The corresponding runs in experiment 6 had a specific methane production of 135 mls $CH_4.gVSS^{-1}$ at day 35, compared to 16 mls $CH_4.gVSS^{-1}$ in this experiment. There were no significant procedural differences between the two sets of runs. The addition of

methanol markedly improves methane production but again recovery of the input COD is low. The reasons for this poor performance are unknown. Resin analysis of some of the serum bottles at the end of the experiments found that the total resin acid concentration in each bottle did not relate well to the calculated total resin acid concentration at the start. While some of this variation may possibly arise from acid degradation, a blank bottle and a 1/1 extract bottle showed similar concentrations. Little activity occurred in either, thus resin acid concentrations should show little change.

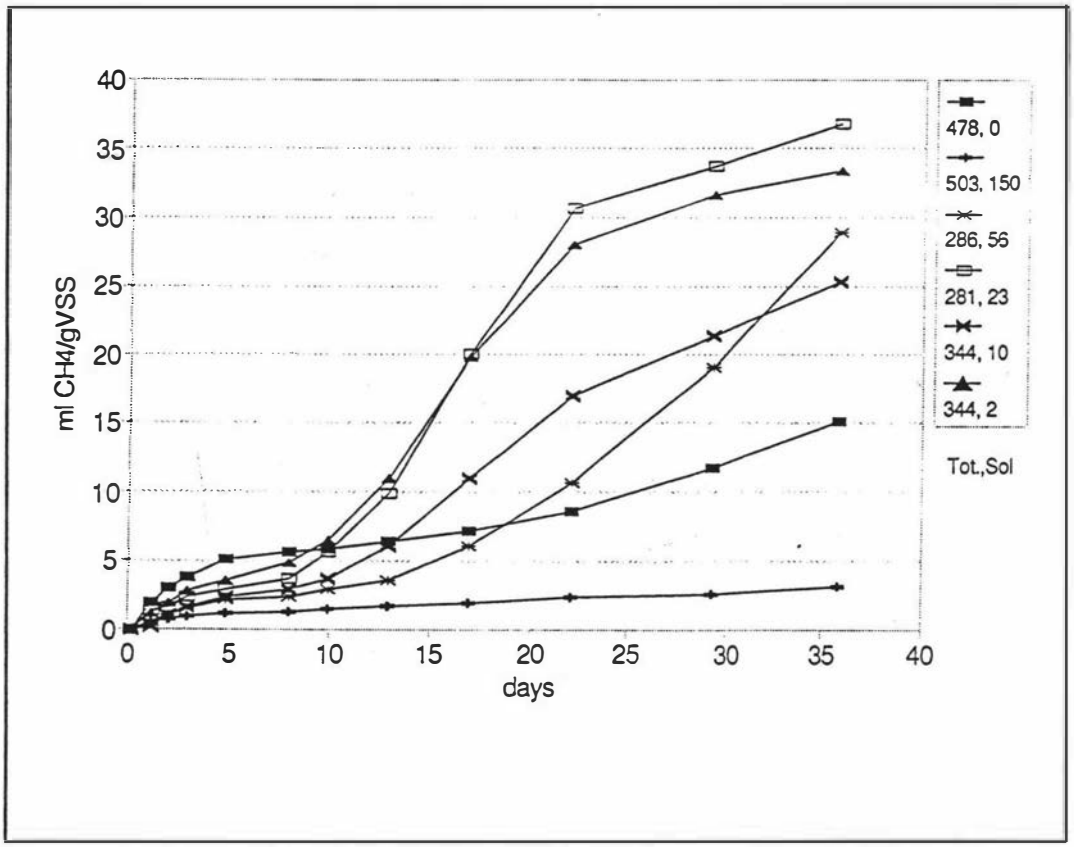

Figure 5.13 Profile of methane production over time with resin acid concentrations.

The most probable explanation for the unexpected resin acids concentrations in the serum bottles is that the seed sludge is very variable in resin acid concentration. This is a function of the composition of each bottle's inoculum. Given the great variation in granule size, the proportions of granule to diffuse sludge to liquor must vary considerably. Granules were calculated to contribute

53 to 56% of the non-extract resin acids. In reality this range must be sufficiently large to contribute as much extra as was added in the 1\1 extract injections. The dissolved resin acid fractions of the blank and the 1\1 extract runs were 1 $mg.\ell^{-1}$ and 8 $mg.\ell^{-1}$ respectively compared to total resin acid concentrations of 343 $mg.\ell^{-1}$ and 491 $mg.\ell^{-1}$. It would seem that the dissolved resin acids do not remain in solution to a great extent. Examination of the specific methane production curves for the measured runs shown in Figure 5.13 shows little evidence of inhibition as a function of total resin acid concentration but a reasonable agreement with the added extract concentration. The trends shown at the end of the experiment however suggests that the final methane production is related to the total resin acid concentrations.

These factors suggest that initially inhibition is a result of the soluble resin acids. In the previous experiments with more concentrated extracts the resins acids were observed to form a hydrophobic gum on injection into the serum bottle. This gum showed a high affinity for any solid surface and was very difficult to clean from the glass serum bottles. The dissolved acids in experiment 8 are presumed to follow this behaviour and to coat the available solids in the serum bottles. Thus, when measured, dissolved concentrations were low. Toxicity of resin acids has been reported to be associated with the bacterial membranes (Sierra-Alvarez *et al*, 1993). This would explain the initial effects to some extent.

In the longer term, as fresh cell growth becomes more significant inhibition may be a function of the total pool of resin acids available for dissolution and surface interaction with the methanogenic biomass. Another factor present is the differences in resin acid composition of the feed and feed extract compared to that of the seed sludge. Dehydroabietic and 13-abieten-18-oic acids are highly concentrated in the seed sludge. Dehydroabietic acid has been reported as one of the more inhibitory resin acids (Sierra-Alvarez and Lettinga, 1991), little has been reported upon 13-abieten-18-oic acid. These acids may have more effect on the long term toxicity in the serum bottles.

The resin acids have shown to be highly inhibitory when added in a dissolved form to sludge that has had long term exposure to predominantly non-dissolved resin acids. A 50% inhibition was observed to occur at 56 mg.ℓ^{-1}. This degree of inhibition is essentially the same as that reported for unacclimated sludges (Sierra-Alvarez and Lettinga, 1991). Thus no acclimation to soluble resin acids is evident. However feed at 10 times this total concentration is treated successfully in UASB reactors with little evident toxicity except when easily digested compounds are depleted. Co-metabolism has been reported for the resin acids (McFarlane and Clark, 1988). It is proposed that this is responsible for the observed inhibition in the continuous reactors. Changes in the resin acids indicate that there is at least some dissolution of the resin acids from the TMP wastewater. This dissolution may be a factor in the longer term toxicity problems observed in serum bottle experiments 2 to 8. In experiment 1 the seed granules had a lower resin acid concentration than those used in the subsequent experiments. This may have been partially responsible for the problems experienced in the later experiments.

5.6 CONCLUSIONS.

These studies have shown that the resin acids are toxic when in a soluble form and that even after four to five years contact with TMP wastewaters no acclimation to soluble resin acids is evident. Thus at the levels found in the mill effluent used as the feed to the UASB reactors, the resin acids must be in an insoluble form and so the UASB reactor has been able to treat the waste.

CHAPTER SIX GRANULES

6.1 Introduction

High performance of a UASB reactor is largely dependent upon development of a granular sludge (Forster, 1991, Goodwin *et al,* 1990). It has been widely suggested that granulation (i.e. the initiation and growth of granules) is inhibited by high suspended solids feed stocks (McFarlane and Tan 1986, Hulshoff Pol and Lettinga, 1986, Vanderhaegen *et al* 1992), where usually the suspended solids referred to are of an inorganic nature. Thermo-mechanical pulp mill wastewaters are generally high in suspended solids. These suspended solids are predominantly wood fragments with very little soil or grit particles. This difference from those cases reported in the literature suggested that granulation during treatment of the TMP wastewater was a possibility. Granulation occurred extensively in some of the reactors utilised in this work. In others little or no granulation occurred. The nature of the granules produced and their apparent effect are examined in this chapter.

6.2 Granular characteristics

6.2.1 The granular environment

The granules found in reactors C and D were contained within a thick sludge. At the bottom of the reactor the sludge becomes so thick that when the QVF glass sections were removed to the 75 mm diameter joint, shown just above S1 in Figure 3.4, the sludge retained all the reactor contents above this with no mechanical aid. Within the sludge the granules were abundant and had a wide size range. This is demonstrated in Figure 6.1. Generally each size fraction of the granules were spread evenly through the bed of the reactor. The exception to this was in the narrow (25 mm nominal diameter) curved inlet sections shown in Figure 3.4. Here masses of wood fibre accumulated and displaced all granules smaller than approximately 2 mm.

6.2

Granules when removed from the reactor still coated in the matrix layer were a uniform deep black, no colour variation was visible. When washed and gently

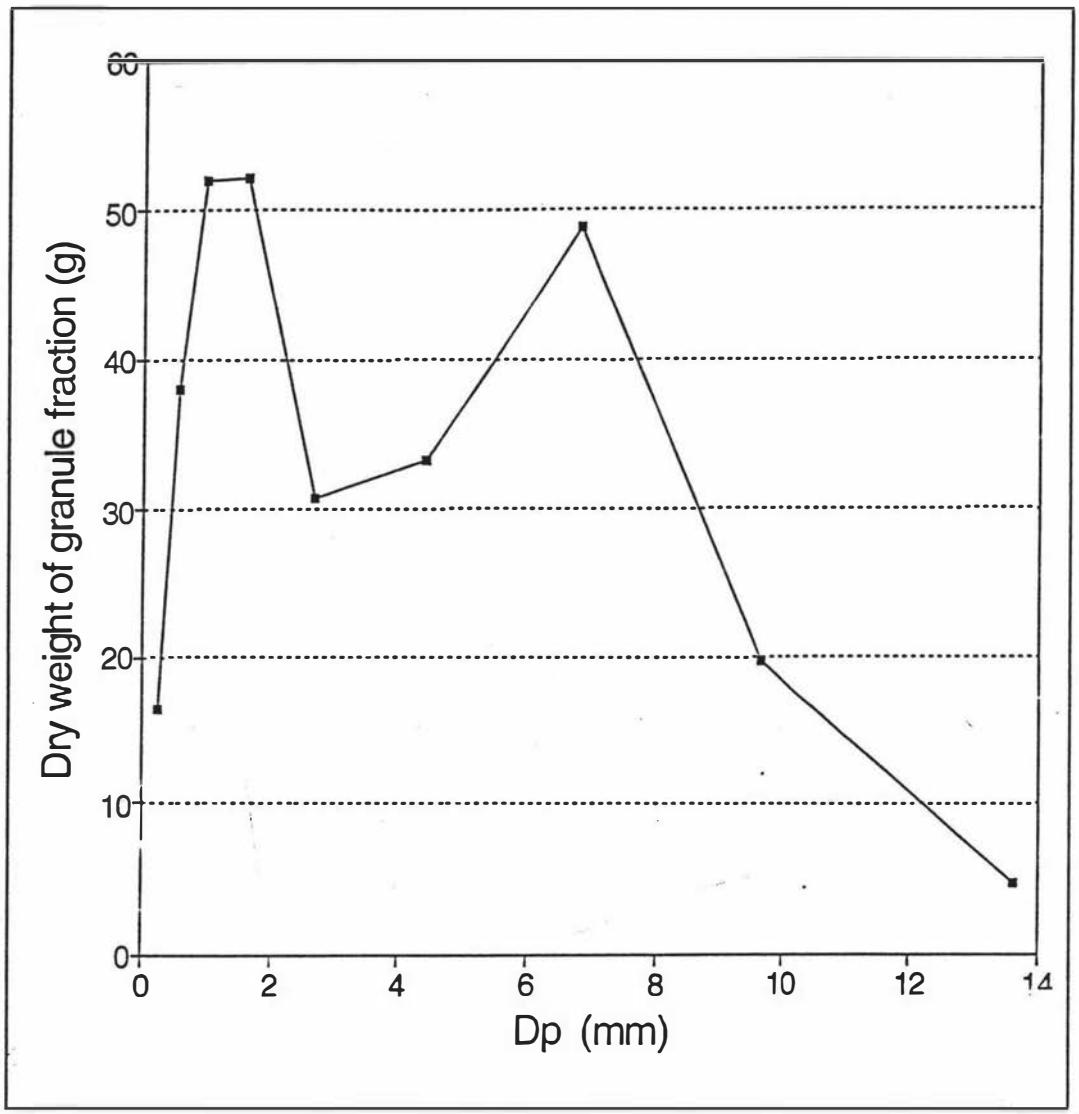

Figure 6.1 Granule size distribution. The dry weight of the granules of a given size fraction are plotted against the nominal size fraction.

blotted dry the granules exhibited a rough pitted surface and variegated grey-green colour as can be seem in Figure 6.2. The brown granule in this figure is a granule that has been air dried at 104 ± 1 °C. Other photographs of the granules from the size distribution analysis were of air dried granules. Drying in the presence oxygen results in conversion of Fe(II) to Fe(III), Compounds of Fe(III) usually have a red-brown colour (Ainscough, 1994). The presence of

water made photography difficult and obscured surface features. The red colour also allowed the use of angled lighting to show surface structure to better effect.

Figure 6.2 Wet granules. The smaller divisions of the scale are 1 mm.

6.2.1.1 Aggregation and the sludge bed matrix

All the sludge bed granules when examined were found to be surrounded by a 2-4 mm layer of fine solids. This layer has been labelled the sludge bed matrix. Close to the actual hard granule surface this layer had a consistency similar to loose potter's clay. The firmness decreased with increasing distance from the granule surface. To observe the granules with any clarity it was necessary to take the reactor apart. The time required to form the granular and bed matrix structures was of such length as to prevent such invasive investigations at intermediate times and, together with time and equipment constraints, to prevent the operation of repeat experiments.

This reactor dismantling must necessarily have some effect upon the physical order of the bed and thus the order and arrangement of the bed during operation cannot be described with complete certainty. However 30-40 mm diameter

aggregations were commonly found when the sludges of reactors C and D were examined. These aggregations consisted of many granules held together with the clay-like matrix found around the single granules. Granules of all sizes were found within the aggregations. Possibly the single granules found were from aggregates broken up during sludge removal, however the completeness of the surrounding layer of fine solids described above suggests that the single granules were originally individual entities. If this were not the case the more diffuse outer layers would be missing on one side of the granule.

Most of the bed appeared to exist in the aggregated form. From the 1.5 litre level down to the 25 mm tube sections of the reactors, aggregation of the bed became so complete that it appeared to exist as a fixed matrix with very little free liquid, 10 - 20 % of the volume of this matrix being entrapped gas. Structural integrity of this matrix was such that a column 75 mm in diameter and 75 - 80 mm in height removed from the QVF fittings was self supporting.

In the first period of operation of reactor C aggregation did not occur with the degree of structural integrity seen in the later phases of operation of reactors C and D. Some clumping together of granules was observed but this was of a more open construction than that described above. Reactor C was in operation for 1357 days before the sludge bed was split, compared to the subsequent operation of reactors C and D for 378 days. When reactors C and D were seeded with the sludge from reactor C any matrix structure as described above was destroyed in the sludge transfers. The granules were washed in a mixture of reactor C supernatant and effluent, mixed and added to each of the two reactors as a two part seed, one granules only and the other a thick liquor containing all the solids washed off the granules. Thus it would seem unlikely that the aggregations were simply a function of the length of undisturbed time. A number of operational differences between early reactor C and the twinned reactors C and D. Reactor C, prior to the sludge split, had periods of operation without external disturbance of the bed for at least as long as the total period of operation of the restarted reactor C and reactor D. However there were no zinc

toxicity problems for these later periods of operation whereas reactor C experienced severe inhibition as discussed in Section 4.2. Feed interruptions were much more prevalent in the early stages of operation of reactor C than for the later reactors C and D. Both reactors C and D were subjected to greater organic and solids loading rates. Reactor C_{early} had a varied OLR with 5 stages ranging from 5 -14 kg $COD.m^{-3}.d^{-1}$. Reactor C_{late} ranged from 7.6 - 19.4 kg $COD.m^{-3}.d^{-1}$ and reactor D 7.2 - 41 kg $COD.m^{-3}.d^{-1}$. As the major components of the matrix of fine solids appears to be feed-suspended solids and reactor bacteria it is postulated that the formation of the matrix layer was dependent upon the solids loading rate, solids retention time and solids degradation rate.

It appeared that the matrix surrounding the granules was dependent upon the provision of the TMP fines in the feed. Reactor D, which had shown the same structure as reactor C, was opened approximately a year after feeding was stopped. Almost all evidence of the matrix was gone, leaving predominantly granular sludge. The matrix composed of feed suspended solids and anaerobic bacteria, as far as can be ascertained , was degraded over the time in which they sat in the quiescent reactor.

6.2.1.2 Bed compression

The degree of compression of the bed and apparent lack of flow channels found upon opening the reactor may be caused by the lack of the upwards force of the feed flowrate. During operation, feed was introduced for approximately 10 seconds every minute. Effluent overflow did not start until after feed introduction had ceased. When the feed inlet section surface of the reactors was clean it could be seen that the feed accumulated in the small gas space in the downflow portion of the U-shaped bottom of the reactor. The gas volume was compressed as feed was pumped in and then the additional pressure in that volume pushed the new feed slowly into the bed. The accumulated wood fibres act as an effective filter. From the structure observed it seems likely that this accumulation of fibre was slowly pushed further into the bed by each successive

inflow of new fibre. The fibre mass did not displace all of the granules of the bed. Neither, in approximately one year's operation did the bed structure become so thick that pump head pressure caused the feed tube joiners to fail. Any failures in this respect were traced to feed tube problems.

6.2.2 Size distribution

Representative sampling of the granules from the relatively small reactors used was a disruptive process and so could only be done at otherwise inactive times. This occurs only at the end of operation for reactors C and D. All the sludge was removed from reactor C on day 378 of operation. The supernatant was decanted off and approximately 800 ml removed for the size distribution analysis. Fairly large granules were present in the reactor and so a sample of this size was necessary to get a representative selection. A layer of the supernatant liquor was kept over the sample in an attempt to keep oxygen from the granules as much as possible. This meant that the suspended solids content of this sample was slightly lower than that found in samples taken from the bed only. The suspended solids of the sample was 460 $g.kg^{-1}$ giving approximately 370 g of suspended solids in the sample.

The size analysis was performed in a wet sieve array constructed by the Food Technology Workshop, Massey University. Wet sieving was used to protect the relatively brittle granules from damage and because of the nature of the sludge sample. The sieve array consisted of nine sieve trays with square mesh plus a solid bottom tray with a water inlet/outlet. A set of wet sieves with a constant ratio of size increase was not available. The apertures of the mesh are shown in Table 6.1.

Sieving the sample was carried out using a variety of procedures. Initially the sample was placed into tray number nine and the rest of the array filled with reactor C effluent. The array was sealed and gently agitated on a modified rotary shaker for twenty minutes. This method was ineffective in separating the

size fractions. A combination of upwards and downwards washing of considerable duration gave acceptable separation. The wet weight and dry weight of the size fractions can be seen in Table 6.1. The difference between the 370 g SS calculated for the original sample and the total dry weight in Table 6.1 is the amount of SS that passed through the 0.14 mm sieve.

Table 6.1 Mesh sizes and weight fractions for solids distribution analysis of reactor C sludge

Tray number	mesh size (mm)	wet weight (g)	dry weight (g)
1	0.14	37.8	16.4
2	0.4	71.8	38.1
3	0.75	89.9	51.9
4	1.3	87.8	52.1
5	2.0	46.0	30.7
6	3.5	49.4	33.3
7	5.5	71.6	48.9
8	8.5	28.1	15.2
9	11.0	8.5	4.7
		==========	==========
Total Weight		491.0	291.3

Table 6.2 shows 'Dp', the average particle diameter for the fraction, the dry weight sum and the number of particles in each fraction. The number of particles was counted for fractions seven, eight and nine. For the other fractions

22 - 500 of the particles were counted out and weighed. In the number 1 tray 2 - 4 % of the particles were wood fragments and other non-granular debris. Dry weight fractions were used for comparison purposes because of the variation in water content of the fractions. A plot of fraction dry weight versus Dp as shown in Figure 6.1 has a bi-modal distribution. Examination of the granule size fractions shows extensive quantities of granule fragments in fractions 1 to 5.

Simple washing of the sludge direct from the reactor shows fragmentation of a similar extent to that seen in the fractions. Solids sampling, reactor cleaning and even the sometimes violent movement of the bed during gas movement were possible causes of this fragmentation. While the sieving action was as gentle as possible it is highly likely that this was the cause of some of the fragmentation observed.

Table 6.2 Average particle diameter and particle numbers

tray number	Dp (mm)	particle number
1	0.24	1890000
2	0.55	791000
3	0.99	76900
4	1.61	14500
5	2.65	1580
6	4.39	300
7	6.84	171
8	9.67	36
9	13.64	5

Fragmentation of the larger granules was the most likely cause for the peak in weights for fractions 1 to 4. Wood fibres and fibre fragments also make some contribution to the weights of these fractions.

It is obvious that granulation was extensive. Granules larger than 0.14 mm made up most of the bed mass. Handling and observation of the fines washed out of the sieve array suggests that a considerable portion of these fines were also granular in nature and settle well.

6.2.3 Solids analysis

Solids analysis of the seed granules for reactors C and D showed total solids (TS) to be 700 $g.kg^{-1}$ and volatile solids (VS) to be 240 $g.kg^{-1}$. These granules had been washed in a mixture of reactor liquor and effluent. A sample of large granules (5 - 8 mm in diameter) from this mixture gave a TS of 840 $g.kg^{-1}$ and VS of 290 $g.kg^{-1}$. Small granules (1 - 2 mm in diameter) gave a TS of 740 $g.kg^{1}$ and VS of 250 $g.kg^{-1}$. For these samples dissolved solids were difficult to separate and measure. The dissolved solids (DS) of a highly granular sludge taken from reactor D was 7.7 $g.kg^{-1}$ and the volatile dissolved solids (VDS) was 3.6 $g.kg^{-1}$. In times of reactor upset the DS and VDS of the liquid on the granules may be somewhat higher than the above values, however it is unlikely that they would be more than the highest values measured in the feed of 8.5 $g.kg^{-1}$ DS and 7.7 $g.kg^{-1}$ VDS respectively. Within the granules it would be expected that DS and especially VDS would be lower than in the bulk liquid due to degradation of these compounds by the bacteria. Thus DS and VDS, for these very high solids analyses, are expected to be negligible. For these very high solids sludges and granular samples the values of TS and suspended solids (SS) are considered to be essentially equal as are those of VDS and volatile suspended solids (VSS). The errors introduced by this approximation are estimated to be less than 3 %.

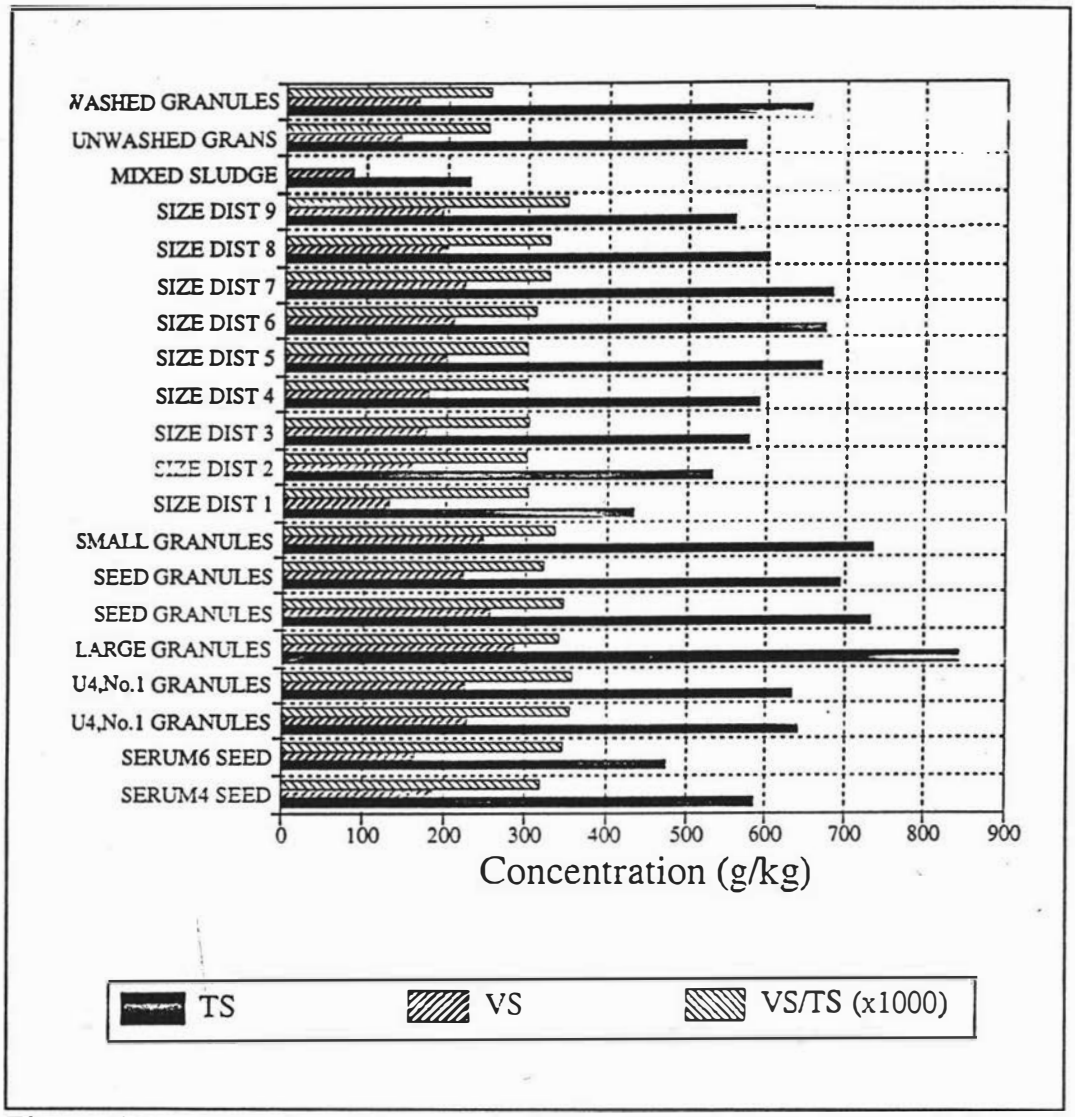

Figure 6.3 Granule solids analysis. Volatile solids, total solids and the ratio (TS/VS x 1000) are plotted from the data given in Table 6.3.

Figure 6.3 shows the solids analysis for the sieved granules. "Size dist 1" is the smallest and "size dist 9" the largest. The curve shown by both TS and VS is probably a function of the surface area and porosity of the granules rather than their age. Surface area to volume ratio increases with decreasing diameter. Thus for the smaller granules, the surface water film plus interstitial water becomes a larger part of the wet weight. Size fractions eight and nine show a progressive decrease in TS and VS. This may be due to the very broken and pitted nature of the outer layer of some of these granules shown in Figure 6.6. Water held in these regions may have reduced the apparent TS and VS values. VS/TS ratio is

practically constant for size fractions one to five and then shows a gradual increase with successive fractions. This suggests that the structure and composition of the granules were similar, especially in fractions one to five. For the larger granules it is suggested that the bacteria rich layer below the outer surface of the granule becomes a larger proportion of the granule volume with increased diameter. Granule structure is discussed in Section 6.2.4.

Table 6.3 Solids analysis for granules

Description and source	sample date	TS $g.kg^{-1}$	VS $g.kg^{-1}$	VS/TS $g.kg^{-1}$
mixed sludge C	4/4/89	642	229	0.36
mixed sludge C	4/4/89	633	226	0.36
large C	5/11/91	843	286	0.34
seed mix C	5/11/91	733	254	0.35
seed mix C	5/11/91	693	222	0.32
small C	5/11/91	737	247	0.34
average s.d D	21/9/92	591	185	0.31
mix sludge D	22/10/92	228	83	0.36
unwashed granules D	22/10/92	573	143	0.25
washed granules D	22/10/92	655	165	0.25
assay seed C	19/11/92	586	187	0.32
assay seed D	19/11/92	609	195	0.32
assay sludge D	22/3/93	474	164	0.35

Granules taken from reactors C and D over a period of four years show a similar VS/TS ratio which suggests a similar granular composition over time. Granules from reactor D sampled on 22/10/92 show a lower ratio but those sampled earlier and later from the same reactor had a higher VS/TS ratio, as shown in Table 6.3. It is uncertain why this has happened. The removal of more than half the sludge bed a month earlier with inevitable destruction of the sludge bed matrix plus a days feed interruption three weeks later may have exposed bacteria in the granules to conditions resulting in cell lysis in the outer layer of the granules. Analysis of the sludge from which these granules were taken shows a VS/TS ratio similar to the higher levels although absolute VS and TS values were much lower. This suggests that the sludge was more diffuse than was usual and that less of the total sludge biomass was contained within the granules than usual. Five months later the VS/TS ratio was back to normal. Indicating that the sludge matrix had been reformed

Both light and scanning electron microscope work suggest that wood biomass does not make up a significant proportion of the volatile solids in the granules. However it is a significant proportion of the matrix surrounding the granules and thus of the sludge as a whole.

6.2.4 Elemental analysis

Inductively coupled argon-plasma emission spectrometry (ICP) was used to determine some of the elemental constituents of the granules, total sludge and feed. Figure 6.4 shows a plot of these two samples. Those elements marked (*) were below the detectibility limit for that element. These elements were plotted as the detectibility limit of that element. The concentrations plotted were on a wet weight basis as the samples came out of the reactor. Iron is obviously the major constituent of the granules. On a dry weight basis iron represents approximately 45 percent of the granule weight. Calcium, sodium and phosphorus make up another 10 percent. The other measured elements a further

4 percent. Volatile solids such as hydroxides and carbonates represents approximately 41 percent. These species were probably complexed with iron.

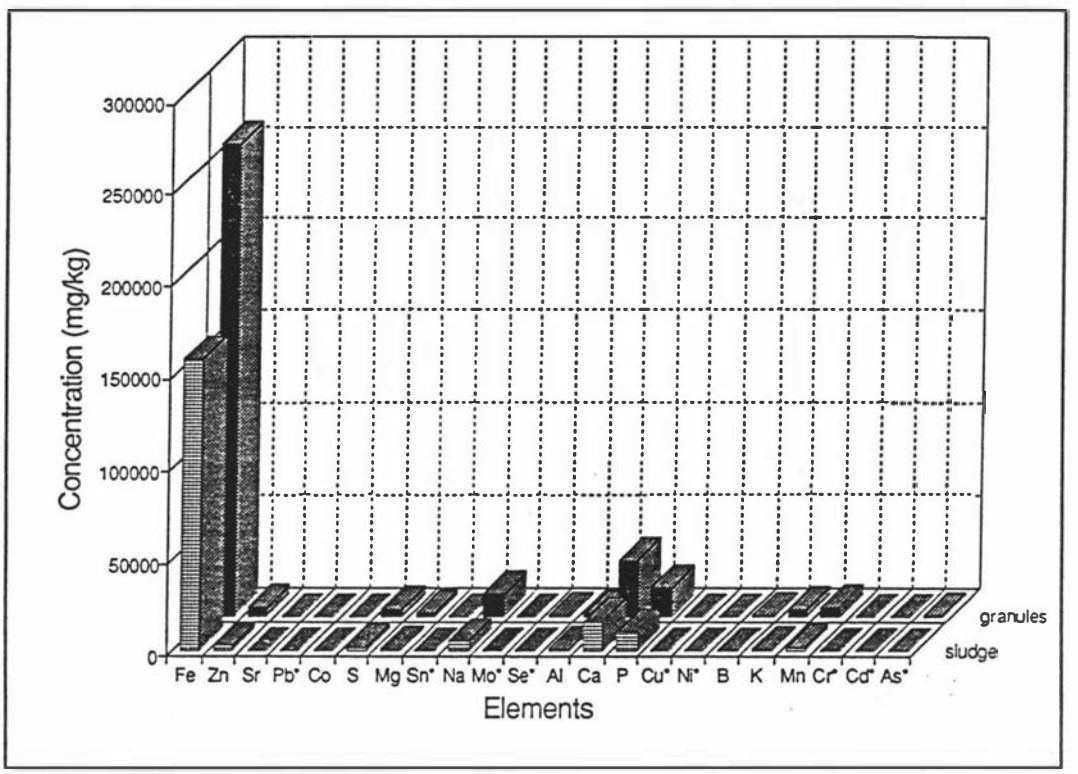

Figure 6.4 Granule ICP analysis. (* indicates plotted at the limit of detection. The actual level of these species is below the detection limit.)

ICP analyses of feed samples are given in Figure 6.5. These show a similar element concentration pattern, if not concentration, to that of the sludge. It should be noted that the samples for 25/2/91 and 26/11/91 had the usual nutrients added. The other two samples were analysed before nutrient addition. Thus sodium, calcium and phosphorus were higher in these samples. The usual elemental concentration of the nutrients added to the feed is also plotted. A sample of whole mill waste was also analyzed. This had been stored in a plastic container and so had no other iron source than those normally expected in a wastewater of this type. This sample had a total solids concentration approximately half that of the screw press effluent but an iron concentration of 1.84 $mg.l^{-1}$ compared to the average of the feed of 650 $mg.l^{-1}$. Thus it seems likely that practically all of the iron in the feed comes from the drum. To give a concentration of 650 $mg.l^{-1}$ of iron in the wastewater requires the removal of

a layer of steel from the inside of the drum 0.01 mm in thickness. Given the amount of rust seen on the inside of the drum this seems reasonable.

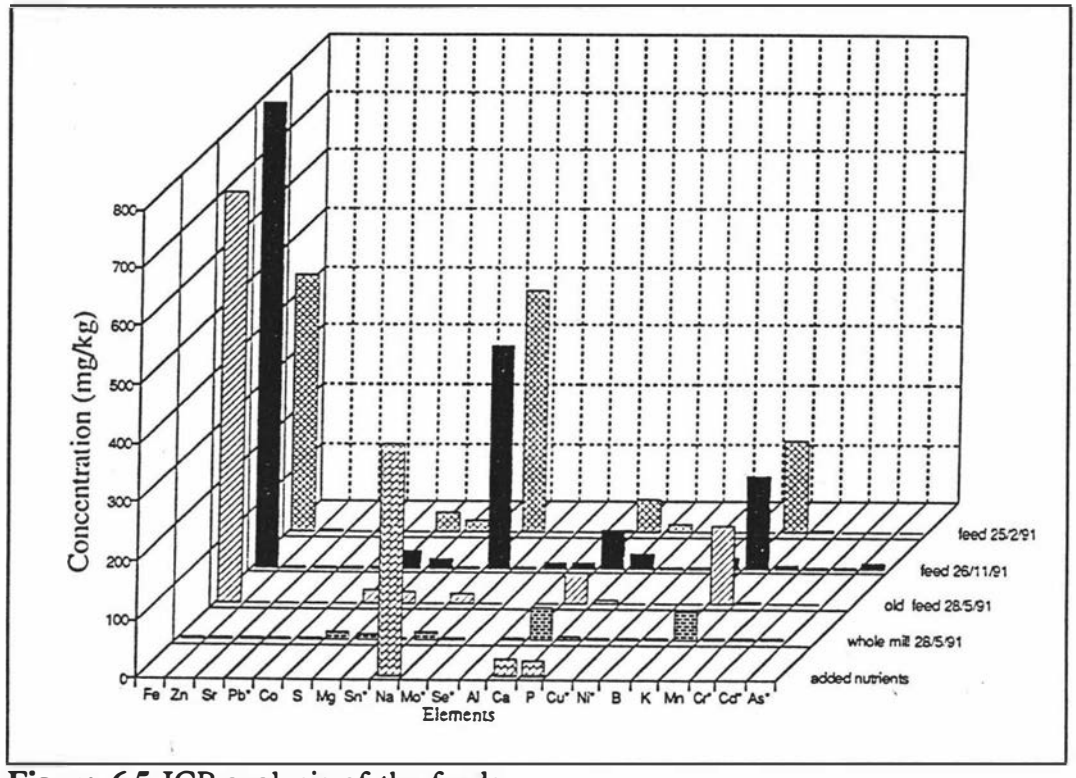

Figure 6.5 ICP analysis of the feeds.

Variation seen in the iron concentration in the three samples seems a function of the length of time the wastewater was in contact with the steel storage drum. Iron concentration probably slowly increased with increased storage time for the batch of feed and then dropped suddenly when the next batch was collected. What effect on the reactors this variation may have had is unknown but granulation had developed under these conditions and suspended solids degradation was occurring. Thus the decision was made to maintain this situation to allow the collection of data to characterise the performance of this reactor. At the time of printing, work was being conducted to investigate the effect upon granulation of iron concentration.

One effect of the high iron concentration of the granules was a high density. The granules average approximately 2000 $kg.m^{-3}$ density, compared to 1000 -

1050 kg.m^{-3} for many granules (Guiot *et al*, 1992; Dolfing, 1986). Thus these granules can be expected to show extremely good settling properties even at very small diameters. The high iron content would seem to form significant structural elements in the granules. This would thus improve granular strength and probably prevent or reduce loss of granulation in adverse conditions. Thus the high iron content makes a significant contribution to granule stability. A similar conclusion was formed from observation of iron sulphide deposits upon *Methanothrix* morphotypes (Shen *et al*, 1993). This paper considers iron as a metabolic supplement rather than as a structural medium.

Given the grey-green colour of washed fresh granules and the reducing environment within the reactor, the iron probably existed as Fe(II) compounds. These were likely to be in the form of iron hydroxide and iron carbonate complexes (Ainscough, 1994)

6.2.5 Structure

The macroscopic surface structure of the granules was variable. Some were relatively smooth while others were rough and pitted. This is shown in Figures 6.2 and 6.6. To some extent this seems a function of the degree of intactness of the granule. Scanning electron micrographs (SEM) of the washed granular surface as seen in Figure 6.7 show a varied surface with some tightly aggregated areas and some more loosely aggregated areas, cracks and pits. The tightly aggregated areas were shown at greater magnification in Figure 6.8. No bacteria or wood fibre were evident. This amorphous grain structure is also seen underlying cells in other areas of the surface and in a much more globular and separate form within granule pores and inside the outer layer of a granule. The more separate form can be seen in Figures 6.9 (shell internal surface) and 6.13 (unwashed granule outer surface) along with a number of blunt ended bacteria. Either this structure is an artifact of drying reactor liquor or it is part

Figure 6.6 Large Granules Each division on the scale is one millimetre

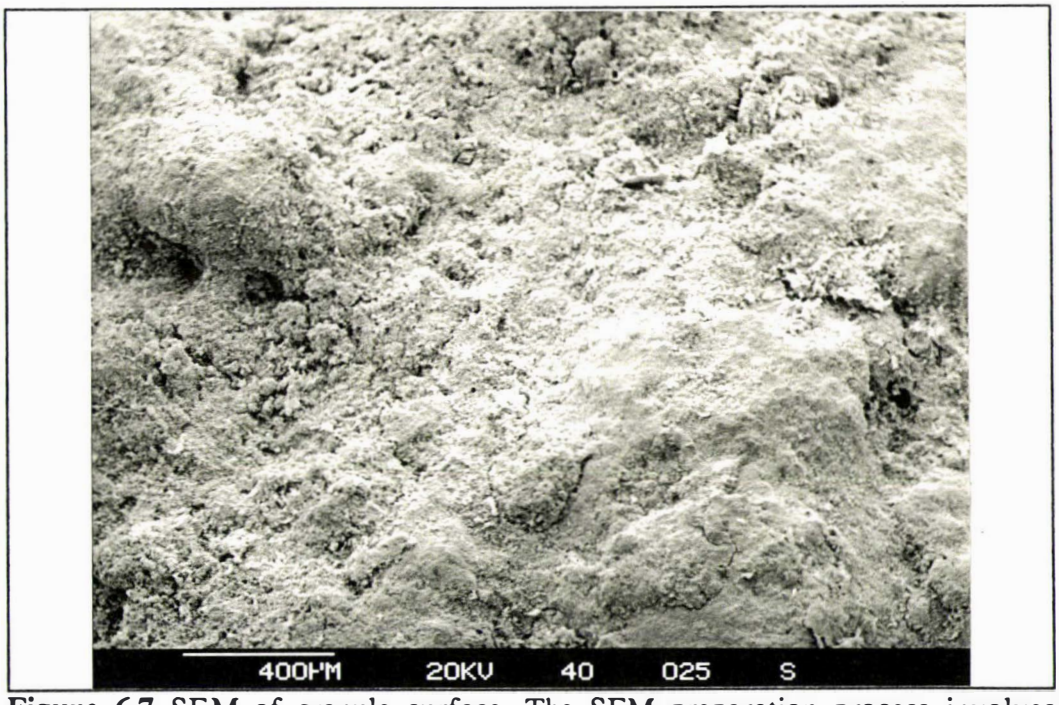

Figure 6.7 SEM of granule surface. The SEM preparation process involves fixing and drying the granule. Magnification (x50).

of the granular structure formed by the inorganic compounds. However it does not resemble any such drying artifact seen previously by the Ag Research

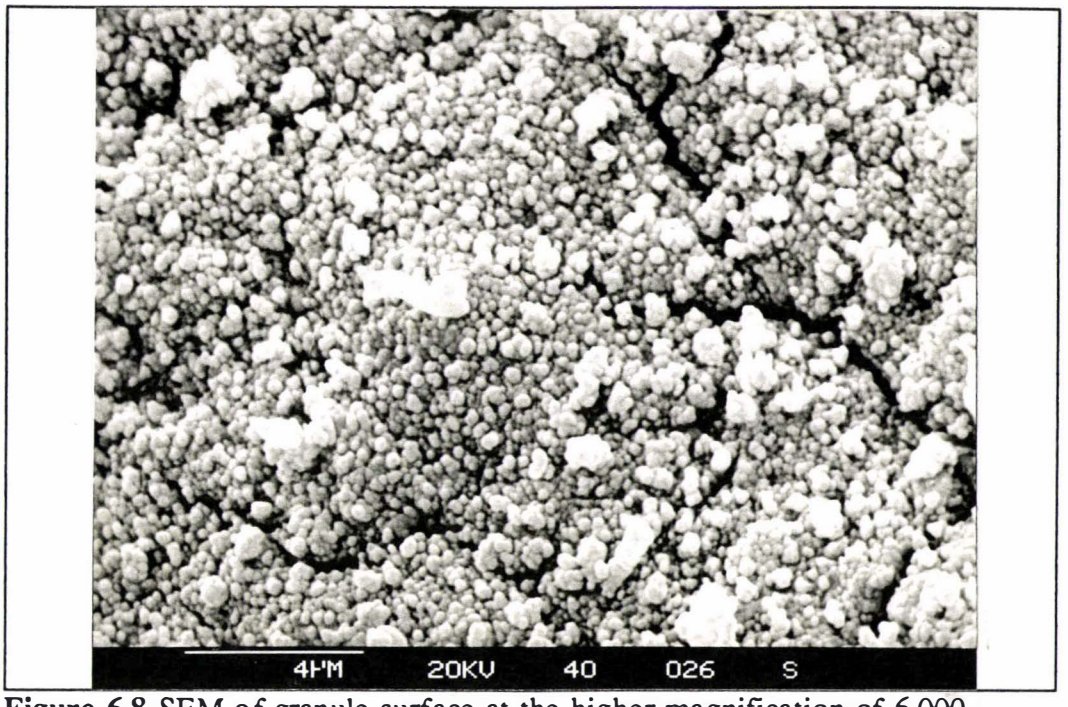

Figure 6.8 SEM of granule surface at the higher magnification of 6,000.

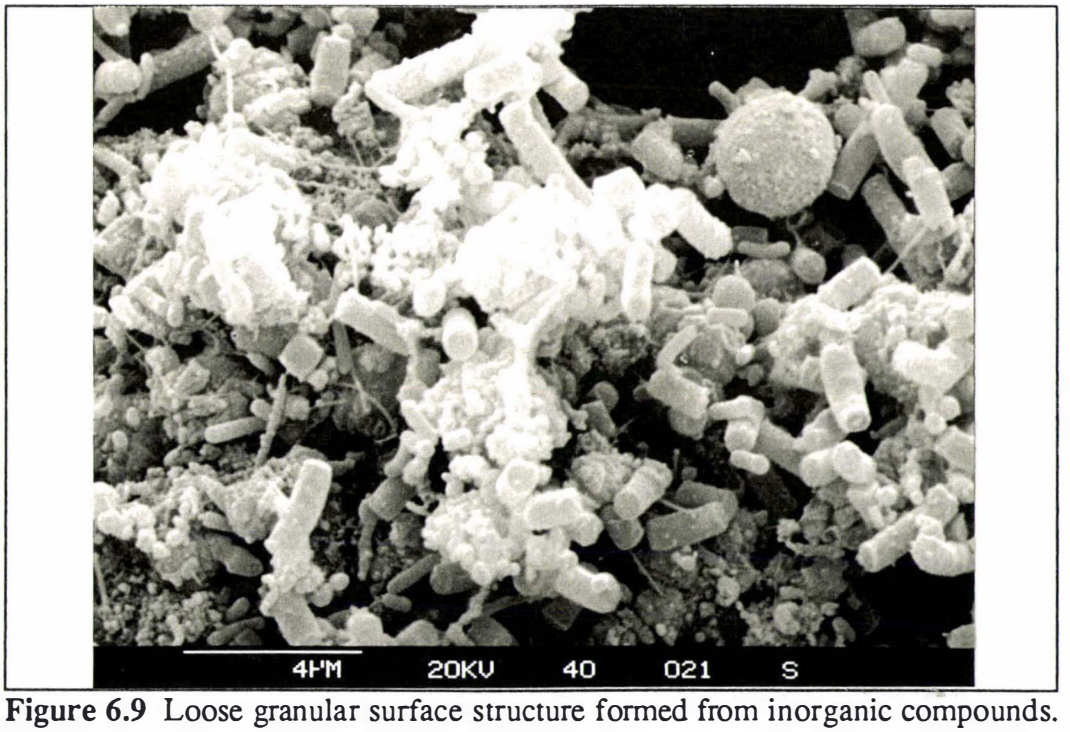

Figure 6.9 Loose granular surface structure formed from inorganic compounds. Some of the blunt-ended rod shaped bacteria present appear to have deposits of the inorganic material upon their surface (x6,000)

Electron Microscope Unit staff (Hopcroft, 1994). The amorphous grain structure was also seen to underlay an abundant scattering of bacteria and the discrete form of the amorphous grain structure as shown in Figure 6.12, this makes it unlikely that this structure is an artifact of the drying process. Given the preponderance of iron in the granules and the discontinuous nature of this structure it probably consists largely of precipitated iron carbonates and hydroxides.

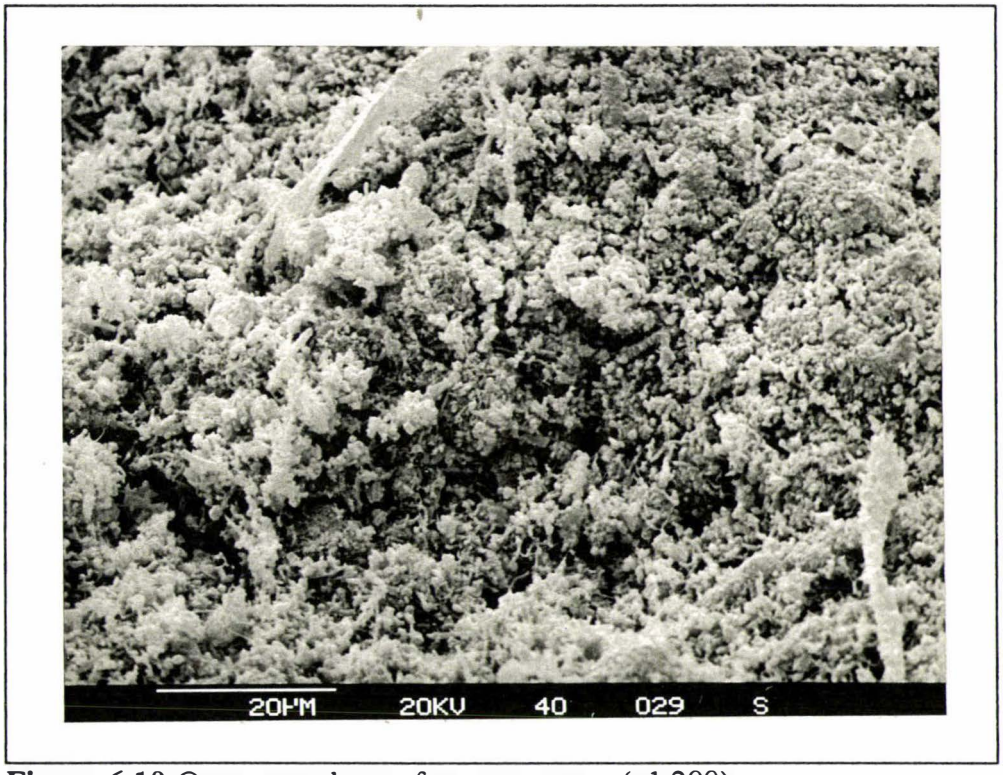

Figure 6.10 Open granular surface structure. (x1,200)

Figure 6.10 shows a more open surface area with a number of pores. Figure 6.11 is an enlargement of the pore in the centre-right of Figure 6.10 and shows what appears to be rod shaped bacteria at the surface. The bacteria shown are of a range of morphotypes. These more open areas may be the remains of the matrix layer fixed to the granular surface by the extensive amorphous precipitate like structure shown in Figure 6.11. These areas, as illustrated, may represent

granular growth by mineralisation and/or fermentative bacteria about a shell layer pore providing flow channels to the interior of the granule. The granules in these photographs were washed in reactor liquor to remove extra-granular solids and thus the observed structures must be fixed to the granular surface reasonably well.

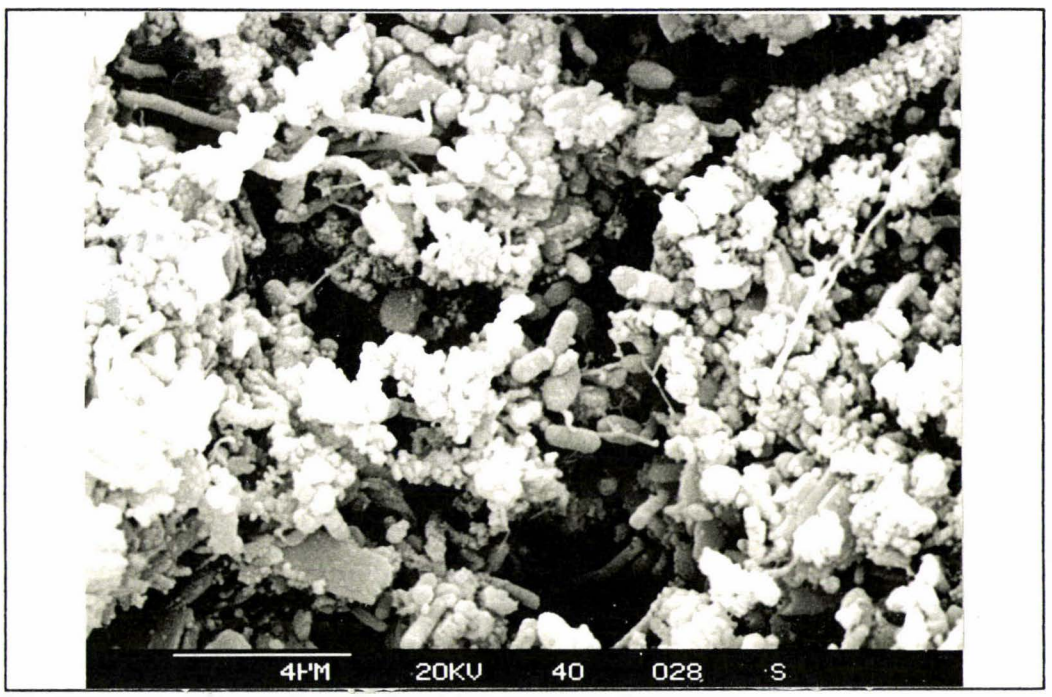

Figure 6.11 Enlarged pore from Figure 6.10 (x6,000)

Figures 6.12 and 6.13 show two magnifications of a point on an unwashed granule. A mixture of bacteria and precipitated solids can be seen to overlay a fairly solid surface. This mixture may be the remains of the sludge matrix, most of which has disappeared from the inactive reactor. This mixture of bacterial cells and other materila does not seem to be affixed to the underlying surface as strongly as that seen in Figures 6.10 and 6.11 The two square objects in the lower right of Figure 6.12 have the morphology sometimes seen in scanning electron micrographs of wood fragments. Other areas of this granule appear similar to the tightly aggregated surface seen in Figure 6.7 but even more tightly aggregated, shown at high magnification in Figure 6.14. A lower magnification view of this surface shown in Figure 6.15 has the appearance of the cracking seen in dried mud. It is uncertain whether this cracking was an artifact of the

fixing and drying process or is representative of the granule in the reactors. If this layer were dried reactor liquor this material would be expected to be spread uniformly over the whole surface of the sample. As it is not uniform it suggests that 2this surface is part of the granule. If the cracking has occurred as a result of drying then this surface must have had a reasonable water content and may well be the interface where the granule surface is laid down. If the cracking is representative of the granules within the reactor it suggests that the surface is under some tension possibly from gas evolution or bacterial growth. The cracking may provide channels for liquor entry to the granule.

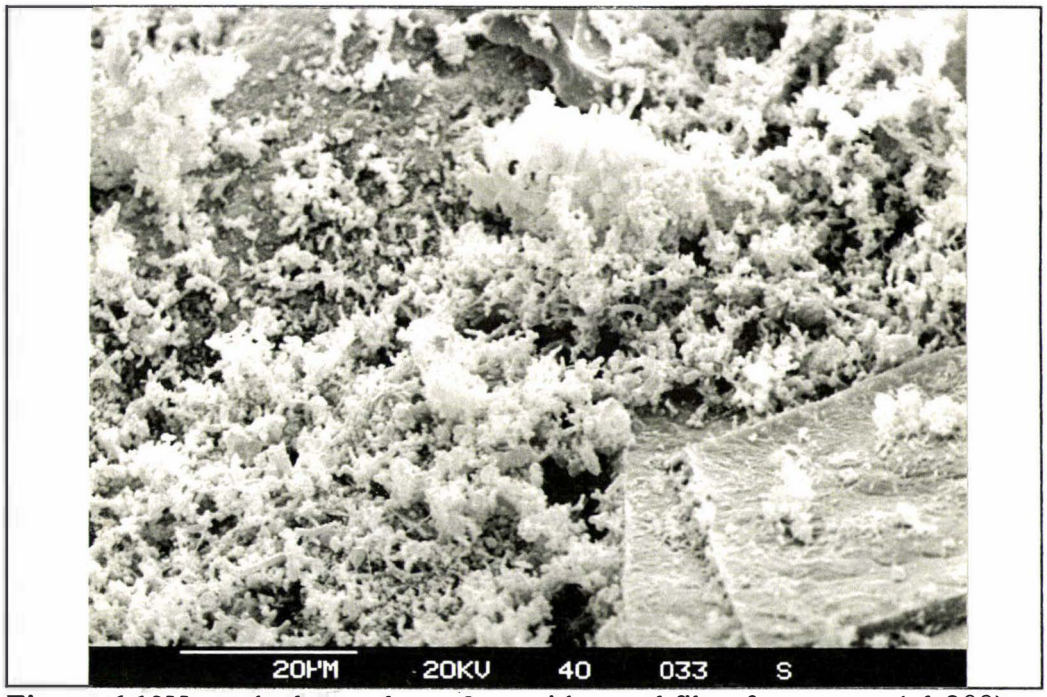

Figure 6.12 Unwashed granule surface with wood fibre fragments. (x1,200)

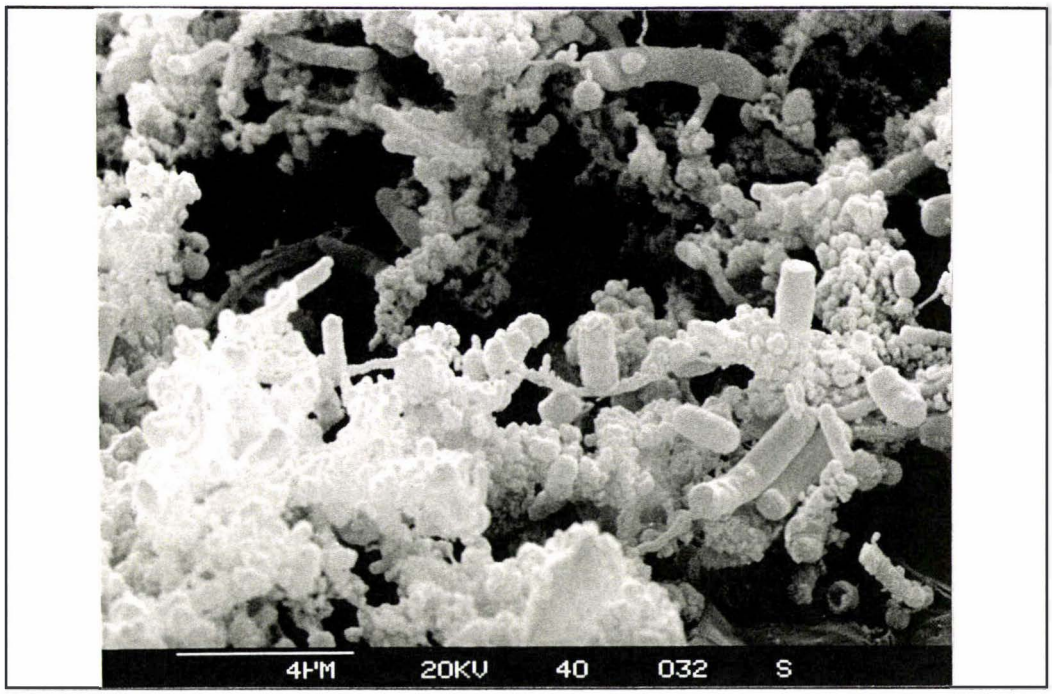

Figure 6.13 An unwashed granule surface (x6,000)

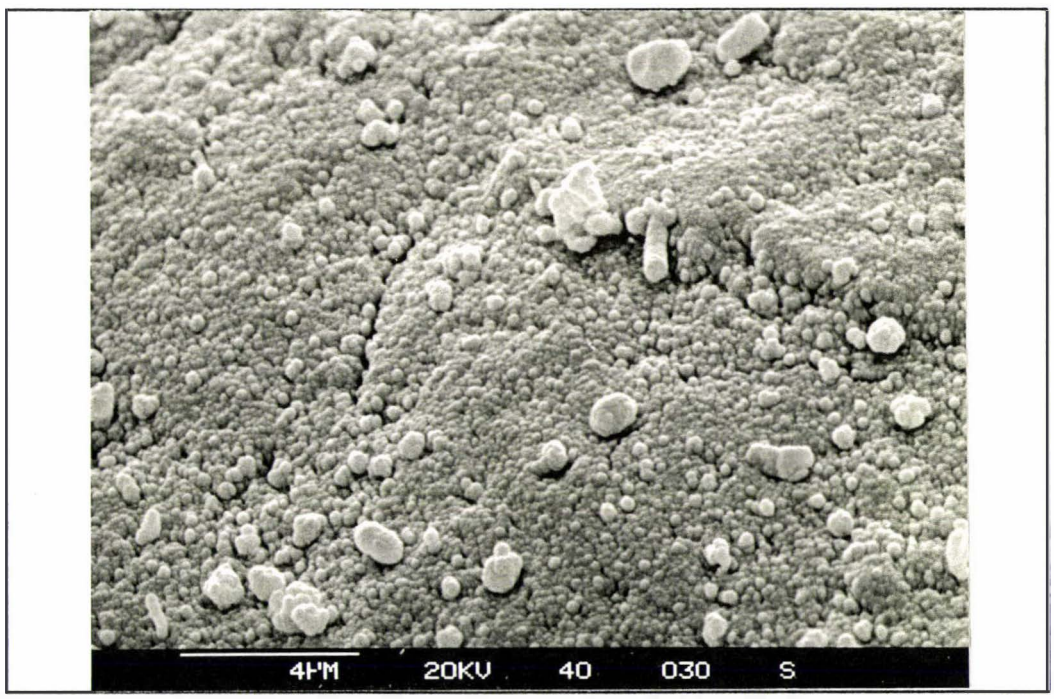

Figure 6.14A tightly aggregated area of a granule. (x6,000)

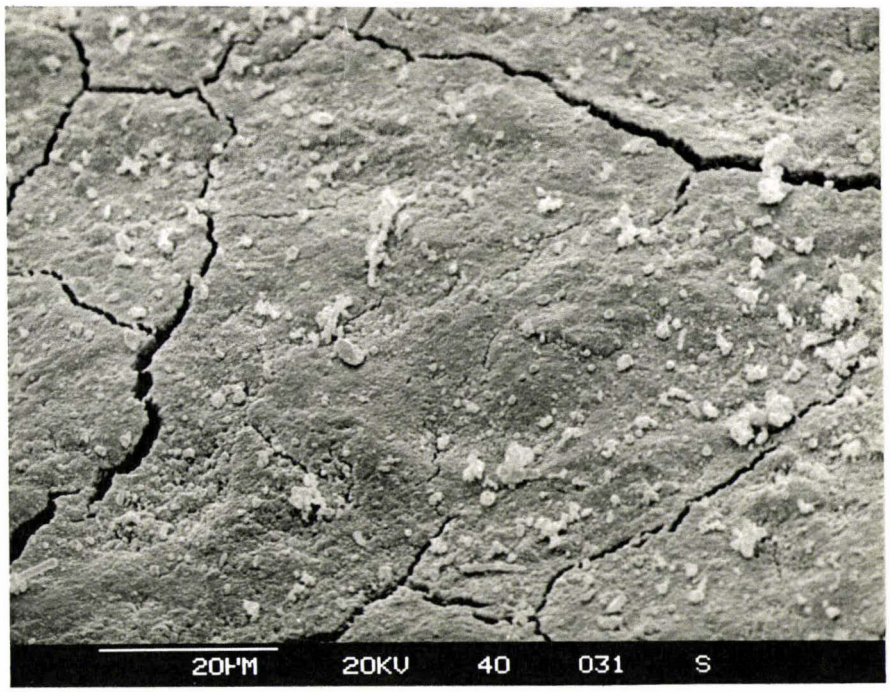

Figure 6.15 A lower magnification of the tightly aggregated area of an unwashed granule (x1,200)

The internal structure of the granules vary. Broadly two types of granule were found. The first, Type I, was a granule of relatively uniform construction. Figure 6.16 shows the broken edge and surface of the outer layers of such a 5 - 6 mm granule. This edge is a fracture rather than a cut surface. The granules were hard but quite brittle and fracture once a knife or scalpel cut is started. The outer layer is reasonably dense and inside this a mixture of dense and less dense zones exist. Micro- and macroscopic observation of fractured surfaces of these granules found that generally this mixture of dense and less dense zones fills the rest of the granule volume. On occasion the very centre of some granules showed a relatively open core in which the solids present were much lighter in colour than the usually black bulk of the granule.

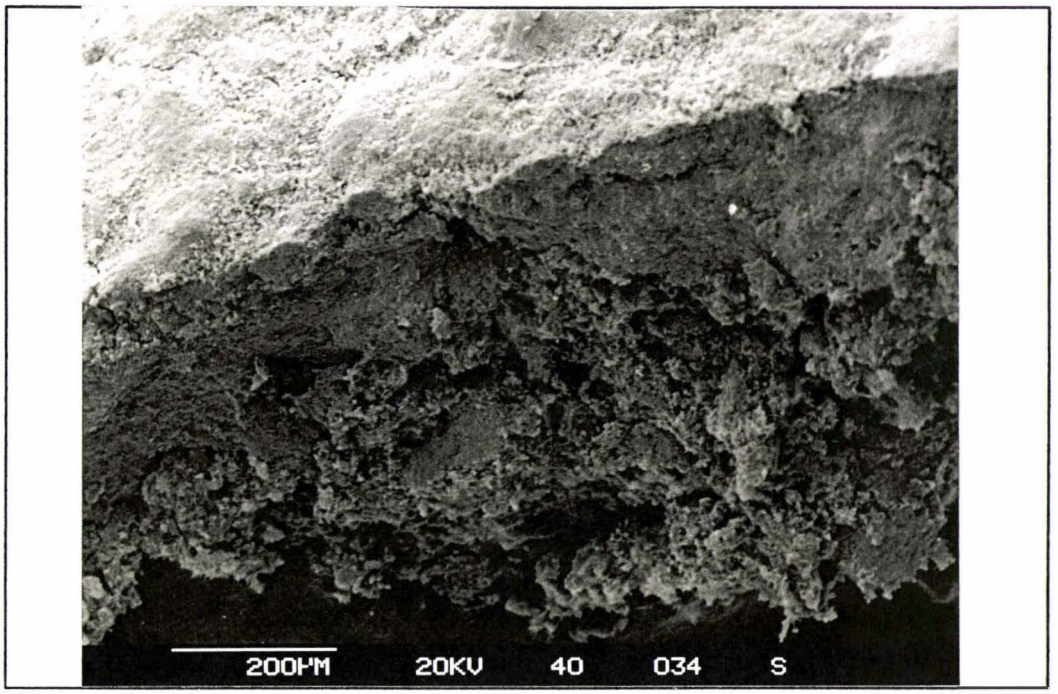

Figure 6.16 The broken edge of a 5-6 mm uniform granule at x111 magnification.

The second type of granule, Type II, appeared to be one of the above granules surrounded by a further layer of growth. This outer layer showed no evidence of attachment to the inner granule and once broken could be removed from the core granule with ease. Between the shell and core was a wet layer of low solids content. This is shown by the gap seen between the inner and outer layers in Figure 6.17. This was a granule air dried at 30 °C. The cut made to divide the granule can be seen as the lighter coloured zone at the bottom of the picture. A distinct gap exists between the shell and the core granule. Two layers can be seen in the shell. The outer layer was denser and reasonably uniform, while the inner layer shows a varied density similar to that seen in the type I granules.

Figure 6.17 A large granule that has been fractured open. The divisions are in millimetres.

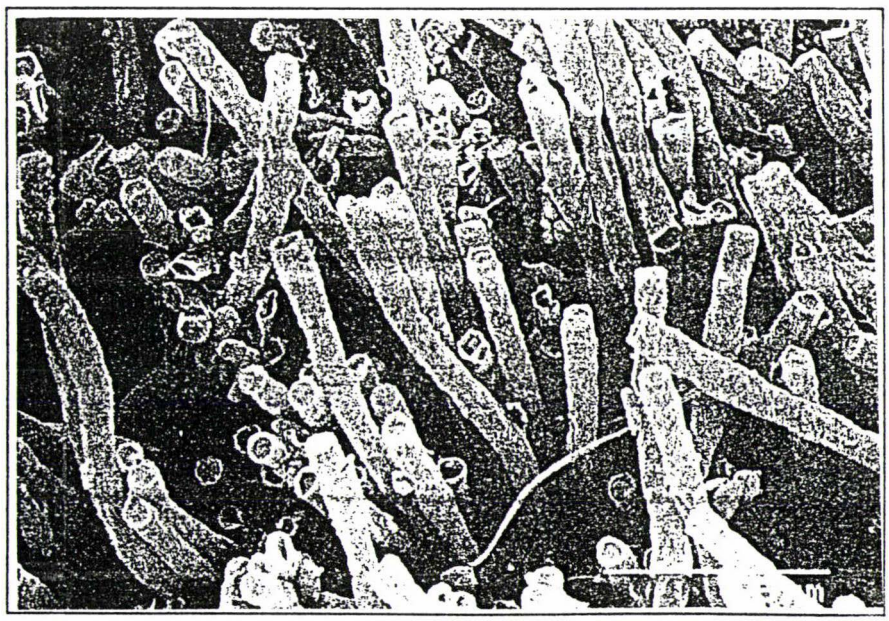

Figure 6.18 *Methanothrix* sp. from an acetate fed granule (Forster, 1991)

Reported examinations of various UASB granules in the literature have almost always found *Methanothrix* species within the granules generally suggest that these bacteria play an important role in granular formation (Guiot *et al*, 1992; Forster, 1991; Huser *et al*, 1982). The morphology typical of *Methanothrix* species is shown in Figure 6.18.

Scanning electron micrographs (SEMs) of the inside of the shell layer, such as shown in Figures 6.19 and 6.20, show extensive layers of bacteria. Most of the bacteria have the flat-ended rod morphology typical of *Methanothrix* species (Guiot *et al*, 1992; Forster, 1991; Huser *et al*, 1982). Many of the bacteria appear to have deposits on the sheath. Ferrous sulphide has been observed to stick to the sheath of *Methanothrix* species and might contribute to stabilization of bacterial aggregates within granules (Dubourguier *et al*, 1985). This suggests that the deposits seen may be ferrous sulphide or other iron compounds. Examination of the SEMs as to whether or to what extent the deposits were contributing to the stabilization of the granules shows a great deal of linking and structural formations at the surface, and within the shell layer itself, of the granules but much less on the inside of the shell layer. These structural and linking formations generally have the appearance of the deposits observed on the *Methanothrix* morphotypes. The network of thin strands seen in Figure 6.19 and others is symptomatic of the drying of a polymer gel as might be formed by extracellular polysaccharides (Hopcroft, 1994). This may be contributing extensively to granular structure given the degree of connectivity shown.

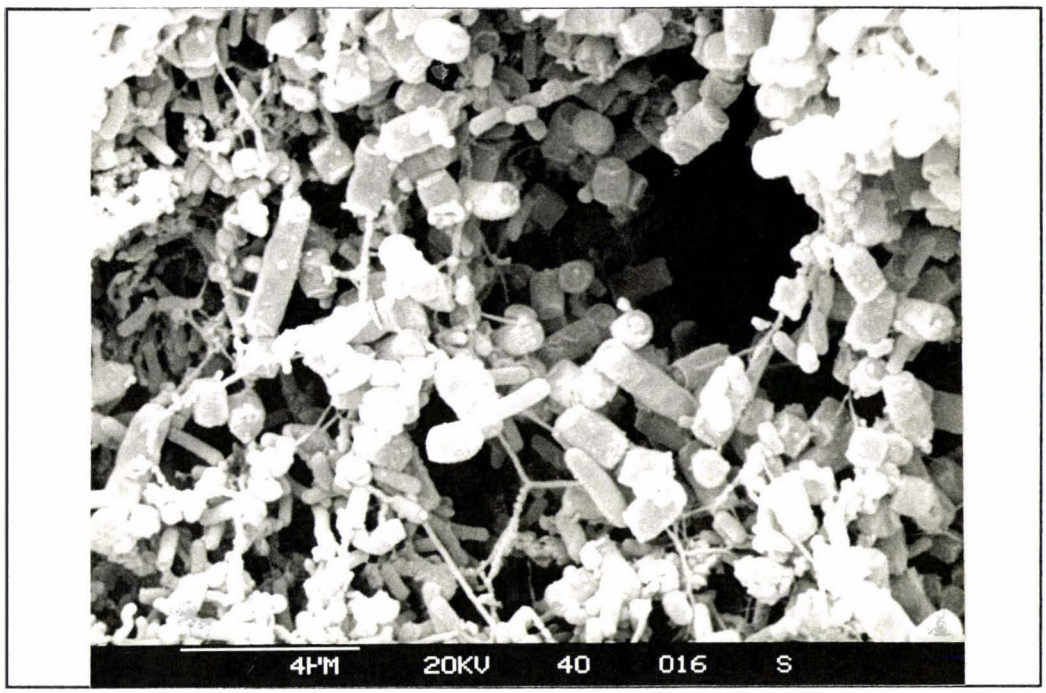

Figure 6.19 SEM of the inside of the shell of a large granule showing the rod shaped bacteria present. (x6,000)

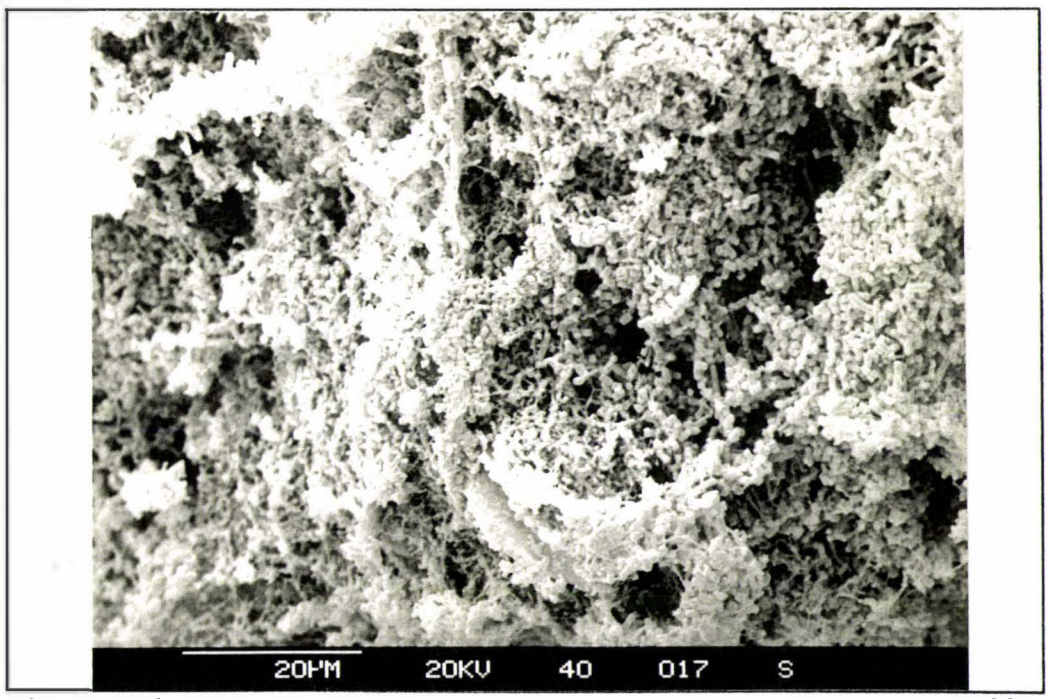

Figure 6.20 SEM of inside of large granule at lower magnification, (x1,200).

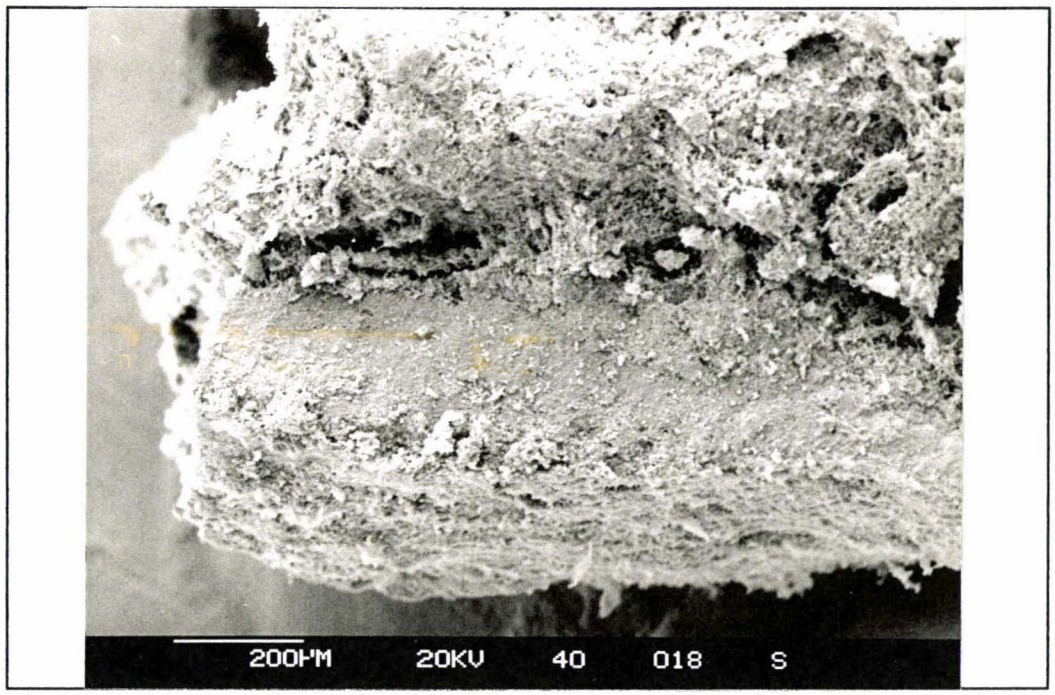

Figure 6.21 The edge of the shell layer of a granule. (x87)

An SEM of the edge of the shell layer is shown in Figure 6.21. The outside of the shell is towards the bottom of the picture. The shell layer seems to occur in two broad layers. The apparent division line between the two layers seen may be an artifact of the breaking of the shell, however the division while usually not entire, was often seen in broken shell edges. The inner shell layer had extensive amounts of bacteria mostly of the *Methanothrix* morphology, but the outer layer was much denser and seemed to contain no bacteria.

Figure 6.22 a-d shows views of the two layers. The denser outer layer was about 0.5 mm in thickness and usually crystalline in nature. The amorphous structure was seen to sandwich this crystalline layer and to be interspersed within the predominantly crystalline area . Observation of many granules showed this layer to be of reasonably constant thickness about the individual granule and relatively independent of granule diameter. Even in core granules and those without shell layers, the outer layer of the granule seems more dense and mineralised than the material further into the granule. The second, inner layer, seemed to be much more variable in different granules. Commonly this layer was found to be approximately 2 mm thick but was much thinner in some, usually smaller,

granules. When ashed large pits and holes were obvious in this layer suggesting that there were zones which were predominantly biomass and others which were predominantly mineralised. Between the shell and the core granule was a third more diffuse layer. This was a thick black paste of volatile solids. Any inorganics present did not appear to have any structure or degree of interconnection and little residue of this layer was left upon ashing.

Electron and light microscope examination of granules showed little inclusion of wood biomass. Figure 6.23, taken from the side of the shell layer shows two wood fibres. Very little of this type of biomass was seen within the granular structure and if seen it was usually on or near the surface where it may have been deposited during drying.

Occasionally very large granules were found to contain two shell layers. The most likely explanation for these shell layers would seem to be removal of the sludge from the reactor and break-up of the sludge structure. This happened when reactors C and D were seeded from reactor C. After removing the sludge from the reactor the granules were separated from the rest of the sludge so that the two very different fractions could be added to the two new reactors equally. Thus the surrounding layer of sludge matrix was largely removed from the granules. During the above processes some exposure of the granules to oxygen was inevitable. When the granules were placed back in the reactor their surface was exposed directly to the reactor liquor without the protection of the matrix layer. Inhibitory effects of the oxygen and some feed compounds may have resulted in the inactivation of surface bacteria. Possibly rather than continue growing the granule simply became a surface upon which bacteria adhered. The granules with two shell layers may stem from similar types of event in the history of reactor C. These occurred at 15 and 31 months before reactor C was split.

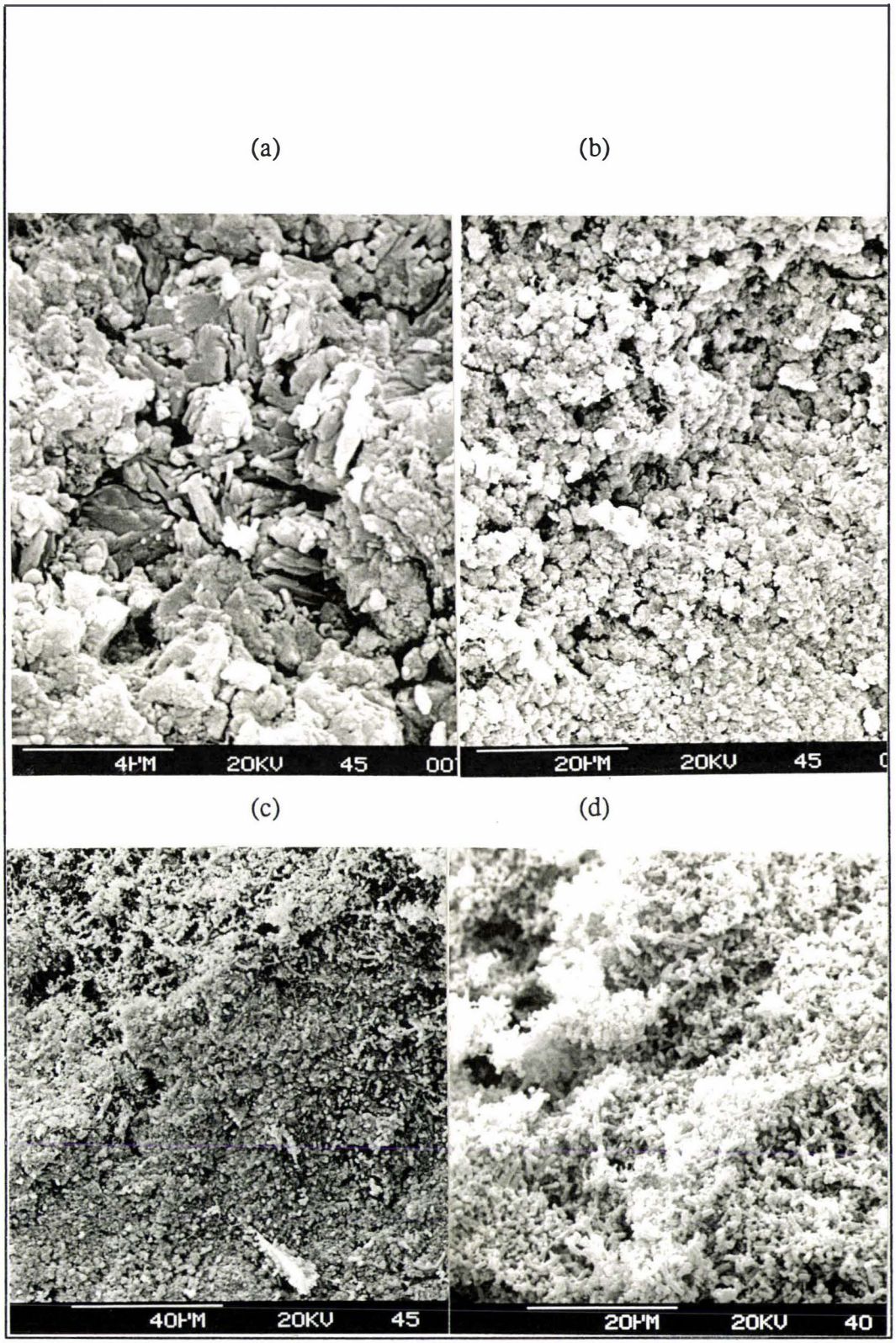

Figure 6.22 Views of the cross-section of the shell layers shown in Figure 6.21 a - (Upper left) Outer layer (x6,000); b - (upper right) Outer layer (x1,200); c (lower left) Transition between layers, outer layer towards the bottom of the photograph (x6,000); d - (lower right) Inner layer (x1,200)

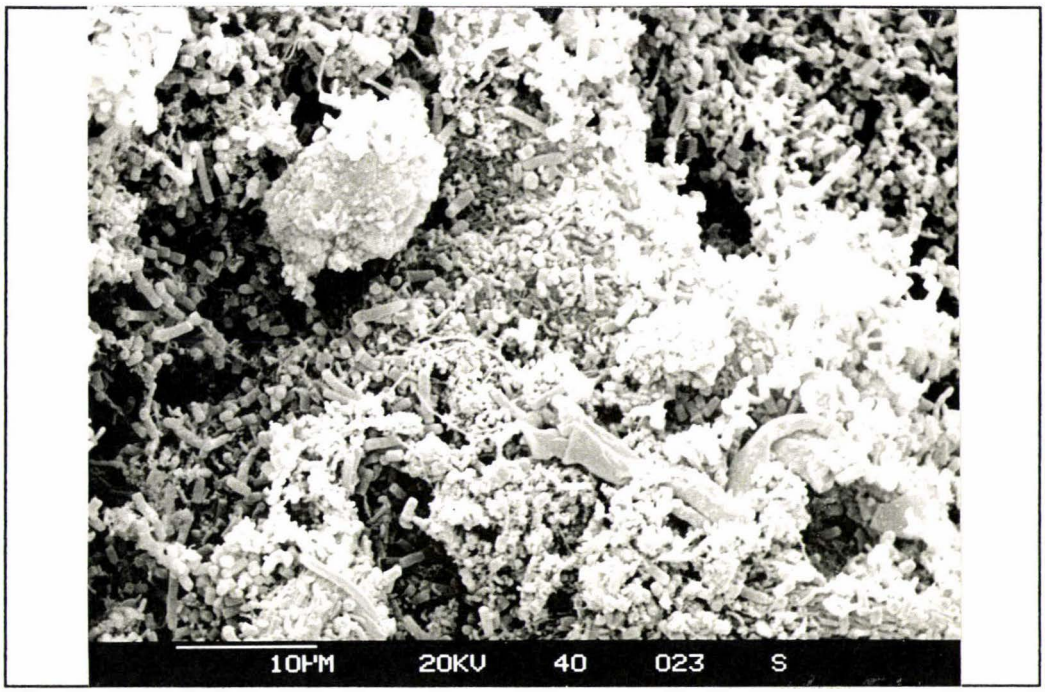

Figure 6.23 SEM of the side of the shell layer showing two pieces of wood fibre. (x1,800)

Wood fibre fines have been reported responsible for prevention of granulation in medium density fibreboard screw press effluent (McFarlane and Tan, 1985) and their removal was deemed essential for successful anaerobic treatment of TMP/CTMP (Habets and de Vegt, 1991). Another report found that successful UASB treatment could occur with fines retained in CTMP effluent, but that reduced performance resulted and granule settleability was greatly reduced. The work commenced with active granules from recycled paper mill. No increase in granular sludge was observed, although this may have been the result of the feed toxicity and the short duration (140 days) of the trial (Richardson *et al*, 1991). Biomass retention was identified as a potential problem.

In contrast this project has produced extensive granulation from a non granular seed sludge. The granules were extremely well settling and have great structural integrity. These granules have retained their structure after storage at room

temperature, under reactor liquor for a period exceeding 18 months. The iron rich granules removed the problems of biomass retention even under severe inhibition reported as a drawback of the UASB reactors for treatment of these types of forestry industry wastewaters. The threshold iron concentration needed for this type of granulation needs to be investigated. Cheap iron sources were also needed such as dust from iron refineries or possibly iron rich soils.

6.3 Serum bottle tests

Granulation in reactors C, C and D was extensive and formed a number of large granules over 1 cm in diameter. At this size diffusional limitations may have affected the methanogenic activity of these granules. To test this possibility and to determine the biological methane potential of the wastewater a series of serum bottle assays was conducted.

6.3.1 General procedure

Experiment one was started when granules were available from the seeding of reactors C and D. This was intended as a quick test of the potential of the method. Reactor feed (screw press effluent) with the usual concentrations of added urea, calcium mono-phosphate and sodium bicarbonate was diluted to 20 % v/v. Additional sodium bicarbonate was added at 2 $g.l^{-1}$ to maintain neutral pH. The diluted feed plus granules were sealed into nitrogen gas flushed serum bottles and incubated at 35±1 °C. Gas production was measured periodically and the methane content of this gas analyzed each time. The methodology is described in section 3.3.

Experiments 2-8 were conducted after the steady state runs for reactors C and D were complete. A defined media was prepared as suggested by Owen *et al.*, (1979) with nickel and selenium added at levels equivalent to the copper concentration. Resazurin was not used. The use of granules as the inoculum prevented the use of the anaerobic transfer apparatus. Defined media, diluted

feed and granules were transferred to the serum bottles under copious nitrogen gas flushing. Despite these precautions, there was considerable inhibition evident in the results of these tests and the use of various concentrations of defined media was attempted to overcome this problem. Provision of micro-nutrients as non chloride compounds gave increased activity. Inhibition from common salt used in tracer studies in both reactors C and D was suspected.

A total of eight experiments involving 137 individual serum bottles were run. Control runs were included with each experiment omitting feed or inoculum as appropriate. Analytical procedures are described in Section 3.3. Only experiments one to three are relevant to this chapter. Experiments four to eight are discussed in Chapter 5. Conditions for experiments one to three are shown in Table 6.4.

6.3.2 Inoculum source and quantity

Experiment 1 used granules from reactor C that had been incubated in a nitrogen flushed flask digester for three days with no feed. Experiments 2 to 8 were inoculated with granules or granular sludge from the more active of reactors C or D at the time of use (judged by gas production). The granular nature of the inocula meant that obtaining equal quantities of inocula in each bottle was impossible with out disruption to the granules and possible exposure to oxygen. The amount of reactor liquor included with the inocula was another source of variation in the quantity of biomass introduced to each bottle.

Table 6.4 Experimental conditions for serum bottle experiments one to three

Expt	Feed COD added	Run	Variable	Granule diameter (mm)	VSS (g)
1	92	1-3	Large granules	4 - 8	0.42
	92	4-6	Medium granules	2 - 3	.07
	92	7-9	Small granules	0.5 - 1	0.20
	92	10-12	Feed blank	0	0
2	144	1-3	Large granules	4 - 8	0.42
	144	4-6	Medium granules	2 - 3	0.26
	144	7-9	Small granules	0.5 - 1	0.29
	144	10-12	Fines	0.1 - 0.3	0.29
	144	13-15	Feed blank	0	0
3	0	1-7	Seed blanks	0 - 6	0.37
	97	8-10	Feed blank	0	0
	97	11-13	Washed granules	1 - 6	0.36
	97	14-16	Unwashed granules	1 - 6	0.34
	97	17-20	Mixed sludge	0 - 6	0.19
	30	21	granules + acetate	1 - 6	0.20
	127	22	granules + acetate + feed	1 - 6	0.29

6.3.3 Results and discussion

Gas production in the serum bottles was slow compared to similar assays described in the literature (Sierra-Alvarez, 1990). Experiment 1 produced approximately 90 % of the final methane within 30 days but was monitored until day 345. Experiment 2 was monitored for 245 days. Experiment 1 shows a range of total methane production from 66 - 88 % of the theoretical yield. Experiment 2 shows a range of 85 - 92 % of the theoretical yield for the samples and 14 - 18 % for the feed blanks. The yields for these two experiments are shown in Table 6.5.

Table 6.5 Actual and theoretical yields of methane from serum bottle runs for experiments 1 and 2

Expt Run	inoculum size (g)	Theoretical yield[a] (ml)	Actual yield[b] (ml)	Recovery percentage
1-1	1.62	34.1	32.0	88
1-2	1.43	34.1	30.6	84
1-3	1.27	34.1	29.3	78
1-4	0.31	34.1	27.7	76
1-5	0.25	34.1	25.2	69
1-6	0.21	34.1	26.7	73
1-7	0.91	34.1	26.9	74
1-8	0.73	34.1	26.1	72
1-9	0.75	34.1	27.0	74
1-10	0	34.1	23.9	66
1-11	0	34.1	25.3	69
1-12	0	34.1	24.4	67

Expt Run	inoculum size (g)	Theoretical yield[a] (ml)	Actual yield[b] (ml)	Recovery percentage
2-1	2.15	53.3	50.7	89
2-2	1.77	53.3	50.0	88
2-3	2.54	53.3	50.9	89
2-4	1.35	53.3	49.7	87
2-5	1.09	53.3	50.1	88
2-6	1.53	53.3	50.7	89
2-7	1.30	53.3	48.5	85
2-8	2.23	53.3	48.8	86
2-9	1.48	53.3	48.9	86
2-10	1.48	53.3	52.4	92
2-11	1.60	53.3	51.8	91
2-12	2.61	53.3	48.3	85
2-13	0	53.3	9.4	19
2-14	0	53.3	9.6	19
2-15	0	53.3	7.7	15

a theoretical yield = substrate COD x 0.351 x (273+35)/273

b all values corrected for gas production from the seed granules with the average of the seed blank runs in experiment 7

The realised mineralisation percentage of experiment 1 is, on average lower than that of experiment 2. This probably occurs because oxygen was not rigorously excluded from the feed of experiment 1 and thus some of the COD is oxidised to carbon dioxide and water rather than giving methane. This only has at most a 2 % reduction in the theoretical methane production. A full micro-nutrient

media was not supplied for experiment 1, possibly a micro-nutrient became limiting preventing full degradation to methane of the feed substrates. Another possibility is that insufficient mixing of the feed during the serum bottle process may have allowed some settling to take place. This could result in variation in the COD added to each bottle. The trend of the percent recovery seen in experiment 1 of an initial decline followed by a levelling out is suggestive of this. In experiments 2 - 8 nitrogen gas sparging of the feed vessel plus hand mixing before each removal of feed largely prevented this problem.

Methane production from the feed blanks in experiment 2 was much slower to start and much less complete than in experiment 1. It seems likely that the number and type of bacteria in the feed differs with each of the batches of feed used in these experiments. Lower cell numbers result in lower activity. In the later experiment it is possible that bacteria capable of using all of the variety of organic compounds found in the feed were not present. Also the suspended solids content of the feed used in the later experiments was higher than that used in experiment 1, approximately 30 % of total solids compared to approximately 25 % for experiment 1. Production of methane from the feed blanks was delayed enough to indicate that methane production by feed bacteria was negligible in the seeded serum bottle runs.

Figures 6.24 and 6.25 show the total methane production and methane production rates for experiment 1. Methane production by the large (4 - 8 mm) and small (0.5 - 1.0 mm) granules shows a similar curve but that of the medium (2 - 3 mm) granules lags markedly behind the other two. Examination of the specific methane production rate curves (ml CH4/day/gVSS inoculated) in Figure 6.25 shows very dissimilar rates. If the quantity of the biomass was rate limiting the specific methane production rate curves would be very similar. This suggests the production rate of methane is independent of the presumed quantity of biomass present. If substrate and/or product diffusion was limiting, the methane production rate (ml CH4/day) would be inversely proportional to the length of the diffusion path. Thus the large granules, having presumably, the longest

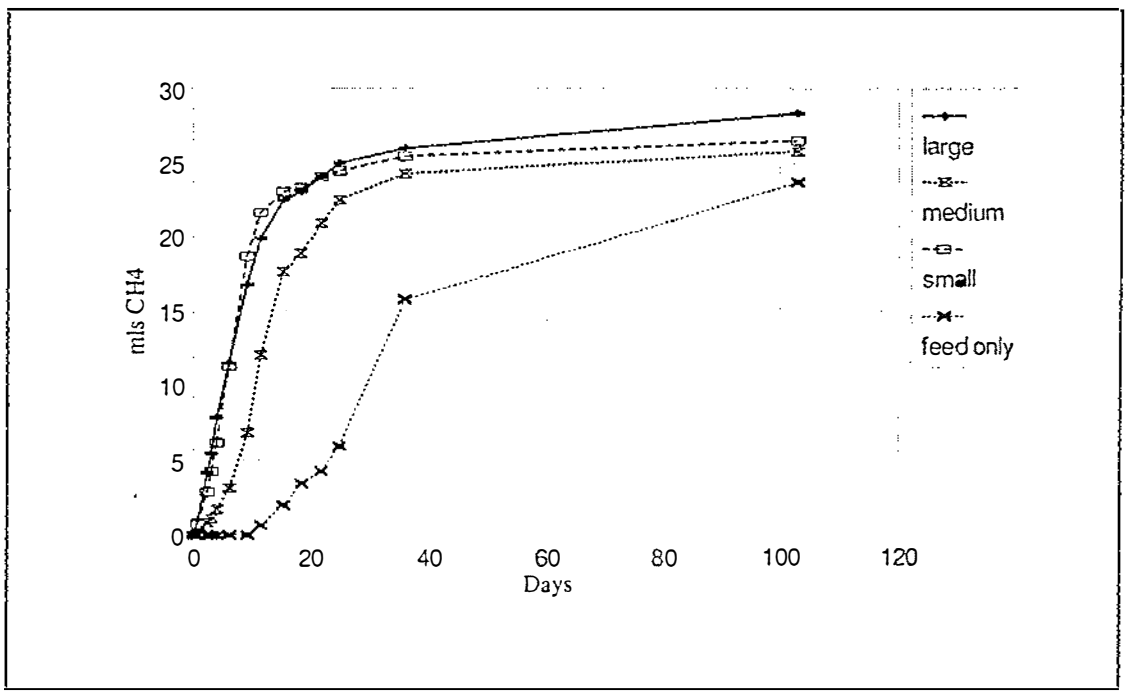

Figure 6.24 Total average methane production for experiment 1 with granule size as a parameter.

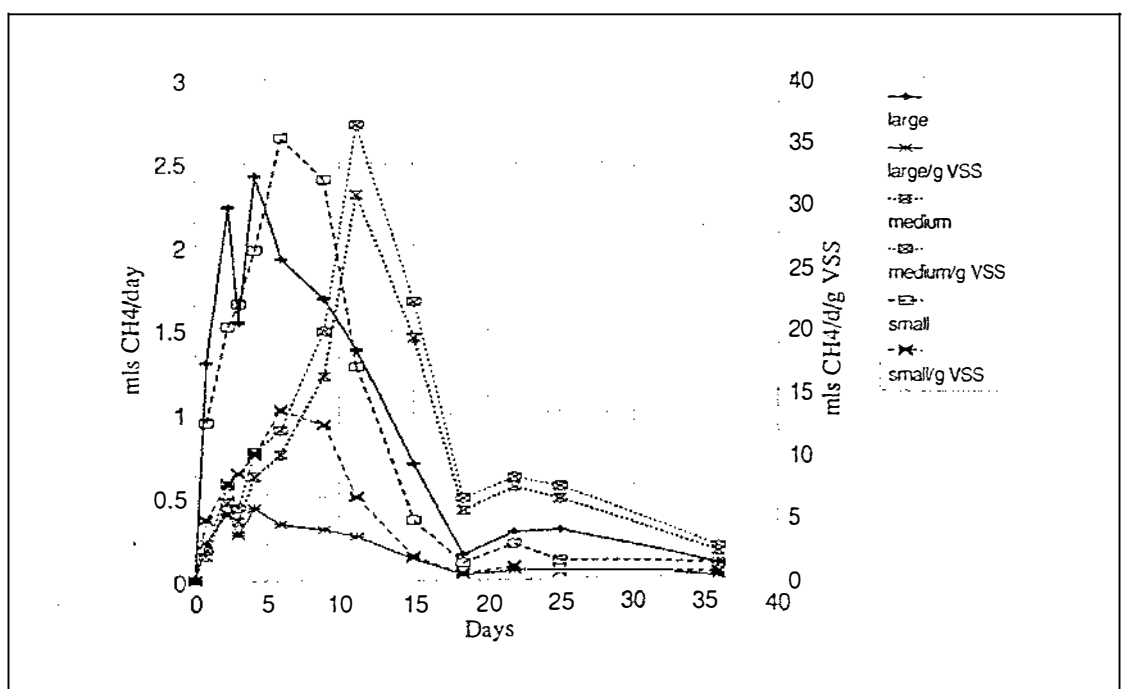

Figure 6.25 Methane production rates for experiment 1 with granule size as a parameter.

diffusional paths, would be expected to show the lowest production rate. However the three granule size fractions show very similar maximum methane production rates. This suggests that any diffusional limitation, if present, is similar in all three size fractions. Greater diffusional limitation in any one of the size fractions should result in a lower maximum methane production rate for that fraction. This is obviously not the case in this experiment.

Examination of the time taken to reach the maximum methane production rate, as shown in Figure 6.25, would seem to indicate that it is an inverse function of the size of the inocula used. This together with the similar maximum methane production rates would seem to suggest that the seed is acting as an inocula only and does not play a rate limiting role in methane generation. To achieve this type of result requires either:-

(1) Such severe diffusional limitation that the granular biomass has no appreciable methanogenic activity and acts only as an inoculum for single cell or flocculated growth. This growth then provides all the methanogenic activity.

(2) that the rate limiting step of the mineralisation of the wastewater is not conducted directly by the granular biomass but by either single cell growth or extracellular enzymes produced by some or all of the granular biomass.

The presence of the granules in the continuous reactors suggests that at least some of the granular biomass, if only that near the granule surface, has significant methanogenic activity. Without this activity the granules were unlikely to form and iron precipitation would be much more uniform throughout the reactor. Great abundance of *Methanothrix* morphotypes inside of the shell layer suggests these cell were or have been active in the continuous reactors. Macroscopic examination of the shell layer of granules suggests that a number of large (up to 0.5 mm in diameter) pores often exist in this layer. A gas lift

type mechanism by exiting biogas may pull fresh substrate into the cell rich inner layer as described in Section 6.2.4. The pores at least, reduce the likelihood of severe diffusional limitation. The extent of change in methane production rate with time suggests a large increase in cell numbers.

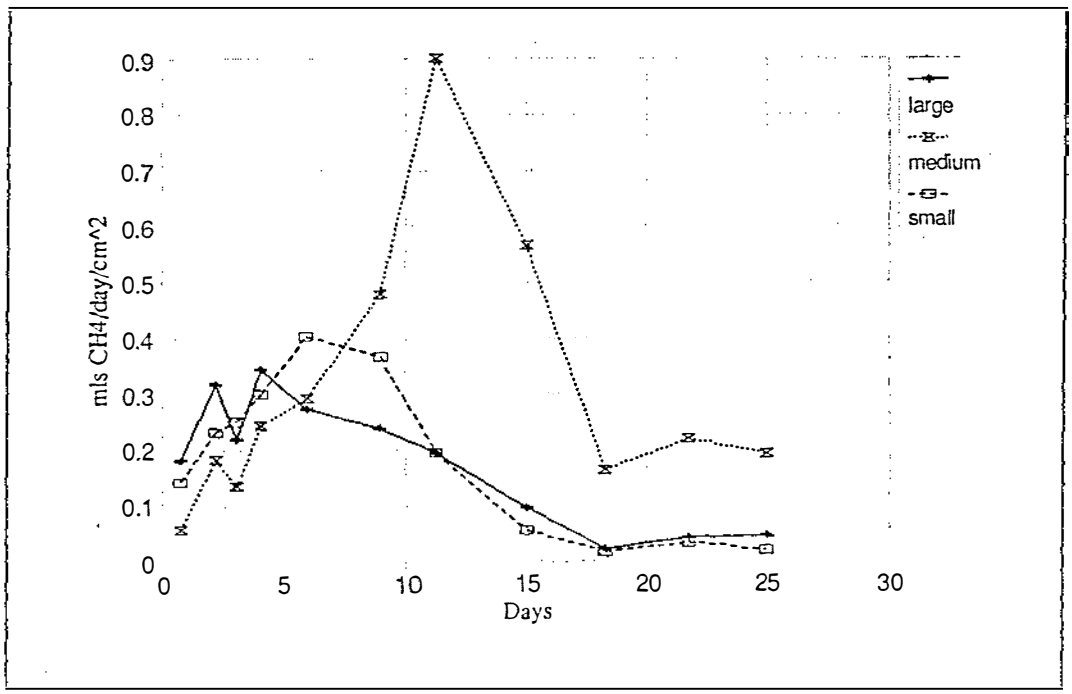

Figure 6.26 Surface area specific methane production rates with granule size as a parameter.

Surface area specific methane production rate curves for the three fractions, as seen in Figure 6.26, do not show enough similarity to suggest a granule surface area mediated mineralisation process. The calculated surface areas for the large and small granule fractions were similar as shown in Table 6.6, that for the medium granule fraction significantly less. The time taken to reach the maximum methane production rate is a simple function of the surface area of each granular inocula of the assay runs.

Table 6.6 Calculated average granule surface areas

Granule size fraction	average surface area (cm^2)	Time to maximum CH4 production rate (days)
large	7.1	4
medium	2.6	11
small	6.6	6

Experiment 6 examined the toxicity of the feedstock. Figure 6.27 shows the effect of feed concentration on the methane production rate. The first three concentration points for each day (i.e. each line on the graph) generally lie in a straight line. This indicates that substrate inhibition does not become significant until the feed concentration is greater than 25 %. The strength of the feed batch used for experiment 6 was approximately 50 % higher than that used for experiment 1. Feed strength in experiment 1 is approximately 14 % of the undiluted feed used in experiment 6. Thus substrate inhibition did not have a significant effect upon experiment 1.

Scanning electron microscope photographs of the inside of granules show an overwhelming preponderance of *Methanothrix* morphotypes as is shown in Figure 6.20. Thus acetate is the predominant chemical species likely to be effected by diffusional limitation. Other bacterial morphotypes were seen almost exclusively on the granular surface or on the sides of pores. These would not experience any diffusion effects, in the serum bottle tests, apart from those resulting from the usually quiescent liquid conditions.

In summary, the proposed mechanisms operating in experiment 1 were fermentation as the rate limiting step with a very low active biomass or

methanogenesis as the rate limiting step again with very low active biomass. Production of extracellular enzymes by the fermentative bacteria can not be discounted with the available evidence.

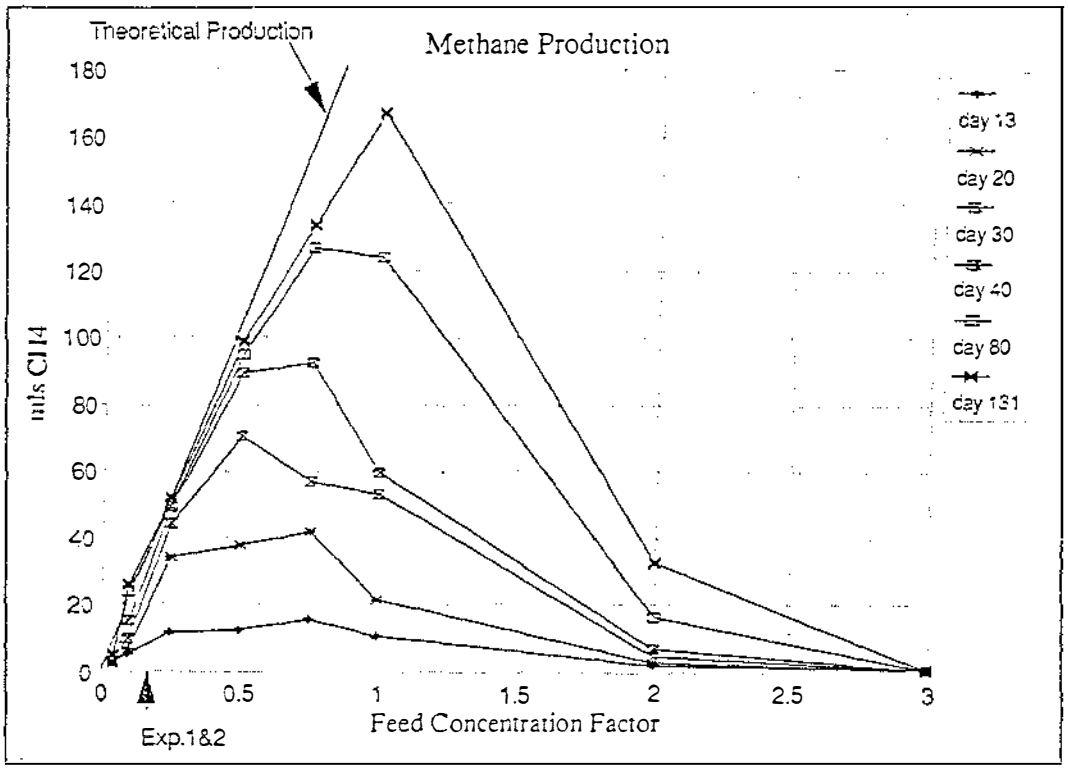

Figure 6.27 Effect of feed concentration factor relative to the full strength feed on the methane production rate in experiment 6.

Shake flask work with a sludge suspension showed that diffuse growth from the reactors had a specific COD removal activity of 350 mg COD.g $VSS^{-1}.d^{-1}$ compared to 150 mg COD.g $VSS^{-1}.d^{-1}$ for the reactor sludge bed and 15 - 100 mg COD .g $VSS^{-1}.d^{-1}$ for the experiment 1 serum bottle tests. What must be considered is the proportion of VSS that was active anaerobic bacteria. It is likely that a significant fraction of the granular VSS will be ferrous hydroxide and ferrous carbonate complexes. For the diffuse growth and non-granular portion of the sludge, wood biomass must have been a significant fraction of VSS. Clearly the sludge bed and, to a greater extent, the granules were limited in their activity, probably by diffusion resistances. The granules do exhibit some

activity and in most of the runs showed an initial burst of activity that corresponded well with the acetate concentration added to the serum bottle. This gives some support to the proposal that the granules are predominantly acetate users.

Calculation of the ratio of granular to non-granular VSS in the later stages of operation of reactors C and D along with the observed activity of these two sludge fractions suggests that the granules contribute approximately 40 % of the COD reduction activity of the reactor. This calculation does not take into account the diffusive resistances experienced by the two fractions.

Experiment 2 was a repeat of experiment 1 carried out a year later with much more rigorous exclusion of oxygen and provision of micronutrients. A fines size fraction (approximately 0.1 - 0.3 mm particle diameter) was included to further test diffusion limitation effects. Methane production rates were considerably lower than those seen in experiment 1. This was assigned to toxicity effects from the use of sodium chloride in earlier tracer studies. Sodium has an inhibitory effect upon *Methanothrix* species giving 10, 50 and 100 % inhibition at 5, 10 and 14 $g.l^{-1}$ respectively. Further to this, at equal sodium concentrations sodium chloride had a somewhat stronger effect on the specific activity than sodium sulphate but can be neglected for design purposes (Rinzema *et al*, 1988). Suspended growth shake flask experiments performed during the serum bottle series showed a 2.8 fold increase in the specific COD reduction rate as a result of replacing chloride based compounds with sulphate based compounds for the basal medium used in the serum bottles and the shake flask experiments. Reported as maximum observed specific methane production rates for comparison with serum bottle rates, the two rates where 47 ml $CH4.gVSS^{-1}.d^{-1}$ before and 132 ml $CH4.gVSS^{-1}.d^{-1}$ after removal of the chlorides. Serum bottle assays also showed an improvement in rates after the new basal media was used. Thus chloride had a significant inhibitory effect in these experiments. This inhibition makes comparison of experiments 1 and 2 difficult and may cause

variation in the responses of the granule size fractions as a result of diffusional effects upon the inhibitory substances.

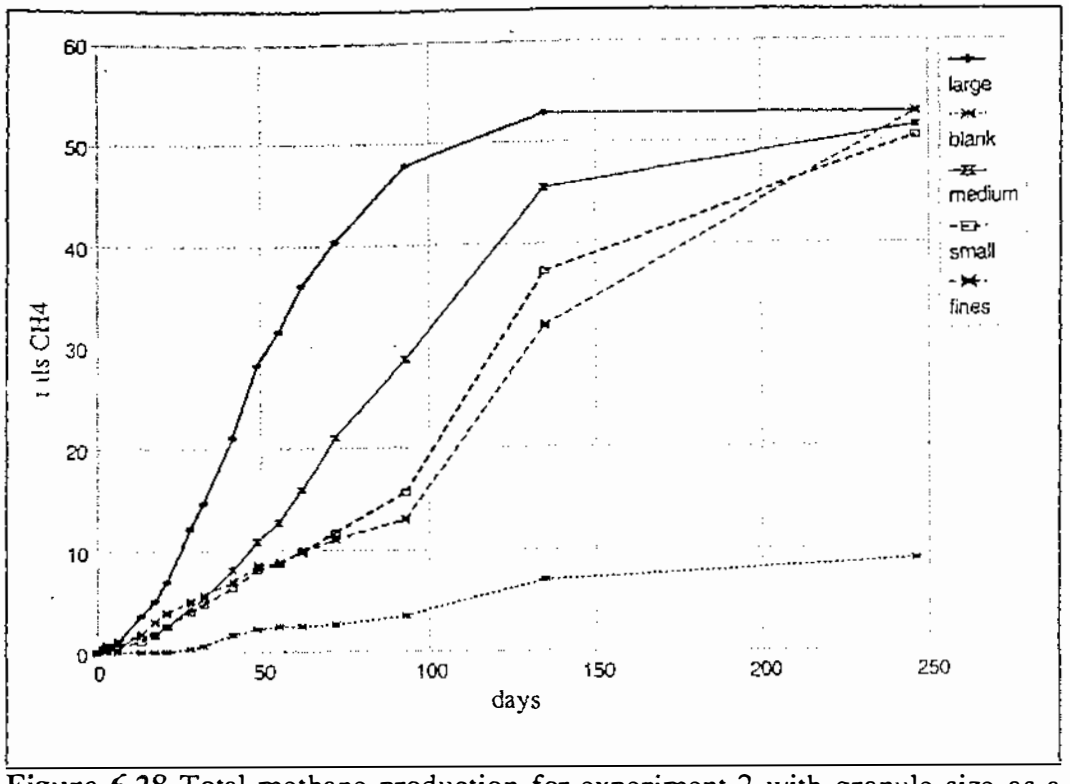

Figure 6.28 Total methane production for experiment 2 with granule size as a parameter.

For the first ten days of experiment 2 methane production was relatively similar for the four size fractions. After this each fraction, in order of decreasing granule diameter, began to show an increased methane production rate. This is shown in Figure 6.28. A faster methane production rate was expected in the large granule runs in light of the experiment 1 results, because these runs had larger seed quantity than the others. Seed quantity in grammes of VSS is shown in Table 6.4. The medium granules had the lowest average seed quantity of the four size fractions but was the second to exhibit a climbing methane production rate as shown in Figure 6.29. Although the three lower size fractions had similar seed masses it was expected that this fraction would have the slowest methane production. The specific methane production rates of all runs show considerable variation from measurement to measurement compared to the relatively smooth curves seen in experiment 1. Some of this variation, that where the trend is the

same at the same sample time as at day 56, is probably due to problems with the temperature control of the incubator in which they were stored. Methane production rates attained were slower than those in experiment 1. Feed quantity was higher, 144 mg COD compared to 92 mg COD. The higher initial concentration should increase reaction rates to some degree and would increase diffusion rates by a factor of 144/92 providing reaction rates for the diffusing species were not close to the original diffusional rate. If this were the case an initially high rate, decreasing with substrate concentration, would be expected. Also seen is variation in production between the replicates of some size fractions. This is a function of the seed quantity of that run and is seen only in the later stages of the experiment.

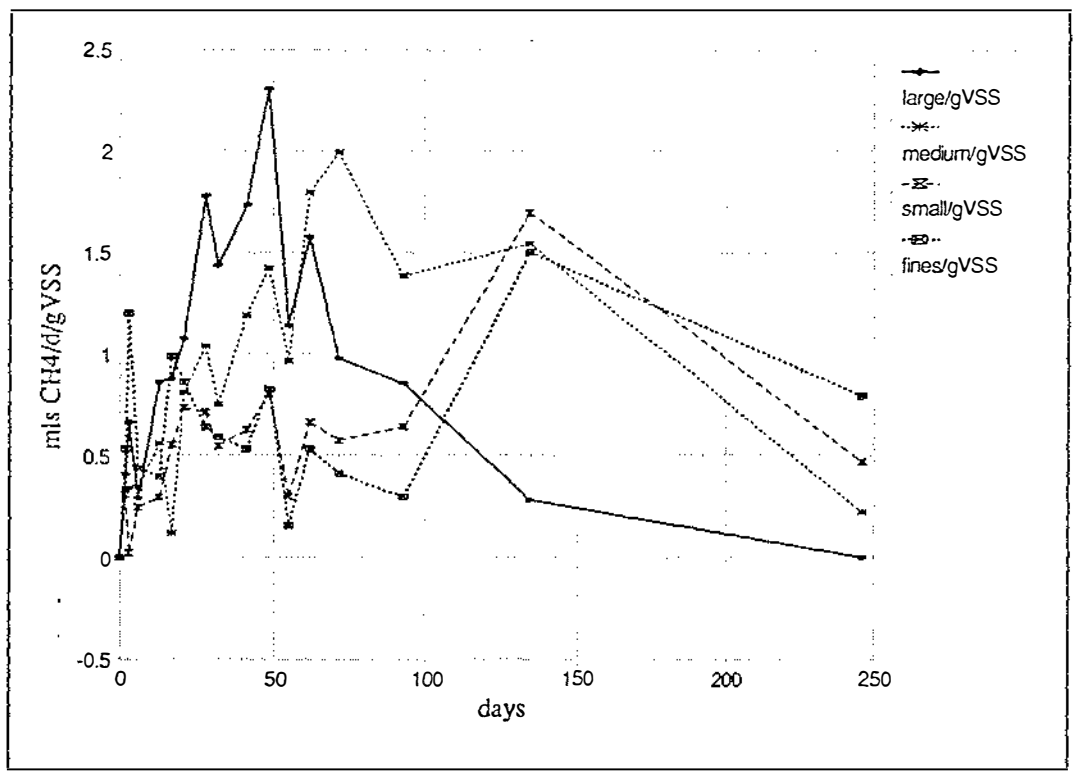

Figure 6.29 Specific methane production rate for experiment 2.

The above evidence suggests some inhibitory substance or substances in the serum bottle liquor. Reduction of chloride concentration reduces this inhibition.

All the granule size fractions were initially effected by this inhibition but recovery is a function of the granule diameter as shown in Figure 6.28.

The serum bottle runs of experiment 3 were aborted after 69 days of very little activity. However the early activity that did occur gives some indication of the influence of the matrix layer upon methane production. In this experiment the effect of the matrix layer was assessed. Although only a small amount of substrate was converted to methane the trends were clear and the test runs differ significantly from those of the blank runs. The test inocula were

1) large washed granules as used in experiments 1 and 2

2) large unwashed granules with the denser layer of the matrix layers intact

3) mixed sludge which was as similar to the state of the sludge in the UASB reactors and consisted predominantly of smaller granules and fines.

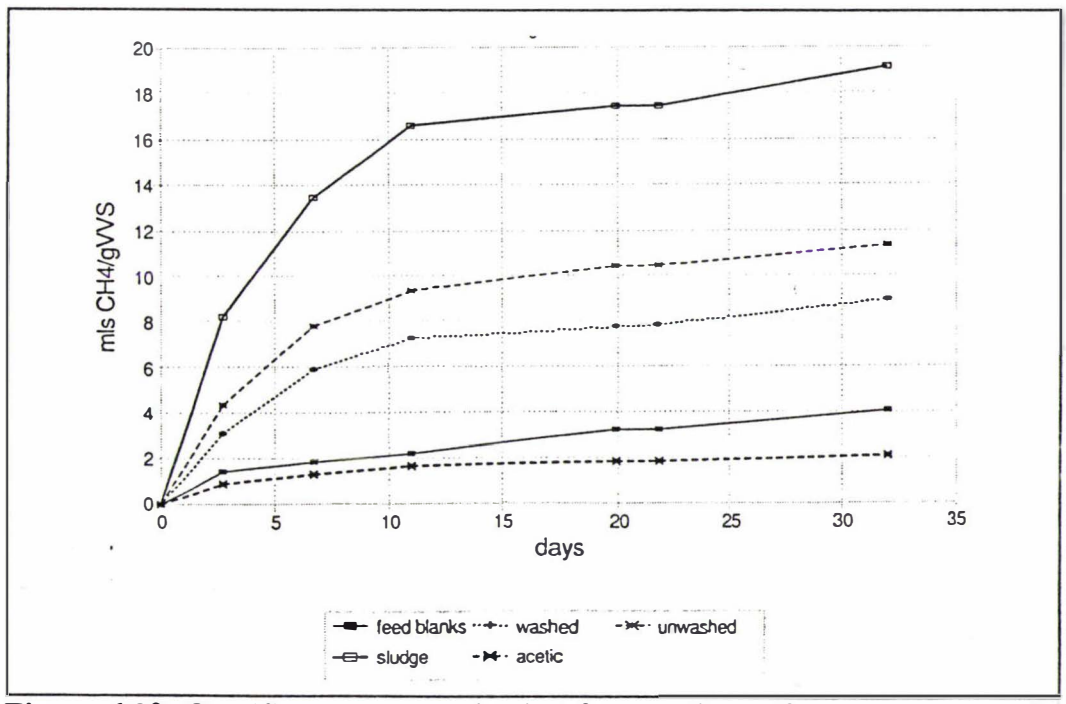

Figure 6.30 Specific methane production for experiment 3.

The experimental data shown in Figure 6.30 demonstrates that the matrix layers improve the methane production of the granules.

Whichever mechanism is operating it is evident that granule size has a function in the determination of methane production rate in the situation of experiment 2. This was not found in experiment 1. The significant difference between experiments 1 and 2 is the presence of some inhibitor in experiment 2. Other experiments have implicated chloride as at least one of the compounds responsible. Sodium concentrations have been calculated to be at least 7.5 $g.l^{-1}$. Sodium has been found to inhibit acetoclastic methanogens at 5 $g.l^{-1}$ and chloride increases the degree of inhibition (Rinzma *et al*, 1988). This suggests that the methanogens were the rate limiting step in the overall process and that diffusional shielding of the methanogens from the inhibitory substances occurred in experiment 2.

6.4 Conclusions

The investigations of this chapter demonstrate that there were large quantities of very dense and strong granules with in the reactors. These granules have a well developed structure and contain a rich layer of bacteria. Most of these bacteria exhibit the distinct morphology of *Methanothrix* species.

The serum bottle experiments showed that the methanogenic activity of the granules is low compared to a suspension decanted from a slurry of reactor sludge probably as a result of diffusional limitation. In serum bottle experiment 1 the granules at least provide the inoculum for suspended cell growth and in experiment 2 the granules provide apparent diffusional protection from an inhibitory substance. Thus the granules must contain a quantity of viable methanogenic bacteria suggesting that some activity occurs within the granules themselves. The maximum activity of the granules could not be assessed accurately.

While the granules appear to have low activity the fact of their existence suggests it is significant. The degradation of wood SS in the granulated reactors but not in floc based growth reactors strongly suggests that the granules play a significant role in that degradation. The high iron content give the granules weight and strength. It would appear that the granules were important in retaining solids within the reactor in such a way that these solids are degraded.

CHAPTER SEVEN FINAL DISCUSSION AND CONCLUSIONS

A number of experiments have been performed to provide information upon the suitability of the anaerobic digestion process for the treatment of thermomechanical pulp (TMP) mill wastewaters in New Zealand. This research was seen as part of an integrated programme to further the understanding and development of suitable wastewater treatment for the forestry industry wastewaters identified as major and/or problem pollution sources. The overall aim of that programme was to facilitate the adoption of integrated waste treatment systems by the forestry industry that are both ecologically and economically attractive.

A number of reasons make the anaerobic digestion process attractive compared to other waste treatment technologies: low nutrient and energy inputs are required, excess sludge disposal needs are low and a useful energy resource, methane, is generated in an easily recovered form. However high treatment efficiency and consistent long term performance in the face of marked variation in organic loading rates, toxicant concentration and environmental conditions must be proved before this technology becomes an accepted part of the forestry industry wastewater treatment scene. For this specific wastewater an understanding of the performance possible from the reactor and the degradation and fate of the resin acids in the wastewater is required.

At the start of this project the decision was made to treat a real industry wastewater with minimal suspended solids removal prior to treatment. The likely high cost of additional solids removal at full scale and reports of 80 % total chemical oxygen demand (COD) removal in the upflow anaerobic sludge blanket (UASB) reactor treatment of a similar wastewater were major factors in this decision. Early work involved the development of a reactor configuration to overcome the many operational problems of this wastewater. It was concluded from this work that for an effective startup of a UASB reactor, an acclimated seed sludge and an organic loading rate (OLR) of 10 kg $COD.m^{-3}.d^{-1}$ quickly

gave a robust reactor able to withstand considerable stress in terms of changes in loading rates. The sludge obtained from the number one pond of the Tasman Pulp and Paper Mill was by far the most active and would be a likely source of seed sludge for start-up of large scale anaerobic digesters for the treatment of pulp mill wastewaters in New Zealand.

The reactor configuration developed gave dissolved COD removals of 65 - 82 % and theoretical total COD degradations of 50 - 67 % for an OLR range of 7 - 38 kg $COD.m^{-3}.d^{-1}$. The methane composition of the gas was from 65 - 85 %. Suspended solids (SS) degradations of 22 - 50 % of influent were achieved over the same OLR range. SS degradation was approximately 50 % better than quoted values in the literature. The apparent COD and SS removals over estimated the degradation by including the accumulated SS within the reactors as degraded SS.

No SS degradation was evident in the floc based UASB reactors A and B nor was any reported in the literature. The iron-rich granules in the final reactor configuration, whilst not demonstrating high methanogenic activity, are the unique factor which appears to give successful SS degradation.

The reactor could withstand OLR step changes from 7 to 28 kg $COD.m^{-3}.d^{-1}$ with little effect but was inhibited after periods of total feed cessation for more than 18 hours. This inhibition was worsened if another feed halt occurred within 8 - 10 days. Little or no inhibition occurred if feed was not completely halted or if the halt was for less than 18 hours. The inhibition was quickly reversed by restarting feed at a lower OLR for a short period of time. The length of time required was dependent upon the degree of inhibition experienced. Thus successful operation required good digester management and planning.

Degradation of resin acids was poor, about 10 -18 % of inflow over a long period of time. Some of the resin acids, especially abietic acid, were removed but it appears that this was predominantly an isomerisation to other acids, mainly

Dehydroabietic and 13-abieten-18-oic, acid both of which accumulated in the reactor sludge at concentrations of up to 11,000 mg.ℓ^{-1} and 8,000 mg.ℓ^{-1} respectively. These high concentrations did not appear to be inhibitory but soluble resin acids were, at concentrations of approximately 50 mg.ℓ^{-1}. No acclimation to soluble resin acids was evident.

Granulation in the reactor was attributed to two major factors the lack of an gas-solids-separator, severely selecting against any poorly settling solids and the organic binding ability of iron, which was present it the reactor feed at high levels (400 -700 mg.ℓ^{-1}) due to the storage method used. This resulted in large robust granules with a specific gravity of 2. Thus the settling ability of these granules was excellent.

There was some accumulation of wood SS in the reactor but this was usually less than 25 % of the bacterial accumulation. Solids wasting from the reactor was relatively easy and generally had little effect upon operational performance. Whilst 40 - 50 %, by mass, of the influent SS passed through the reactor the effluent SS exhibited a considerable improvement in settling ability.

Overall, the reactor configuration gave higher removal efficiencies than other systems reported. The disadvantage is the need to avoid halts in the provision of feed.

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