ASPECTS OF THE ANAEROBIC DIGESTION OF WATTLE TANNINS

By

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1. INTRODUCTION

1.1 General

The aqueous extract from the bark of the black wattle tree, (Acacia mearnsii De Wild)[1], is used extensively in the tanning industry for the production of sole and other heavy leathers as well as in lighter leathers and skins[2]. The commercial extract is available in an easily dissolvable spray dried form and in deference to its genus name Mimosaceae is referred to in the trade as Mimosa[3]. Mimosa extract consists primarily of high molecular weight polymeric flavonoid units which also constitutes the active tanning ingredient. Lower phenolics, gums and sugars or the so called "non-tannins" are present as impurities and contribute up to 40% to the total mass of the extract[3].

Effluents from tanneries using Mimosa extract contain as a consequence non-tannin components as well as tannins in their wastewaters. Vegetable tannins in effluents exhibit low biodegradability and contribute to high carbonaceous oxygen demand (COD) and biological oxygen demand (BOD) levels[4]. The significance of these components in the total tannery effluent and resulting disposal strategy depends on a number of factors[5,6]. The type of tannery concerned and the unit processes employed determine the volume and composition of the total effluent. Sole and heavy leather effluents are strong in spent extract which is however fairly well depleted of tannins. Tanneries which employ Mimosa for lighter leathers, pretannages, retannages, fillers, etc. are usually less efficient in Mimosa extract depletion but the relative concentrations of tannins in the total effluent are usually very low due to dilution by wastewater originating from other parts of the tannery. Legislation governing effluent disposal is usually tailored to suit local conditions[5,7] and factors such as a suitable municipal treatment facility, municipal discharge rates, proximity to rivers or dams, availability of irrigation land and the sensitivity of the area to certain pollutants also has an effect on determining the treatment strategy employed.

Much work has been done on the treatment of vegetable tanning wastes. This includes investigations into physico-chemical methods, biological treatments as well as other novel treatments which are more fully described in Section 1.4. An aesthetic problem persistent in all these methods however relates to the fact that small residual levels of tannins or oxidised products impart an unacceptable brown colour to receiving waters[8-12]. Some of these methods are also not cost effective.

Anaerobic digestion of industrial wastes is a relatively new area of interest and its "hot topic" status arises predominantly from favourable energy considerations[13,14]. Available reports on anaerobic treatment of vegetable tannery waste indicate a certain biodegradable potential and in some cases, a decolourized product[15,16].

The purpose of this investigation was therefore to study aspects of the anaerobic degradation process as it pertains to wattle extract.

1.2 Mimosa Extract

Tannins are present in relatively very high concentrations (approximately 30% dry weight) in mature (7 to 10 years) black wattle bark[1,17,18]. The commercial preparation of Mimosa extract involves hot (100°C) aqueous leaching of freshly stripped and chipped bark and concentration of the resulting leachate. This is followed by spray drying. The dedusted powder has a polyphenolic content around 70% with the remainder consisting of gums and sugars with a minimal amount of starches and waxes also present[19].

1.2.1 Classification of Wattle Tannins

Traditional classification of vegetable tannins as hydrolysable or condensed was based on the behaviour of the tannins under acidic conditions[17,20,21]. Essentially, the hydrolysable tannins yield a phenolic acid (e.g. gallic acid) upon acid hydrolysis whereas the condensed tannins undergo progressive polymerisation to yield phlabophens or tannin "reds". Wattle tannins belong to this latter group of compounds but more accurate chemical classification places them in the class proanthocyanidins which is part of the ubiquitous group of naturally occurring phenolic compounds - the flavonoids. The flavonoids comprise 15 classes[22] of structurally unique compounds, of which the polymeric or condensed flavan-3-ols or flavan-3,4-diols are of interest as the active tannins in Mimosa extract[19]. These subunits have a 15-carbon-atom skeleton and are linked by direct C-C bonds[23].

1.2.2 Structure of Wattle Tannin Components

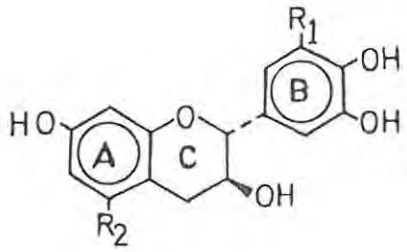
It is only at the trimeric level of flavonoid polymerization that the wattle components exhibit sufficient affinity for collagen substrate to be classed as tannins[24]. Adsorptive effects increase rapidly with higher levels of polymerization and the range includes tannins of up to approximately 3 000 a.m.u. which represents a decameric flavonoid[25]. The

number-average mass of the total extract is approximately 1 250 a.m.u.[26].

The polymeric wattle tannins are built up exclusively from flavan-3-ols and flavan-3,4-diols and the structure of the monomeric compounds isolated from fresh wattle bark are shown in Fig. 1 together with absolute stereochemistry[19,26]. The environment of the 4-hydroxyl group in these flavan-3,4-diols is conducive to solvolysis of the hydroxyl group in acidic media[27,28]. The resulting benzylic carbocation is resonance stabilized (ortho and para oxygenated substitution pattern) and may become involved in electrophilic condensation reactions with available nucleophiles (see Fig. 2).

Resorcinol- and phloroglucinol-type structures undergo nucleophilic substitution reactions in the ortho and para positions with the latter compound having very strong nucleophilic character[23]. The presence of such structures in the aryl A ring of the flavan-3-ols and flavan-3,4-diols allows substitution by the carbocation which is formed by the flavan-3,4-diols to produce C-C bonded dimeric flavonoids which are linked in the 4,6 or 4,8 positions, (both are meta to the carbocation), [Fig. 2]. Model studies have shown[29] that in the phloroglucinol type structure the 4,8 linkage is predominant over the 6,8 linkage while the reverse is true for resorcinol type structures. This is thought to arise as a result of the accessibility of the respective nucleophilic sites. The natural abundance of the phloroglucinol type subunits, e.g. (+)- catechin, is a prognosis for the predominance of naturally occurring linear 4,8 linked flavonoids over the angular 4,6 linked flavonoids[27]. Formation of higher oligomeric flavonoids is thought to occur according to the ionic mechanism described above. Compounds which lack a 4-hydroxyl group, i.e. the flavan-3-ols, are thought to act as terminators of chain polymerization as the formation of a carbocation at the 4 position of such groups is unlikely. The multiplicity of diastereomeric and rotational isomeric compounds theoretically possible obviously complicates the isolation and characterization of higher oligomers many fold although the structure and stereochemistry of certain bi-, tri- and higher flavonoids has been elucidated by ^{1}H NMR and confirmed by synthesis[26,27]. The complexity and high degree of structure variation found in these compounds is further

Flavan-3-ols:



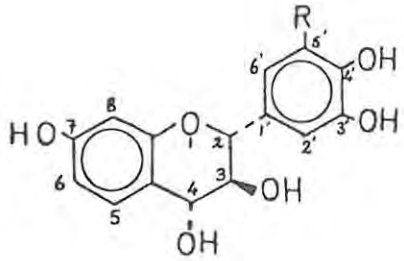
R_1 = H, R_2 = H : (-)- Fisetinidol

R_1 = OH, R_2 = H : (-)- Robinetinidol

R_1 = H, R_2 = OH : (+)- Catechin

R_1 = OH, R_2 = OH : (+)- Gallocatechin

Flavan-3,4-diols:



R = H : (+) Leucofisetinidin

R = OH : (+) Leucorobinetinidin

Figure 1. Typical monomeric flavonoids isolated from wattle bark which undergo polymerization to form wattle tannins

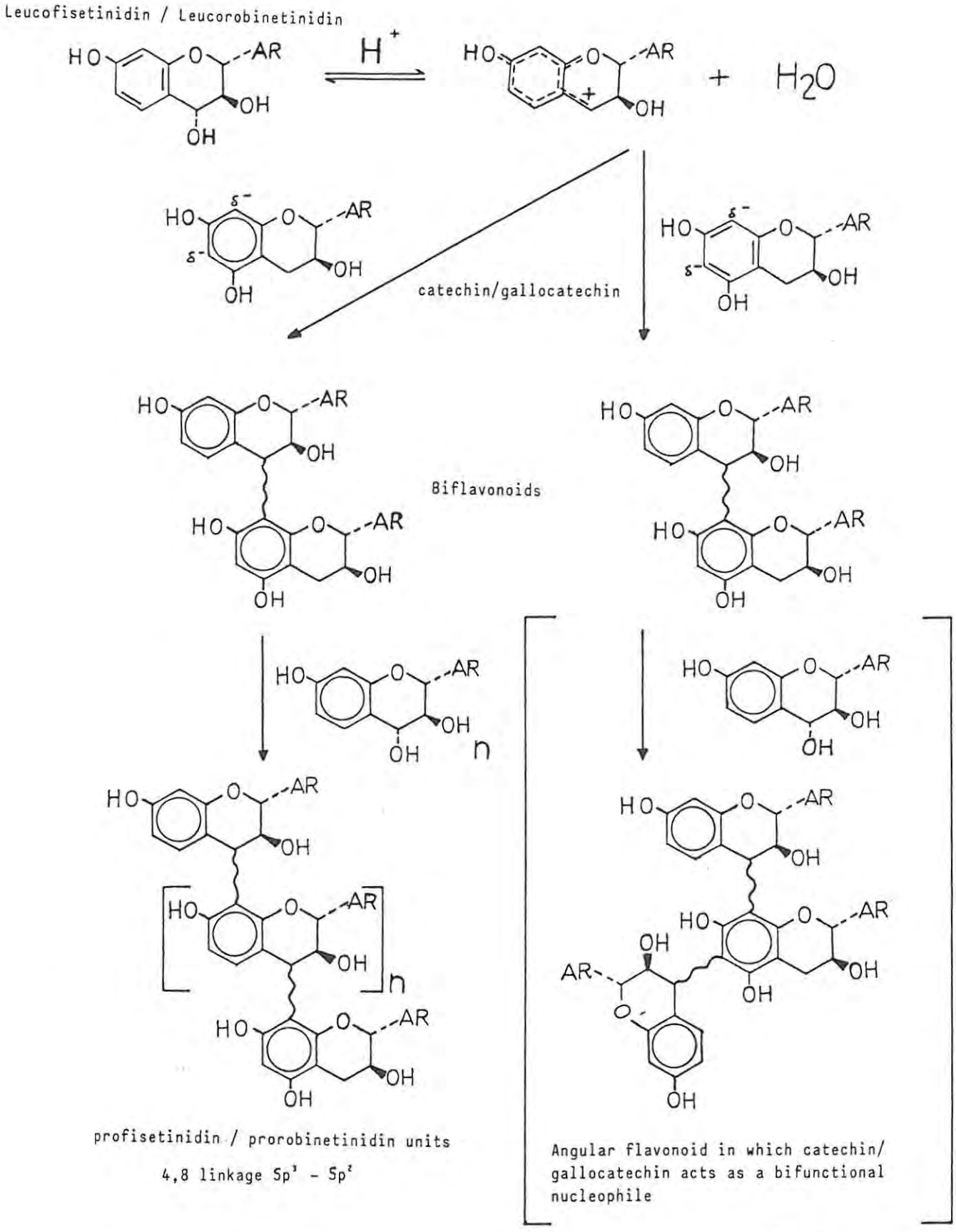


Figure 2. Polymerization schemes for oligomeric flavonoids[27,29].

complicated by the oxidative reactions occurring during commercial production of Mimosa extract as well as acidic and light catalysed oxidation which occurs subsequently in the final product[30,31]. The ready oxidation of these compounds is well known in tanning circles as leather products tanned with Mimosa exhibit colour reddening and deepening in the finished article upon exposure to light and this is one of the major drawbacks of vegetable tannage in comparison to a mineral tannage.

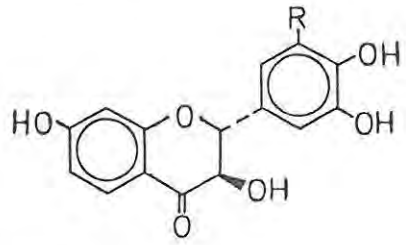
1.2.3 Wattle Non-Tannin Components

The mono- and dimeric flavan-3-ols and flavan-3,4-diols which are classed as non-tannins are depicted in Figures 1 and 2. Other flavonoids that have been identified in wattle extract[19,32,33] contain a carbonyl group in the 4 position which decreases the nucleophilicity of the aryl A ring and precludes carbocation formation and hence dimerization[29]. These compounds are shown in Figure 3. The monomeric phenolic compounds only make up about 3% of the total extract[29].

Carbohydrates and traces of amino and imino acids are also present in the total extract[33,34]. The carbohydrates consist mainly of glucose, fructose, (+)-pinitol and sucrose as well as large molecular weight hydrocolloid gums. This latter group may constitute between 6% to 12% of the total extract[19]. The amino acids present include arginine, alanine, aspartic acid, glutamic acid, serine[35] as well as albizziine[36]. The imino acids present comprise proline, (-)-L-pipecolic acid and (-)-L-4-hydroxypipecolic acid[35]. These latter two groups of nitrogenous compounds constitute approximately 3% of the total extract.

Very small amounts of insoluble material, starches and waxes, are also present[19]. Figure 4 depicts some of the above non-tannin components.

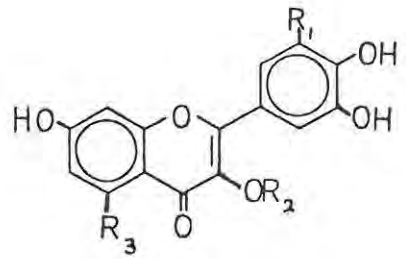
Dihydroflavanols:



R = H : (+)-Fustin

R = OH : (+) Dihydrorobinetin

Flavonols:



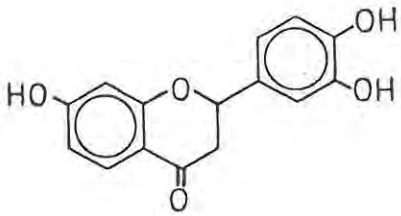
R_1 = H, R_2 = H, R_3 = H : Fisetin

R_1 = OH, R_2 = H, R_3 = H : Robinetin

R_1 = H, R_2 = Rhamnose,
R_3 = OH : Quercetrin

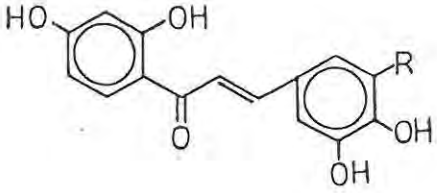
R_1 = OH, R_2 = Rhamnose,
R_3 = OH : Myricitrin

Flavanones:



(±) Butin

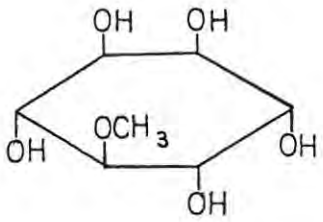
Chalcones:



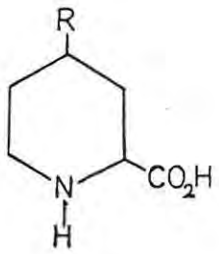
R = H : Butein

R = OH : Robtein

Figure 3. Momomeric flavonoids present in wattle extract

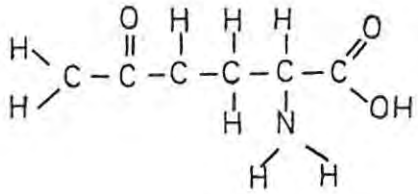


(+)-pinitol

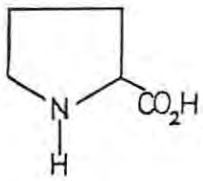


R = H : (-)-L-pipecolic acid

R = OH : (-)-L-hydroxypipecolic acid



Albizzine



(-)-L-Proline

Figure 4. Some of the compounds present in wattle extract in trace quantities

1.2.4 Metabolism of Wattle Tannins

A hypothesis for the biosynthesis of procyanidins is given in a simplified form in Fig. 5[37-40]. It is postulated that the flav-3-en-3-ol undergoes stereospecific proton transfer followed by hydride addition to give the stereoisomeric flavan-3-ols. The latter step is mediated by the biological reductant NADPH. It is proposed that scarcity of this reducing agent can lead to condensation of the activated carbocation with flavan-3-ols to form the procyanidins as depicted in Section 1.2.2. Analagous biosynthetic routes are suggested for prodelphinidin, prorobinetin, profisetinidin and other chemically similar oligomers.

The view that monomeric flavonoids are dormant waste materials stored in plant tissues has, from radioactive labelling experiments, given way to the concept of a dynamic system with continual synthesis, accumulation, interconversion and dissimilation of material within the plant tissues[22,41]. Variations in concentration and composition are found to occur with age, disease, environment as well as diurnal and seasonal fluctuation[1,22]. This *in vivo* catabolism and interconversion is thought to occur in the same organelles of the plant cell as the anabolic processes. Pulse labelling experiments have been used to measure the velocity of catabolites through the flavanoid pool and many of these routes have been elucidated[22]. No proof has been forwarded for the *in vivo* degradation of oligomeric material however, although diurnal concentration fluctuations have been recorded[22]. These fluctuations could be due to catabolism but may also occur as a result of further polymerization, oxidation or mass transport as there is little evidence that higher plants have the ability to cleave extended aromatic ring systems[42].

Extensive research has been done on the mammalian metabolism of monomeric flavonoids[28,43,44] where the urinary metabolites are analysed after oral ingestion of monitored doses of the respective flavonoids. *In vitro* experiments with anaerobic bacterial incubations of gut microflora however were shown to produce metabolites identical to those isolated from mammals. It has been conclusively shown, in subsequent experiments employing germ-free rats, that the intestinal microflora are wholly responsible for

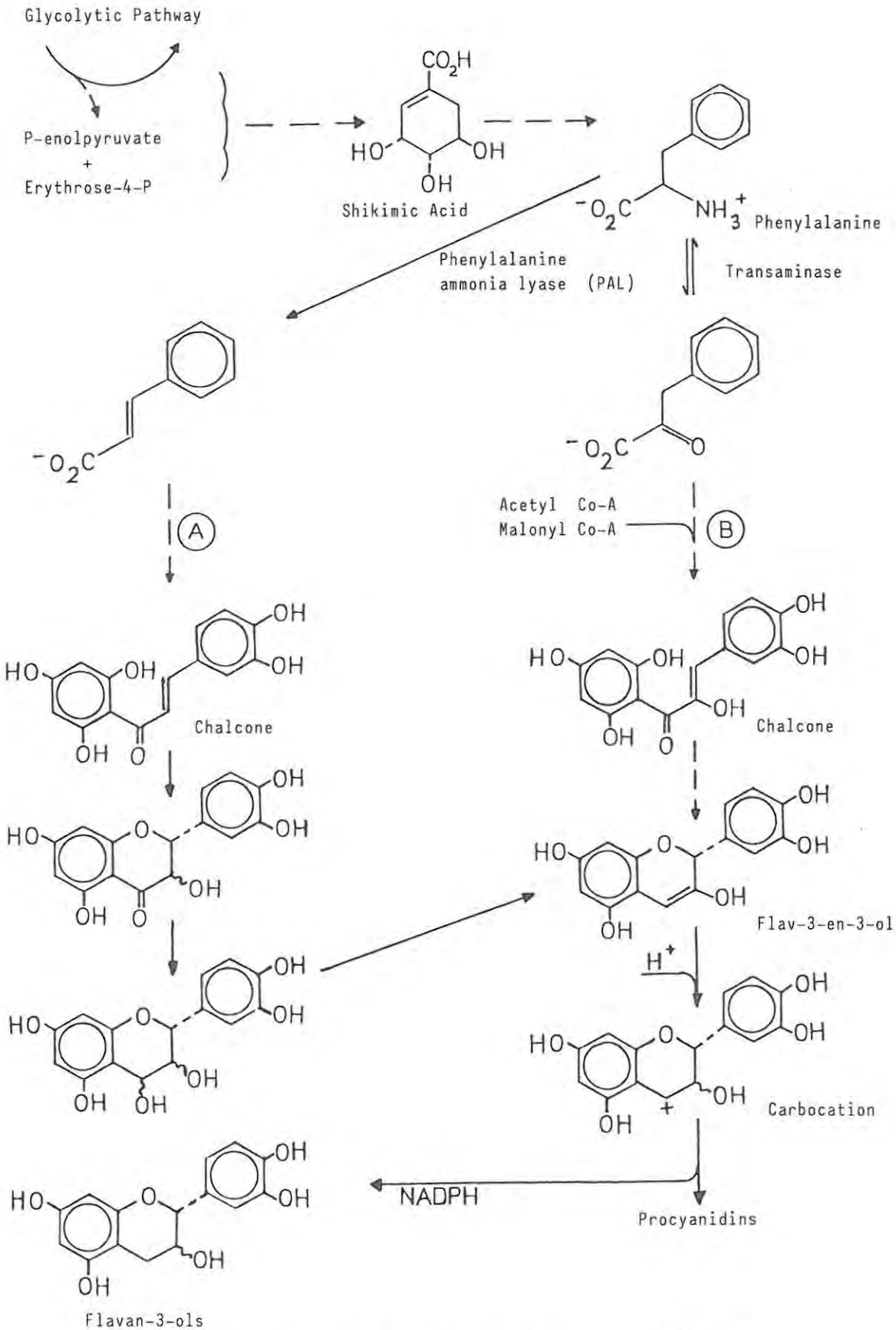


Figure 5. Hypotheses for the formation of procyanidins[37-40].

the ring fission products produced and the participation of mammalian enzymes has been completely ruled out[45,46]. Soil and sewage micro-organisms are responsible for most phenolic breakdown in nature and these aspects will be further covered in Section 1.4.

The physiological functions[22,39,47,48] of flavonoids within the plant remains an area of open debate and few generalizations are possible. An obvious function of the flavonoids is their ability to act as attractants for pollinators owing to intense pigmentation. Their proven antimicrobial and antifungal properties implicates a defensive role in protecting against phytopathogens. A further protective role - as antioxidants - is indicated as a result of their ability to chelate metals and act as UV absorbers. Flavonoids are known to inhibit certain enzymic activity and may act as plant growth regulators and are also known to play a part in disease reaction as phytoalexins. Little is known about the physiological role of oligomeric flavonoids. They are known to render plant material bitter and astringent and hence less palatable to grazing animals[39] but their presence in some plants makes beverages and fruit more palatable to humans[49,50]. They also seem to act as a soil conditioner, where decaying plant matter makes the soil more suitable for growth of subsequent generations of that particular species[50]. An alternate and fairly common view sees their production as a waste material or as an efficient detoxification mechanism for surplus organic matter[51].

1.3 Effect of Vegetable Tannins on Water Quality

The removal of vegetable tannins from wastewaters is necessitated primarily because of the high pollution load these organics represent. They also have a discolouring effect on receiving waters and accounts of tannin toxicity are also noted.

Sole and heavy leather enterprises produce a low volume effluent[52] which is strong in spent vegetable extract despite innovative techniques such as the LIRITAN minimum effluent[53,54], flip-flop[55,56] and dry drum systems[57,58] which utilize high exhaustion methods to minimize both the volume and concentration of tannins in the wastewater. Differing values are given for the pollution load of vegetable wastes by different researchers and this in part may be ascribed to the tanning processes employed. An average COD value of 26 500 mg/ℓ is given by Parker[12] for spent vegetable tannin effluent with total solids of 1,36%. Briggs et al[59] give a COD value of 10 000 mg/ℓ for typical waste, with a tan content of 0,5 - 0,8%, non-tans of 1,0 - 1,5% and suspended solids of 500 mg/ℓ. Hendry[60] et al gives a value of 0,64% (6 400 mg/ℓ) tans for typical LIRITAN colour vat effluent with 5,35% non tans and suspended solids content of 2 810 mg/ℓ. The high values of the latter two parameters are due to the nature of the LIRITAN system which allows a build up of sugars, gums and neutral salts in the tanning liquor. Ludvik[61] et al put the concentration of tanning material in median vegetable effluents at 0,7%. Corning[8] estimates the waste to contain 0,5% tans. He reports[52] that a 0,1% solution of mimosa extract has a COD value of $\sim$ 1 390 mg/ℓ, (this is corroborated by Hendry[62] who found 1,4 mg COD/ℓ/mg mimosa), and a COD/BOD_5 ratio of 10,7. He found furthermore that 84% of the mimosa tan solution COD is taken up by hide powder resulting in a COD/BOD_5 ratio of only 1,5. A high COD/BOD_5 ratio is indicative of biologically recalcitrant material. Carré et al[4] found a COD value of 1 280 mg/g mimosa with a COD/BOD_5 ratio of 100. They classed mimosa in a category of compounds having a low potential for biodegradation.

Aqueous solutions of mimosa tannins are highly coloured and vegetable tannin effluents impart an aesthetically unacceptable reddish-brown

discolouration to receiving waters[61]. This colour deepens as alkalinity increases[63]. In the presence of iron salts blue-black inks are formed[59]. The browning discolouration was shown by Atluru[10] to persist through fungal decomposition of spent vegetable tannins. The work of Parker[12] on lagooning of these tannins showed that a facultative-aerobic system does not remove colour and although blending with brine and chemical coagulation does reduce the colour of the influent, the sludge produced is bulky and has poor settling characteristics. Hendry[64-69] did extensive work on aerobic treatment of wattle containing wastes. He ascribes the browning of effluents after biological treatment to residual non-tan polyphenolic material and registers their discolouring effects at influent wattle levels of 100 mg/ℓ (i.e. 0,01%). Eye[59] was able to remove the colour after aerobic biological treatment by adding lime to pH 12 and then precipitating the coloured compounds with anionic polyelectrolytes. Attempts to precipitate these compounds prior to biological treatment however resulted in voluminous sludges which settled poorly. Young[63] removed the colour from vegetable tannery effluent by adding ferric salts, scrubbing with hot flue gas and then precipitating out the oxidized, red tannin compounds with a polymeric flocculant. Chlorine dioxide has been used by Simoncini[70] et al to purify vegetable tanning effluents and, in series with an activated charcoal column, removes all traces of colour. Substantial decolourizing was also obtained by Šiška[71] who absorbed vegetable tannins onto waste activated sludge. Tannage of bacterial cells is confirmed by Eye and Kruse[72] who found red, "tanned" bacterial cells upon microscopic examination of aerobic flora digesting vegetable tannins. Conflicting reports on colour removal by anaerobic systems treating vegetable wastes have been published. Gates and Lin[73] report no colour removal, Arora et al[16] report decreased colour in the effluent while Eye and Ficker[15] report complete colour removal.

The majority of plant phenolics have no reported toxicity to man or animals but some flavanoid toxicity is noted in fish life[22,74-76]. The degradation products of heterogenous polyphenols may exert toxic effects however[52] and this may be compounded by antiseptic chlorination which can result in the formation of toxic and persistent organochlorine compounds[77,78]. Fungitoxic properties have been ascribed to naturally

occurring polyphenolic compounds however and has in fact been cited as a possible functional role of tannins[39,79]. For example, the polymeric condensed tannin fraction from an acacia species - Acacia arabica - has shown toxicity at a concentration level of 0,1 g/ℓ, (i.e. 0,01%), with reference to the fungus Piricularia oryzae[79]. In contrast, spent vegetable tannin effluents have been shown by Atluru[10] to be amenable to biological treatment with fungal dominant systems, (Genera include Penicillium, Fusarium and Sporothrix). A 5 day retention time removed 66% of total organic carbon (TOC) and the bulk of the remaining refractory material appeared to be oxidized non-tannin phenolics such as quinones and semi-quinones. Bacteriacidal action by several anthocyanins and leucoanthocyanins has been demonstrated by Powers[80] although the action of these compounds on different bacterial strains is diverse. Orlita et al[61,81-83] have looked specifically at the toxic effects of certain chemical components in tanning effluents. Pyrocatechol and pyrogallol are shown to have LD 50's, (i.e. lowest dose causing 50% of organisms to perish within the test period), of 3 and 5 mg/ℓ respectively on Bacillus Megatherium. Wattle solutions had LD 50's of 29, 60 and 208 mg/ℓ on B. Megatherium, Staphylococcus Aureus and a mixed flora, (i.e. psychrophilic bacteria ex municipal waste), respectively. Mixed cultures showed greater resistance to toxicity. In another experiment, mixed vegetable tanning wastewater, (0,7% tannins), was shown to reduce the total number of psychrophilic bacteria in a sample by 37% over a period of 14 days incubation while sporulation was completely inhibited. (It should be noted that so-called mixed vegetable tannery wastewaters included synthetic tannins which are known to exhibit greater toxicity[9,61,84] or inhibition[8,52,85] than natural phenolics). The authors speculated further that toxicity could be due to the tanning action of the phenolics on bacterial protein. Such binding forms the basis of the treatment strategy proposed by Siska[71] and was corroborated by Eye and Kruse[72] as mentioned in the previous paragraph.

At lower concentration levels of vegetable tannin substrates in bacterial systems, many authors have found their effects to be more inhibitory and recalcitrant than directly toxic[8,9,12,52,64-67,69,85]. The effects of vegetable tannins on anaerobic systems will be discussed in section 1.6.2.

1.4 Treatment Strategies for Vegetable Tanning Wastes

The strategies employed for treating vegetable tanning wastes are aimed at reducing oxygen demand and removal of colour. Minimum effluent or recycling methods reduces the scale of the problem and the resulting effluents may be treated separately or in admixture with beamhouse, chrome tanning or domestic sewage effluents. The most commonly used methods involve physico-chemical or biological treatments although pre- and/or post-treatments are usually required to comply with disposal regulations. Some recent findings and novel treatments are also briefly reviewed here.

1.4.1 Classical Physico-chemical Treatment

One of the most effective forms of treatment involves the simple coagulation and precipitation of the tannin material. The use of aluminium sulphate as a coagulant is recommended as the cheaper iron salts form discolouring inks with tannins[5,9]. Hendry[67,69] recommends pH adjustment with lime to pH 7 $\pm$ 0,5 for optimum floc formation. He also found air flotation to produce a better sludge (10% solids) than simple precipitation although further treatment in the form of filter pressing or centrifuging would be required. He achieved 90% removal of polyphenols from typical LIRITAN effluent with a 2% (m/v) addition of alum and 1,3% (m/v) addition of lime (to pH 7,5). Straight admixture of spent vegetable tanning liquors with alkaline beamhouse effluent results in a voluminous sludge precipitate with a low solids percentage. Neutralization and air flotation however produces a sludge with $\sim$ 10% solids and 80% polyphenol removal. Corning[52] added 0,5% lime (no alum) to mimosa tannin solutions of 0,1% and 0,01% and found COD removal efficiencies of 77% and 67% respectively. The COD/BOD_5 ratio dropped from approximately 11 to 5 indicating preferential precipitation of biologically recalcitrant tannins. He makes no comment about the nature of the sludge however.

Roets[86,87] has developed a simple flotation system, (i.e. SILFLO®), for treating mixed chrome/vegetable tanning waste. Following balancing and aeration the waste is dosed with a coagulant (e.g. $FeCl_3$), and a polymer

and the solids are removed by foam induced air flotation. On a full scale operational plant he reports 92% removal of organic matter and 98% solids. The sludge contains up to 14% solids and becomes spadeable in 24 hours.

1.4.2 Lagooning

Lagooning of wastes relies on the spontaneous growth of micro-organisms and wind induced oxidation to remove pollutants. The land area required, inefficiency and the rapid formation of sludges are the major drawbacks of direct lagooning of vegetable tanning waste[4,5,88]. Parker[12] found the tannins degradable in an aerobic - facultative lagoon and achieved 76% removal of COD with a hydraulic retention time (HRT) of 36 days. Efficiency dropped to 37% at HRT of 4 days however and colour removal was not effected. Gates and Lin[73] used an anaerobic-aerobic lagoon for treatment and their results will be discussed in sections 1.6.1 and 1.6.2.

1.4.3 Biological Filtration

Laboratory scale treatment of a mixture of beamhouse and sole leather (vegetable tannins) wastes was done by Humphreys et al[89] using a biological filter system. After 6 months continuous running, close to 100% BOD removal was achieved at a loading rate of 0,12 kg BOD/m^3/day. Pilot plant work was done by Bailey et al[90] on vegetable containing wastes, (i.e. leather dressers effluent), and they achieved BOD reductions of 40% at a loading rate of 0,75 kg BOD/m^3/day. Bailey[88] recommended the removal of remaining traces of colour with activated charcoal. The major drawback of these systems is initial capital outlay.

1.4.4 Activated Sludge Plants (ASP)

A great deal of work has been done using laboratory scale activated sludge plants but little information is available on full size plants. Corning[85] used laboratory ASP's to determine the biodegradability of

tanning chemicals, including vegetable tannins. The units were started on sewage sludge and incremental additions of up to 1 000 mg/ℓ (0,1%) mimosa were made. From direct COD reductions it would seem that approximately 25% of Mimosa was degraded and this was confirmed by using Roux's[91] spectrophotometric techniques for tans. At lower tan additions of 300 mg/ℓ mimosa, a HRT of 2 days and using synthetic sewage[52] up to 50% degradation of Mimosa was obtained as calculated from direct COD reductions. Similar work by Hendry[64-69] using sewage fed ASP's on laboratory scale gave 80% wattle removal at a feed concentration of 300 mg/ℓ, which dropped to 50% at 1 000 mg/ℓ. The HRT was one day. HPLC chromatograms showed almost all the identifiable wattle products were removed although the reddened, oxidized wattle degradation products still registered high COD levels. Further work involved the addition of up to 1 500 mg/ℓ wattle to completely mixed chrome tanning wastewater in the same ASP units. Although COD removals greater than those of the blank were obtained, this was ascribed in part to physical precipitation as permanganate value (PV) measurements rose significantly and the characteristic reddening of wattle was present in the effluent. Tanaka et al[92] treated mixed chrome and vegetable tanning effluents in a full size activated sludge plant at a loading rate of 2,6 kg BOD/m^3/day. He reports BOD removal efficiencies of 96%. Ludvik et al[9] report up to 80% BOD_5 removal of vegetable tanning effluents in admixture (2:1) with domestic sewage, but recommend prior physico-chemical treatment.

1.4.5 Anaerobic Digestion

This will be covered in section 1.6.2.

1.4.6 Other Treatments

Some alternate approaches used to reduce the oxygen demand or colour of vegetable effluents are very effective but not economically feasible at present due to the low cost and reliability of older and simpler methods of treatment. Still other approaches have not been successful, but all are enumerated for completeness.

A method investigated by Šiška et al[71] and which is most likely operative in most biological treatments[68,72] involves the adsorption of tannins to biological sludge. Approximately 50% reduction in organic matter was reported. Gajdusek and Kupec[93] recommend sorption onto superfluous activated sludge. Pollio and Kunin[94] put forward the idea of removing industrial waste phenols by sorption onto resins such as styrene - divinylbenzene copolymers. Briggs et al[59] used the nonionic resin Amberlite XAD-2 to remove 90% of vegetable tannins from a vegetable tan waste stream containing 0,7% tannin at a volumetric flow rate of 1,5 bed-volumes/hour. Column regeneration was achieved by stripping with acetone and tannin recovery by distilling off the acetone. A tannin purity of 82% was obtained using standard tannin analysis but no mention is made of oxidation resulting from the heat treatment.

The use of fungal dominant biological systems by Atluru has already been described in section 1.3[10]. Direct spray irrigation following screening of sole leather effluent is described by Eick[95] but this method is unlikely to find acceptance by modern pollution control authorities.

Okamura et al[96] proposed the utilization of methylated collagen webs (80% Collagen + 20% Tetoron) for eliminating vegetable tannins from waste streams. The development of membrane technology has inevitably led to it being recommended for recovering vegetable tans. Kleper[97] has done pilot plant work where he was able to concentrate spent tannins from 2,7% to 4,1% and projects favourable economies for such systems.

Young[63] advocates scrubbing vegetable tannery effluents with flue gases following aeration with ferric and manganese catalysts in an alkaline equlization tank. He claims complete removal of colour, 60% BOD reductions and 97% reduction of suspended solids.

Hydrogen peroxide treatment of waste containing phenolic residues is a well documented[76,98-100] but expensive[67] form of treatment requiring a 3:1 ratio of peroxide to total phenol[76]. Perchlorates[99] or chlorine dioxide[70] may also be used but can result in the formation of toxic organochlorines[78] or traces of residual free chlorine requiring further

treatment[70]. The use of catalysts such as iron, (Fentons reagent)[52,99-101], or enzymes such as horseradish peroxidase[52] with oxidants have also been investigated but are not economically feasible. Oxidants combined with UV irradiation and ultrasonics is known to effectively cleave phenolic residues[52,102] but again expense would be of paramount consideration.

Solvent extraction techniques were attempted at BLMRA but with little success[52].

1.5 Anaerobic Digestion (AD)

The first application of anaerobic digestion as a technology was for the liquefaction of suspended organic solids in primitive septic tanks, (Mouras, circa 1818)[103]. The treatment of sewage sludge remains the most common form of applied AD although the advent of heating (1927)[103] and stirring[104] has subsequently dramatically improved the process efficiency since then. Improved reactor design[103,105-107] has led to increased biomass retention. This was achieved either by entrainment of biomass on solid support material or by separation of the supernatant and biomass and recirculation of the latter. A better understanding of the microbiological aspects[106,108,109] of AD have resulted in improvements to the microenvironment within reactors and hence to optimal functioning of the entrapped biomass. These advances have led to the extension of AD to the field of industrial wastewater treatment where it has now replaced traditional aerobic treatment as an economic alternative for certain wastes. A comparison of aerobic versus anaerobic treatment for industrial wastes is given in Table 1. Obviously the characteristics of a particular waste will determine which treatment strategy is preferred but generalizations are possible. Anaerobic bacteria have the ability to degrade certain complex organic substrates which are not satisfactorily degraded by aerobes[110-112]. They are also known to detoxify wastes which cannot be detoxified aerobically[113]. The primary advantages of AD remain however their low sludge and favourable energy yields.

1.5.1 Microbiology and Biochemistry of Anaerobic Digestion

The ability of a bacterium to utilize material other than oxygen as an electron acceptor classifies it as anaerobic. Facultative anaerobes can function in the presence or absence of oxygen while the obligate anaerobes cannot utilize oxygen and may find its presence in a medium toxic[109,114]. A functioning anaerobic digester usually contains a whole range of micro-organisms growing in close association, forming a symbiotic community which automatically regulates its own pH, redox potential and free oxygen tension[115]. The composition of a mixed anaerobic community within a digester is dependant on the type of waste present as some

Table 1. Comparison of aerobic versus anaerobic digestion[14,108]

Parameter	Aerobic Digestion	Anaerobic Digestion
Oxygenation	. Various methods to oxygenate. Usually the largest running cost (1 to 1,5 kg O_2 per kg BOD removed)	. Requirement for preventing O_2 from entering system (i.e. enclosed system)
Type of waste	. Can handle dilute or relatively concentrated wastes	. Traditionally for sludges but new methods of bacterial entrapment can handle dilute wastes (High organic throughput)
Toxicity	. Fast turnover time means that it can rapidly adjust to toxic stress	. Many materials toxic and slow turnover and start up times increases the systems vulnerability
Retention time (R_T) (Dependant on waste)	. Very short	. Traditionally very long but new methods have reduced it to ~ aerobic R_T
Surge loads	. Rapid adaptation	. Slow adaptation (can lead to digester souring)
Sludge production (Excess biomass)	. High. Disposal problem altered from liquid to solid waste (Sludge yield coefficient ~ 50 - 100% of BOD removed)	. Very low (~10 - 20% of the volume of aerobic sludge formed)

Table 1 (Continued)

Parameter	Aerobic Digestions	Anaerobic Digestion
Start up	. Fast	. Slow. However, ability of biomass to lie dormant for long periods and then start up quickly, (i.e. operate on stop-start basis)
Nutrients	. Low	. Low
Useful end products	. Sludge as fertilizer	. Methane gas (0,35 m^3/kg COD removed - theoretical). Stabilized sludge useful as fertilizer
Environmental nuisance	. None	. System is potentially odorous but anoxic requirement ensures odour control
Heating	. None	. Required for optimal functioning (usually $\sim$ 35°C)
External control required	. Little	. Temperature, loading rate, pH (should be self regulating)
Land requirements	. Large	. Small
Experience	. Much	. Little (Reputation for failing)

bacteria often have very strict substrate requirements. Attempts to isolate and characterize pure cultures of the different species of bacteria present have met with difficulty because of the fastidious nature of the obligate anaerobes as well as the symbiotic relationship that exists between the species. Four trophic groups have however been shown to function in 3 stages to effect complete breakdown of complex wastes to carbon dioxide and methane gas[108,116]. Fig. 6 gives a general scheme of the breakdown process. It should be noted that although the majority of work done on the microbiology of the anaerobic process has been carried out on sewage sludge the underlying principles in operation have direct application to industrial waste systems.

The acidogenic or hydrolytic population is usually the largest trophic group present by one or two orders of magnitude[116] and is composed of both facultative and obligate anaerobes. Numerically, the predominant bacteria are gram-negative, non-sporing obligate anaerobes. Many of the species have large substrate ranges. Proteolytic bacteria (e.g. Clostridium sp)[109] utilize extracellular proteases to convert protein into oligopeptides and amino acids which can then pass through the bacterial cell wall for further metabolism. Not much is known concerning the lipolytic or cellulolytic genera although certain strains (e.g. Bacteriodes sp and Clostridium sp) have been isolated and characterized from sewage sludge[109,116]. The major end products of the hydrolytic/fermentation stage are simple sugars and alcohols, volatile fatty acids (especially acetic), ammonia, sulfide, aromatics (derived from amino acids), carbon dioxide and hydrogen. Hydrogen acts as an electron acceptor and is characteristic of this step. It should be noted that in the absence or inhibition of H_2 utilizing bacteria, (e.g. methanogens), the increase in partial pressure of H_2 inhibits its own production - (i.e. conditions become thermodynamically unfavourable) - and other electron acceptors are used such as propionate, butyrate, succinate and ethanol. The accumulation of these end products depresses the pH, (which further inhibits methanogen functioning), and is an indicator of digester failure[108]. Doubling times for acidogenic bacteria are reported to be in the order of 2 - 3 hours under optimal conditions[106].

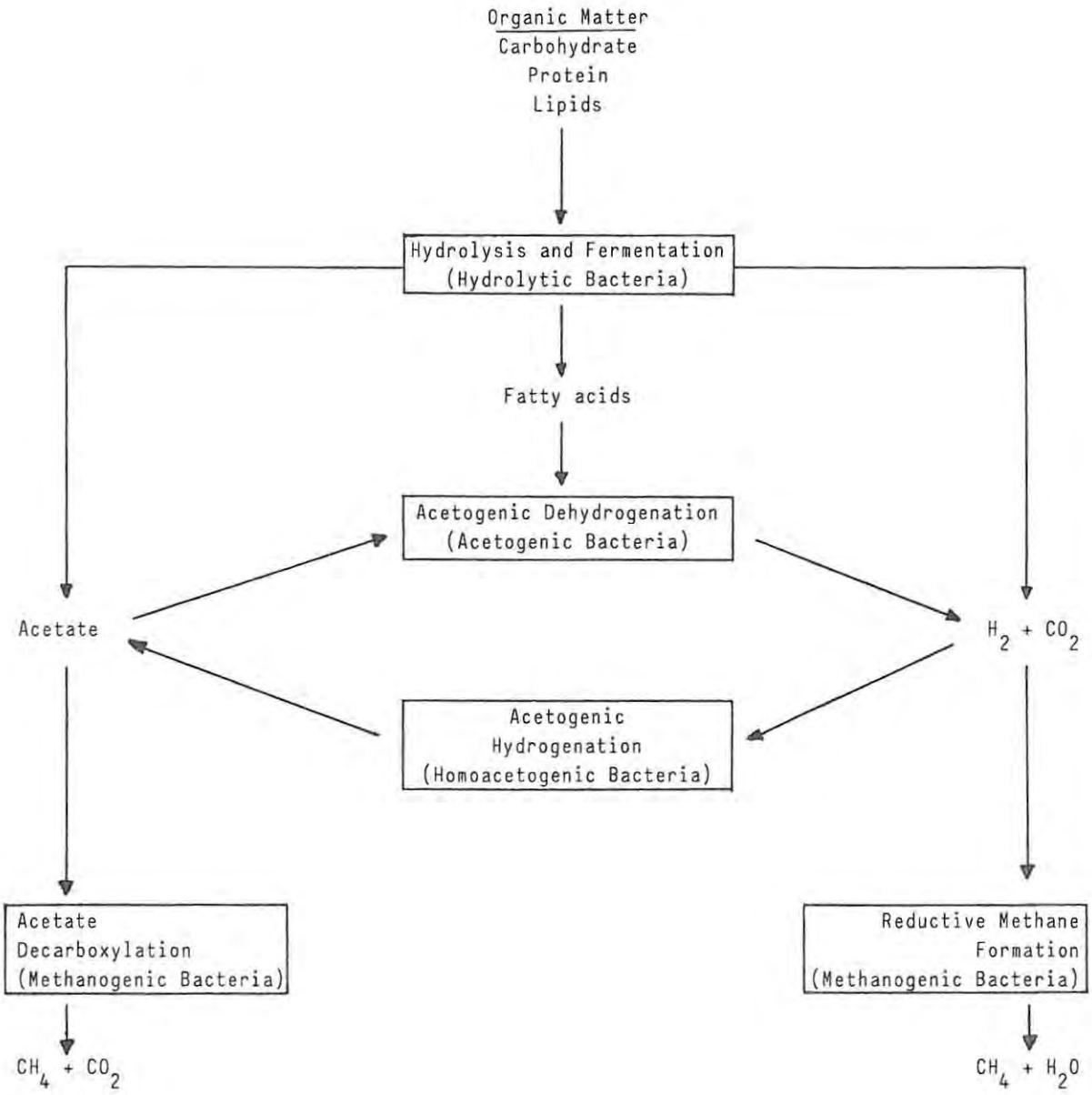


Figure 6. Anaerobic digestion of complex wastes[106,108,116]

The acetogenic bacteria degrade organic acids and alcohols to essentially acetic acid, ethanol and hydrogen gas. In contrast to the acidogenic bacteria, they have an obligate requirement to dispose of electrons as hydrogen gas and are therefore even more sensitive to the partial pressure of H_2 (pH_2) in the digester than the acidogenic species. Inhibition of functioning is reported at pH_2 around 10^{-5} atmospheres[108]. These bacteria have a very slow growth rate, (minimum 2 - 3 days doubling time at 35°C)[115]. They have only been isolated (e.g. the "S" organism)[115] with H_2 utilizing bacteria and hence not much detail is known concerning their physiology.

Clostridium and acetobacterium are the only recognised genera of the homoacetogenic bacteria[116]. They catabolize carbohydrates and H_2/CO_2 while some species are able to convert H_2 and CO_2 to acetate. They are unable to compete with the methanogens for hydrogen but, at times when the methanogens are temporarily inhibited, they may play a part in maintaining low H_2 pressures within the digester. One species, (i.e. Acetobacterium woodii), has been shown to degrade aromatic compounds[117].

It is the obligately anaerobic methanogens who are responsible for the real stabilization of waste[118]. Morphologically they are an extremely diverse group of organisms having rod, spiral and spherical shapes. They also show considerable intra-species variations in cell size and organisation[108]. They reduce one carbon compounds, (e.g. CO_2, CH_3OH, CO, HCO_2H), and acetic acid to methane and carbon dioxide[116], both of which are inert under anaerobic conditions and non-toxic. These products also represent a phase change of the waste and can escape as gases from the digester. Methanogens have a very high affinity for hydrogen and act as the major H_2 sink. They also remove acids from the digester to help maintain the pH balance but are severely inhibited below a pH of 6. Representative genera include methanobacterium, methanobrevibacter, methanococcus, methanomicrobium, methanogenium, methanospirillum and methanosarcina[119]. All these genera are able to reduce CO_2 to CH_4, (i.e. $CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$, $\Delta G°' = -135{,}6$ kJ mole^{-1}), but only the latter genus is able to degrade acetate to methane and CO_2,[119] (i.e. $CH_3CO_2H + H_2O \longrightarrow CH_4 + CO_2 + H_2O$, $\Delta G°' = -31{,}0$ kJ mole^{-1}).

A final group of bacteria that require mention are the sulfate reducers. They catabolize multicarbon compounds in the presence of excess sulfate which acts as an electron sink and is thus converted to sulfide[13]. This reaction is a nuisance in wastes containing sulfates because a reduction in CH_4 production is realized and the alternate product H_2S is highly corrosive to treatment works, has a noxious odour and is inhibitory to methanogenesis[13]. Desulfovibrio and Desulfotomaculum are genera representative of these organisms.

1.5.2 Process Characteristics and Control

In order to optimize an anaerobic digestor system, the temperature, pH and alkalinity must be controlled, toxic substrates must be absent, essential growth minerals and adequate nutrients must be present, surge loads must be prevented and the reactor design must allow for the adequate retention of active biomass as well as efficient biomass to feed contact.

These requirements necessitate a level of expertise and sophistication in the design, monitoring and control of such systems and a lack of expertise has, in fact, been seen as one of the major drawbacks of AD systems to date. Fortunately, a great deal of experience and knowledge has been accumulated over the last few years which allows for the economical implementation of anaerobic treatment systems for many different wastes.

1.5.2.1 Temperature

The categorization of anaerobic systems according to the temperature range in which they function, (e.g. psychrophilic 20°C, mesophilic 30 - 40°C and thermophilic 50 - 60°C)[108,120] is, for the large part, academic. Although bacterial activity increases with temperature[121], it is not feasible, in general to run a digester in the thermophilic range because of the extra heat input and thermal insulation required[122]. The majority of digesters are therefore run at 35°C with the temperature held within a 1°C range. Temperature consistency is required for digester stability as a sudden increase in temperature can precipitate a cycle of events leading to digester souring. The temperature dependence of bacterial activity may, over limited ranges, be described by the Arrhenius expression:[123]

$$\ln \frac{k}{ko} = {}^{-E}/_{RT} \qquad \text{... Equation 1}$$

where k = reaction velocity

ko = frequency factor

E = activation energy (cal/mol)

R = gas constant (1,98 cal/mol °k)

T = absolute temperature (°k)

From plots of ln $^{k}/_{ko}$ vs $\frac{1}{T}$ one can approximate the activation energy E. Values for anaerobic bacterial systems vary between 2 and 40 kcal/mole depending of course on the design of the system and substrate concentration and composition[123].

Q_{10} values are also of interest where Q_{10} is defined as:

$$Q_{10} = \frac{\text{biodegradation rate at (T°C + 10°C)}}{\text{biodegradation rate at (T°C)}} \quad \text{... Equation 2}$$

Values as low as 1,1 have been reported[123] between 20 and 35°C which indicate a diffusion limited growth rate and little temperature dependence. On the other hand, different reactor designs report Q_{10} values of up to 2 which indicates bacterial growth rate limitation and strong temperature dependence[123,124].

1.5.2.2 pH, Alkalinity and Volatile Fatty Acids (VFA)

The methanogenic bacteria are sensitive to pH fluctuation and optimum functioning is reported to be in the range pH 6,8 to 8,0[121]. Methane formation is severely inhibited below pH 6,3 although lower pH's are reportedly not bacteriacidal[122]. The hydrolytic and acidification steps are not as susceptible to pH variation. In a steady-state system pH is usually self-regulatory. pH control is therefore only necessary during start up and surge load conditions or when the nature of the waste influent is such that it requires pH adjustment.

The alkalinity present in a digester acts as a buffer against sudden pH changes. Alkalinity is derived mainly from dissolved bicarbonates, (i.e. $H_2CO_3 \longrightarrow HCO_3^-$, pKa = 6,4), and to a lesser extent from ammonia, (i.e. $NH_4^+ \longrightarrow NH_3$, pKa = 9,3), sulfide, (i.e. $H_2S \longrightarrow HS^-$, pKa = 6,9), phosphates, (i.e. $H_2PO_4^- \longrightarrow H\,PO_4^{2-}$, pK = 7,2) and volatile fatty acids, (i.e. $HAc \longrightarrow Ac^-$, pKa 4,8 to 4,9)[108,121]. At the pH ranges of interest to anaerobic systems, it is only the bicarbonates which are of sufficiently high concentration to be of any significance as buffer material. Measurements of alkalinity are performed either by measuring the

increase in partial pressure of CO_2 on an acidified sample[125] or from direct titration with acid[126]. In the latter method, interferences due to volatile fatty acid titration have to be corrected for. A high level of alkalinity is obviously advantageous as it stabilizes against pH fluctation by neutralizing influent acids or by combining with the VFA produced in the digester as a result of acidogenic activity. Significant increases in VFA concentration - which may be brought about by increased acidogenic activity, methanogenic inhibition or both - which exceeds the buffering capacity of the bicarbonate alkalinity results in a lowering of digester pH and hence further inhibition of the methanogenic or acid removing bacteria. It is the unionised form of VFA which are toxic to methanogens and at lower pH's it is this form which predominates[127]. A further factor of interest is that the VFA toxicity is found to increase with the length of the carbon chain, i.e. butyric $>$ propionic $>$ acetic[108].

In stable digesters, VFA are quickly broken down to harmless end products and the levels of VFA measurable are usually, therefore, very low. Acetic acid predominates although propionic, lactic and butyric acids are also usually detectable. Digestor imbalance is usually indicated by an increase in VFA concentration and high levels of especially n-butyric and propionic acids indicate inhibition of the acetic acid degradation pathway. Measurement of VFA concentration and distribution therefore assists in determining the true stability of the digester. Titrimetric methods for measuring total VFA expressed as acetic acid include direct titration with acid, selective adsorption onto silicic acid and distillation of the acids followed by titration[128,129]. Measurement of individual acids (i.e. C_2 to C_6) is done by gas chromatography[130,131].

1.5.2.3 Toxic Substrates

Heavy metal ions are required in very small quantities in biological processes, are tolerated at higher concentrations and become toxic above critical levels. It is the free ion level which is important here, as a number of compounds precipitate out at biological pH levels or in the presence of suitable ligands. The precipitated ions do not interfere with the biological processes. Sulphide and carbonate ions, which are both

formed by anaerobic processes, are known to be important complexing agents for the removal of a number of different potentially toxic heavy metals[13,108]. A number of experiments have been performed to determine at what level heavy metals become inhibitory to anaerobic digestion[132,133], but these results do not fully take into account the potential of a particular waste to form inert complexes, acclimitization, synergism, antagonism and differences in other digester operating variables[120]. As a result, reported inhibitory and toxic levels for various heavy metal cations vary widely. Methods for controlling heavy metal inhibition usually involve the addition of a suitable complexing agent, the most common being sulphide or its precursors[120].

The lighter alkali and alkali-earth metals are usually stimulatory to methane production at low levels but inhibition occurs at higher concentrations. Sodium for example has reputed[134] stimulatory effects up to 200 mg/ℓ; shows moderate inhibition at 4 000 mg/ℓ, and exhibits strong inhibition at 8 000 mg/ℓ. Control of these toxic materials may be effected by dilution of the waste, the formation of insoluble complexes or by antagonizing the toxicity by adding other metals. Sodium and potassium are reputedly the best primary antagonists when added at stimulatory levels (i.e. 150 mg/ℓ and 300 mg/ℓ respectively)[134].

Ammonia is present in wastes as a dissolved gas (i.e. NH_3) or the ammonium cation (i.e. NH_4^+) and may be derived from proteinaceous breakdown or directly from wastewaters high in N content. The dissolved gaseous form of ammonia is more inhibitory than the cation[134] and the predominance of either species is pH dependant[108], (e.g. $NH_4^+ \rightleftharpoons NH_3\ (g) + H^+$, pKa = 8,9)[13]. Here too, acclimitization to high levels of ammonia does occur and toxicity is affected by synergistic and antagonistic effects.

The presence of sulphates, sulphites and sulphides in industrial waste streams is one of the major problems encountered when dealing with industrial wastes[14]. The problem is threefold. Firstly, the sulphur-reducing bacteria compete with the methanogens for electron donors, (e.g. free hydrogen or acetate), to reduce oxidised sulphur compounds to sulphide[13].

They are more successful than the methanogens (CH_4 : H_2S :: 1 : 5) and methane formation is therefore suppressed[110]. In the second place, free sulphide concentrations above 200 mg/ℓ (as S), are strongly inhibitory on methanogenic cell function resulting in a decline in the methanogen population[14]. Thirdly, the methods involved in dealing with the presence of sulphur all have economic implications. Small amounts may be precipitated with heavy metals, (e.g. iron salts), or the waste stream may be separated and contained or diluted[128]. Alternately, H_2S may be stripped from the digester gas by scrubbing[13] if the gas is recycled. All these methods however result either in loss of methane due to preferential production of H_2S or require expensive capital outlay to protect against the corrosive effects of H_2S. Recent developments at Biomass International reportedly have produced a stable anaerobic microbial association which allows high methane yields in the presence of sulphate. Free H_2S is not detected in waters containing up to 7 500 mg/ℓ SO_4 but further details pend patent approval[14]. In another development, biological sulphate in mine waters may be removed by reduction to elemental sulphur via sulphide by a symbiotic relationship involving sulphur-reducing (i.e. Desulfovibrio desulfuricans), and photosynthetic, (i.e. Chlorobium thiosulfatophilum), bacteria[135].

The classification of an organic compound as toxic is dependant on its concentration and microbial acclimitization. Many common organic compounds, (e.g. alcohols and long-chain fatty acids), are inhibitory at high levels to unacclimitized digesters but are easily degraded by continuously fed digesters[134]. Other highly toxic compounds, (e.g. $CHCl_3$, CCl_4 and CN^-)[136,137], are tolerated at lower concentration levels. Given sufficient time, anaerobic bacterial associations have in fact been shown to degrade phenols[111,138-140], chlorinated phenols[141-143] and polycyclic N-aromatic compounds[112].

1.5.2.4 Nutrition and Essential Growth Requirements

A pre-requisite for optimal anaerobic functioning is a wastewater of balanced composition regarding both C, N and P ratios as well as macro- and micronutrient levels[121]. An average chemical formulation for biological cells may be assumed to be $C_5H_9O_3N$[118] giving a nitrogen requirement of just over 10% of cell volatile solids. Phosphorus requirement amounts to approximately 1/5 of the N requirement[118]. Obviously bacterial cell formulation can only give an indication of the required CNP ratios as the dynamic aspects of cell decay, the escape of gaseous products, soluble alkalinity and effluent waste composition is not taken into account. Optimum CNP ratios have in fact been shown to vary for different influent substrates[121].

Waste streams requiring alkalinity or buffering are usually supplemented with lime (e.g. CaO or $Ca(OH)_2$), bicarbonates (e.g. $NaHCO_3$), or phosphates (e.g. K_2HPO_4 and KH_2PO_4). The inhibitory effects of higher concentrations of free, light metal cations limits the degree of alkalinity or buffering that may be achieved by adding the abovementioned compounds. They are however, together with magnesium and iron, stimulatory to methanogenesis and are supplemented when required to deficient influent waste streams[122].

Reported additions of other minerals and trace elements varies widely with some researchers relying on tap water to supply some of the necessary micro-nutrients[123,144]. Table 2 gives some of the concentration levels of elements added by different research workers for a variety of different wastes. Nickel is known to form part (i.e. Factor F_{430}) of a tetrapyrrole enzyme required for methanogenesis[103] and cobalt has also been shown to form part of a co-enzyme (methylcobalamin) system[103]. Enhanced methanogenesis has been shown to occur by the addition of a cocktail of trace elements[145] although the full effect of the individual metals is not known. Research into these effects is complicated due to the heterogenous nature of the bacterial populations present, synergistic and antogonistic effects occurring between metals as well as the effects of the precipitation of metals, for example by sulphide.

Table 2. Mineral additions to anaerobic systems by various research workers

Trace Element	Nel et al[1]	Khan et al[2]	Fogh et al[3]	Brune et al[4]	Shelton et al[5]	Wong et al[6]	Kelly et al[7]
B	H_3BO_3 $1{,}24 \times 10^{-2}$ mg/ℓ	$Na_2B_4O_7.10H_2O$ 0,19 – 1,14 mg/ℓ	H_3BO_3 2,8 mg/ℓ	H_3BO_3 0,69 mg/ℓ	H_3BO_3 0,05 mg/ℓ	$Na_2B_4O_7.10H_2O$ 0,94 mg/ℓ	
Ca	$CaCl_2$ 36 mg/ℓ	$CaCl_2.2H_2O$ 22,5 – 133 mg/ℓ	$CaCl_2.2H_2O$ 100 mg/ℓ	$CaCl_2.2H_2O$ 250 mg/ℓ	$CaCl_2.2H_2O$ 75 mg/ℓ	$CaCl_2.2H_2O$ 148 mg/ℓ	
Mn	$MnSO_4.5H_2O$ 0,24 mg/ℓ	$MnCl_2.4H_2O$ 0,8 – 4,7 mg/ℓ	$MnCl_2.4H_2O$ 6,0 mg/ℓ	$MnCl_2.4H_2O$ 1,4 mg/ℓ	$MnCl_2.4H_2O$ 0,5 mg/ℓ	$MnCl_2.4H_2O$ 3,9 mg/ℓ	
Zn	$ZnCl_2$ 0,20 mg/ℓ	$ZnCl_2$ 0,54 – 3,25 mg/ℓ	$ZnCl_2$ 10,0 mg/ℓ	$ZnSO_4.7H_2O$ 0,5 mg/ℓ	$ZnCl_2$ 0,5 mg/ℓ	$ZnCl_2$ 2,7 mg/ℓ	
Mg	$MgCl_2.6H_2O$ 24 mg/ℓ	$MgCl_2.6H_2O$ 40 – 203 mg/ℓ	$MgCl_2.6H_2O$ 600 mg/ℓ	$MgCl_2.6H_2O$ 600 mg/ℓ	$MgCl_2.6H_2O$ 100 mg/ℓ	$MgCl_2.6H_2O$ 206 mg/ℓ	$MgCl_2.6H_2O$ 694 mg/ℓ
Co	$CoCl_2$ $9{,}1 \times 10^{-2}$ mg/ℓ	$CoCl_2.6H_2O$ 0,5 – 2,9 mg/ℓ	$CoCl_2.6H_2O$ 1,7 mg/ℓ	$CoCl_2.6H_2O$ 0,5 mg/ℓ	$CoCl_2.6H_2O$ 0,5 mg/ℓ	$CoCl_2.6H_2O$ 2,4 mg/ℓ	$CoCl_2.6H_2O$ 30 mg/ℓ
Mo	MoO_3 $6{,}6 \times 10^{-2}$ mg/ℓ	$(NH_4)_6Mo_7O_{24}.4H_2O$ 0,4 – 2,1 mg/ℓ	MoO_3 0,8 mg/ℓ	$Na_2MoO_4.2H_2O$ $2{,}6 \times 10^{-2}$ mg/ℓ	$NaMo_4.2H_2O$ 0,01 mg/ℓ	$(NH_4)_6Mo_7O_{24}.4H_2O$ 1,7 mg/ℓ	
Al	$AlCl_3$ $8{,}1 \times 10^{-2}$ mg/ℓ						
Se	H_2SeO_3 $9{,}2 \times 10^{-2}$ mg/ℓ			$Na_2SeO_3.5H_2O$ $2{,}6 \times 10^{-2}$ mg/ℓ	Na_2SeO_3 0,05 mg/ℓ		
Si	SiO_2 $3{,}5 \times 10^{-3}$ mg/ℓ						
W	$Na_2WO_4.2H_2O$ $1{,}67 \times 10^{-3}$ mg/ℓ			$Na_2WO_4.2H_2O$ $2{,}6 \times 10^{-2}$ mg/ℓ			
Ni	$NiCl_2$ $6{,}04 \times 10^{-3}$ mg/ℓ				$NiCl_2.6H_2O$ 0,05 mg/ℓ		

Table 2 (Continued)

Trace Element	Nel et al[1]	Khan et al[2]	Fogh et al[3]	Brune et al[4]	Shelton et al[5]	Wong et al[6]	Kelly et al[7]
Fe		$FeCl_3.6H_2O$	Fe-citrate	$FeCl_2$		$FeCl_3$	$FeCl_3.6H_2O$
		3 - 19 mg/ℓ	2,0 mg/ℓ	1,5 mg/ℓ		16 mg/ℓ	723 mg/ℓ
Cu				$CuSO_4.5H_2O$	$CuCl_2$		
				1,5 mg/ℓ	0,03 mg/ℓ		
Na	NaOH	$NaH_2PO_4.2H_2O$	$NaHCO_3$	$NaCl+Na_xH_yPO_4$	$NaHCO_3$	$NaH_2PO_4.2H_2O+Na_3$ Citrate	$NaHCO_3$
	?	2 699 mg/ℓ	5 000 mg/ℓ	200 + 1800mg/ℓ	1 200 mg/ℓ	205 + 146 mg/ℓ	5 000 mg/ℓ
K	KH_2PO_4	K_2HPO_4	$K_2HPO_4.3H_2O$ + K_2HPO_4		$K_2HPO_4 + K_2HPO_4$	K_2HPO_4	KCl
	500 mg/ℓ	3 919 mg/ℓ	600 + 230mg/ℓ		270 + 350 mg/ℓ	337 mg/ℓ	198 mg/ℓ

1. Reference 145 : Petrochemical waste
2. Reference 138 : Phenols
3. Reference 146 : Carbohydrates
4. Reference 147 : Cellulose manufacture
5. Reference 148 : Various
6. Reference 112 : Polycyclic N-aromatics
7. Reference 123 : Whey powder

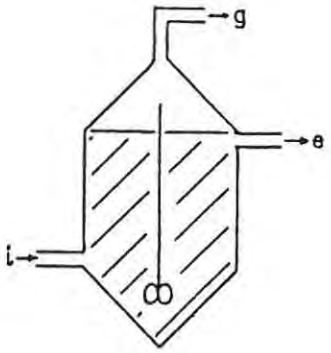
1.5.3 Reactor Design

Progress in the understanding of the physiology and microbiology of AD led the way to improved reactor design which in turn resulted in the fast rates of digestion and the wide ranges of AD application found today. A very large number of reactor designs are in full scale operation at the present time and a much larger diversity occurs at laboratory and plant scale. The reactors can however be classified according to the underlying principles of operation and a summary of the different types is given in Figure 7. Table 3 gives summarized examples of certain wastes that have been treated by the different systems as well as loading rates and efficiencies.

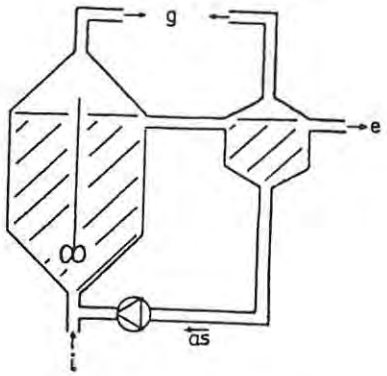
The conventional or classic reactor was originally designed for treating sewage sludges. It is still in use today and comprises a completely mixed, one step process without solids recycle. The normal mode of operation entails batch loading of the reactor, slow mixing for the full retention period, (sometimes up to 60 days)[107], and then unloading of the stabilized sludge. A part of the sludge remains behind as innoculum for the next batch. A long retention time is characteristic of this reactor due to the slow specific growth rate of methanogens and the reactors therefore tend to be large in size. They are susceptible to toxic element or chemical shock and are therefore not practicable for industrial wastes. They do however handle high organic loadings, are relatively cheap to build, require little maintenance and are often run at ambient temperatures[149].

The activated sludge reactors were designed to counteract one of the major drawbacks of the conventional digesters, namely, loss of biomass. The key here was the addition of a solids separator/clarifier to separate sludge or biomass from the effluent stream and to return these solids back to the reactor. This results in a more stable system, resistant to toxic or surge load shocks with a much shorter residence time due to the high relative concentration of viable bacteria in the system. Simple settling of solids in the clarifier does not work too well because entrained gas bubbles formed by continuing methanogenesis lifts the solids to the surface. Thermal shock and vacuum degassing are two methods used to improve settling characteristics[150].

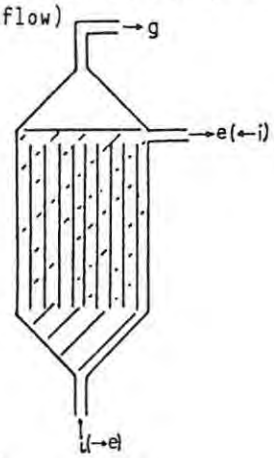
(1)
Conventional or Classic Reactor



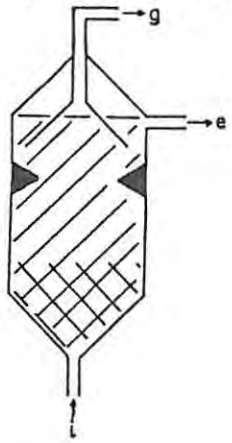
(2)
Activated Sludge Reactor



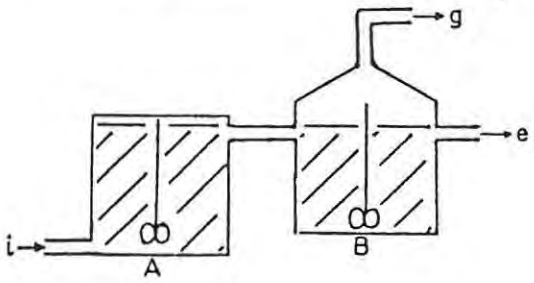
(3)
Anaerobic Fixed Bed Filter - upflow (or downflow)



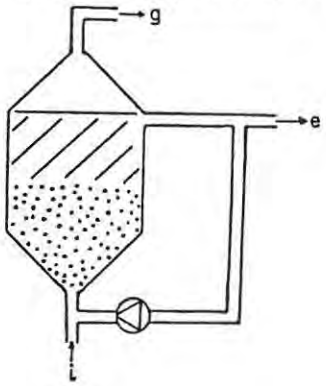
(4)
Upflow Anaerobic Sludge Blanket Reactor (UASB)



(5)
Two Phase Reactor



(6)
Anaerobic Expanded Bed Filter



i = influent waste
e = effluent waste
g = gas
as = activated sludge recycle
s = solids separator/clarifier
A = acidogensis
B = methanogensis

Figure 7. Reactor designs for anaerobic digestion

Table 3. Comparison of performance characteristics for various anaerobic reactors

Type of Reactor	Waste Treated	Loading Rate (max)	% COD Removed	Ref.
Conventional	Domestic Sewage	(8,0 kg DS/m^3 d)	-	107
Activated Sludge	Pharmaceuticals	6 kg COD/m^3 d	95%	151
UASB	Sugar beet	20 kg COD/m^3 d	88%	152
	Pharmaceuticals	30 kg COD/m^3 d	95%	151
	Potato processing	6 kg COD/m^3 d	80%	153
	Thermomechanical pumping effluent	5 kg COD/m^3 d	50%	154
Fixed Bed Filter	Pharmaceuticals	10 kg COD/m^3 d	95%	151
	Piggery slurry supernatant	20 kg COD/m^3 d	88%	149
	Food and drink manuf.	25 kg COD/m^3 d	70%	155
	Sugar refinery	6 kg COD/m^3 d	76%	156
	Carbohydrate (synthetic)	16 kg COD/m^3 d	97%	14
	Phenols (synthetic)	5,6 kg COD/m^3 d	88%	111
	Petrochemical	7 kg COD/m^3 d	83%	157
	Petrochemical	4,7 kg COD/m^3 d	93%	158
Expanded Bed	Whey permeate	12 kg COD/m^3 d	63%	106
	Glucose (synthetic)	16 kg COD/m^3 d	70%	106
	Pharmaceuticals	20 kg COD/m^3 d	87%	151
	Cellulose	8 kg COD/m^3 d	90%	103
	Molasses waste	24 kg COD/m^3 d	-	159
2 Phase Reactor	Pig manure	20 kg COD/m^3 d	88%	103
	Sugar beet	40 kg COD/m^3 d	70 - 95%	160
UF System*	Whey permeate	16 kg COD/m^3 d	95%	161

* Essentially an activated sludge reactor but incorporating an ultrafiltration (UF) membrane for solids recycle

The upflow anaerobic sludge blanket reactor (UASB) is a potentially inexpensive but elegant design which entraps biomass by means of a gas/solids separator at the top of the reactor. Feed is distributed at the bottom of the reactor and is gently mixed by rising gas bubbles. Flow through the reactor is essentially plug flow. Microbial flocs which are entrained and taken to the surface by gas bubbles are released only to fall back down into the active fermentation zone. Little activity occurs in the upper settling zone which acts as the clarifier. Large scale use of UASB reactors is found in the treatment of sugar beet and starch wastes where the phenomenon of biomass pelletization has greatly increased the settling characteristics of the sludge and hence the throughput of the reactors[106,162]. Pelletization is thought to be substrate specific[162].

Anaerobic filters make use of the propensity of anaerobic bacteria to stick to inert solid support material. The design may be upflow or downflow but relies on the principle that attached bacteria do not wash out easily and these reactors are therefore well suited to treat weak or strong soluble wastes at high throughflow rates such as those found in industrial streams. Support media may be glass, plastic, stainless steel, ceramic or other inert material[149]. Activated carbon has also been used for treating phenolic wastes[111,138]. The initial capital outlay required to fill the reactor with the support material may be quite high and the presence of support material also decreases the available volume within the reactors. The reactors are limited by the amount of suspended solids tolerated in the waste stream as these solids tend to clog and block the flow through the reactor. A periodic backwash is sometimes used to clean the system. A long start up time is also a drawback of these systems. They are however very tolerant to both organic and hydraulic surge loads and many systems are in operation worldwide.

A limiting factor in fixed bed filters is the amount of available surface area for microbial attachment. The use of smaller solid support material increases both surface area and the chances of blockage. Filters using small support media therefore have to be expanded or fluidized by high recirculation rates which prevents clogging. The support material used includes sand, aluminium oxide, tiny porous plastic beads or other fine

material[106,163]. These systems require very long start up times, are susceptible to blockage and have high power input requirements, (i.e. in running the recirculation pump). Initial capital costs are also high. Functioning systems do however report very high loading rates with excellent COD removal efficiencies and high stability with regard to shock loadings[164].

Attempts to optimize both the acidogenic and the methanogenic phases has led to the development of the two-phase anaerobic reactors. pH adjustments or retention times may be used to separate the phases[165,166]. Criticism of such systems revolves around the lack of interspecies transfer of hydrogen between acidogens and methanogens in two phase systems which would lead to the accumulation of higher fatty acids such as propionate and butyrate. The degradation of these latter species requires the participation of the acetogenic bacteria in the methanogenic phase. Results to date indicate that these acids are in fact rapidly degraded in the methanogenic phase and furthermore that an increase in maximum specific COD conversion rates of up to 3 fold is experienced[167]. The two-phase reactors are also more stable under shock load conditions[166] and shorter cell regeneration times allow a reduction in reactor volumes to be realized[165]. Incomplete separation, that is a partial pre-acidifcation step, has also been shown to result in higher rates of waste stabilization[167].

The different types of reactor described above allow a choice to be made between plug flow or completely mixed systems, suspended growth or fixed growth, upflow or downflow, through flow or recycle reactors and a whole host of systems between these extremes. The so-called "anhybrid" reactor[110] or "upflow blanket filter"[168] for example comprises a suspended growth colony in the bottom half of the reactor with fixed bed media present in the top half to act as a polishing step. Other novel concepts include the anaerobic baffled reactor[169] which is essentially a number of UASB reactors in series and the membrane anaerobic reactor system[161] which incorporates an ultrafiltration membrane on the effluent discharge of a suspended growth reactor to retain the biomass.

1.6 Application of Anaerobic Digestion Technology to Tannery Wastewater

Tannery wastewaters and sludges are extremely difficult to treat anaerobically relative to municipal wastes due to both the presence of very high levels of organic matter as well as the recalcitrant and/or inhibitory nature and quantity of chemical additives used in the tanning processes. Table 4 gives typical gross pollutant characteristics of raw tannery effluents as well as pollutant characteristics of various process areas in the tannery. A treatment strategy is usually tailored for an individual tannery's requirements and sometimes involves separation of individual waste streams from various parts of the tannery. Anaerobic digestion has been applied to some of these effluents with various degrees of success and admixture of total tannery waste with domestic sewage wastes has proven advantageous[170,171].

Table 4. Typical Gross Pollutants of Final Effluents of Full Tanneries and Tannery Sectors[6,172]

Parameter	Wet Blue Tannery	Chrome Tannery with Full Finishing	Chrome and Veg Tannery with Full Finishing	Chrome Tannery Full Finishing			Chrome and Veg Tannery with Full Finishing			USA Tanneries	
				Limeyard	Tanyard	Dyehouse	Limeyard	Tanyard	Dyehouse	Chrome + Full Finish	Beamhouse only
pH	9-11	9-11	9-11	12	5	7	12	5	7	-	11-12,5
Total COD (mg/ℓ)	10 000-20 000	7 000	6 000	30 000	10 000	3 000	30 000	8 000	3 000	8 000	-
Suspended Solids - SS (mg/ℓ)	5 000-10 000	2 000-4 000	2 000-5 000	6 000	1 000	2 000	6 000	1 000	2 000	5 000	3 700-5 500
Total Dissolved Solids-TDS (mg/ℓ)	20 000	15 000-20 000	10 000-15 000	18 000	20 000	6 000	18 000	8 000	6 000	16 000	8 000-14 000
Sulphides (mg/ℓ)	500	100	300	-	-	-	-	-	-	300	500-1 500
Chromium (mg/ℓ)	500	100	50	-	-	-	-	-	-	150	-
BOD_5 (mg/ℓ)	-	-	-	-	-	-	-	-	-	3 000	2 800-4 555
Oil & Grease (mg/ℓ)	-	-	-	-	-	-	-	-	-	600	-
Total Kjeldahl Nitrogen (mg/ℓ)	-	-	-	-	-	-	-	-	-	600	2 800-4 200

1.6.1 Anaerobic Treatment of Beamhouse and Chrome Tannery Effluent

From Table 4 it is seen that the beamhouse wastes have the highest organic pollution load. They also account for the largest volume of wastewater[172] and are characterized by a high pH, high organic loading, high sulphide levels and relatively high salt concentrations.

pH levels are self adjusting in anaerobic systems[170] but most beamhouse wastes that have been looked at are usually neutralized - often with acid tanning liquors - before introduction to anaerobic systems[88,172].

Sulphides are present in beamhouse anaerobiosis mainly by direct introduction but also by reduction of oxidised precursers (e.g. keratin protein and inorganic sulphates). The amount of soluble sulphide present is directly proportional to pH[5] and inversely proportional to the amount of gas produced as well as the concentration of heavy metal ions[134]. According to McCarty[134] sulphides escape with digester gas and are precipitated by heavy metal ions. Young[173] found that soluble sulphide levels of up to 200 mg/ℓ may be tolerated with acclimation. This finding was confirmed by Maeda et al[174] who found sulphides inhibitory below 500 mg/ℓ. Oelthoff[110] et al show that sulphide reducing bacteria and methanogens can function side by side if the former species are at sufficiently low concentration. Friedman et al recommend[173] pretreatment of beamhouse wastes by acidification and mixing which reduces the residual sulphide concentration to below 300 mg/ℓ before introduction into anaerobic systems.

Sodium is inhibitory at high concentrations (8 000 mg/ℓ)[134] but would be sufficiently diluted in total beamhouse wastes so as not to present a problem in anaerobic treatment.

Research into the inhibition of anaerobic digestion by chrome has led to many contradictory findings. Some of these are shown in Table 5. Hexavalent chrome is generally found to be toxic at relatively low concentrations (i.e. $<$ 50 mg/ℓ). Tannery effluents only contain trivalent chrome however and oxidation of Cr^{3+} to Cr^{6+} under a reducing anaerobic enivronment is not feasible[175,176]. Some researchers have found trivalent chrome inhibitory at levels of 25 mg/ℓ[177] while others have found it not inhibitory up to levels of approximately 10 000 mg/ℓ (10 g/kg)[4]. These discrepancies may in part be resolved by solubility considerations. Cr^{3+} solubility is inversely proportional to pH and at the pH levels of interest, a large amount of chrome would precipitate out as the inert hydroxide[61,134]. The high lime content of beamhouse wastes (cf sewage sludge) would furthermore tend to buffer the digester in the alkali range which would increase the Cr^{3+} precipitate. Sulphides present, or formed by the reduction of sulphates and sulphites would also tend to form insouble complexes with chrome[178]. Bailey[88] reported that sodium salts increased chrome solubility as did organic masking agents (e.g. formates and phthalates). Tannery effluents treating salt cured hides or ones that use masking agents in the tanning process would therefore experience chrome toxicity at lower levels than other effluents. The problem of chromium toxicity in tanning effluents is in any case being circumvented by legislative and economic pressures. The low levels of chrome stipulated by legislative bodies has led to the development of high uptake chromes, alternate tanning procedures and chrome recycling methods; some of which also have economic benefits[179-181].

Pilot plant work at Centre Technique de Cuir (CTC) has demonstrated the anaerobic biodegradable potential of a mixture of tannery effluent sludges (65%), fleshings (25%) and untanned wastes (10%)[5]. Using 50 ℓ and 70 ℓ completely mixed, mesophilic digesters they achieved a 33% breakdown in

Table 5. Inhibition of Anaerobic Digestion by Chromium

Substrate	Level of Inhibition	Comments	Reference
-	3 - 6 mg/ℓ Cr^{6+} 25 - 50 mg/ℓ Cr^{3+}		177
Sewage Sludge	500 mg/ℓ Cr^{6+} 3,5% Cr^{3+}	 (dry weight)	182
Tannery Sludge	5% Cr^{3+}	(dry weight)	183
Tannery + Domestic Sludge	> 3 200 mg/ℓ Cr^{3+}		171
Sewage Sludge	530 mg/ℓ Cr^{6+} 450 mg/ℓ Cr^{3+}	 	184
Tannery Sludge + Fleshings	> 1% Cr^{3+}	(total weight)	4
Sewage	> 300 mg/ℓ Cr^{3+} 100 mg/ℓ Cr^{3+} 50 mg/ℓ Cr^{3+}	(HRT = 21 days) (HRT = 17 days) (HRT = 14 days)	88
Synthetic (Gelatin)	< 50 mg/ℓ Cr^{6+} > 1 000 mg/ℓ Cr^{3+}	 	174
Tannery Sludge	> 5 800 mg/ℓ Cr^{3+}	(Precipitates out) (as Hydroxide)	170

total waste with 500 ℓ of biogas (CH_4 ~ 75%) being produced per kg volatile solids added at a hydraulic retention period of 20 days. The biogas contained less than 1% H_2S which was attributable to the high pH, (9,3), and buffering capacity of the lime rich influent. The high levels of soluble sulphide present resulted in a low redox potential which fosters methanogenic growth. Tolerance of the system to high influent chrome concentrations, (i.e. up to 10 000 mg/ℓ Cr^{3+}), was attributed to the alkalinity present but could in part be also attributed to precipitation by sulfide.

Maeda et al[174] have done fairly extensive work on the effects of ammonia, chrome and sulphide on anaerobiosis of synthetic tannery waste. They used gelatin as feed source. Ammonia was found to be progressively inhibitory regardless of pH. Gas production was inhibited at 4 000 mg/ℓ and was virtually halved at an NH_4 concentration of 8 000 mg/ℓ. Chromium VI was found to inhibit gas production at 50 mg/ℓ and virtually halt gas production at 100 mg/ℓ while Cr^{3+} was found not inhibitory up to concentrations of 1 000 mg/ℓ. Sulphide was found progressively inhibitory and halved gas production at 2 000 mg/ℓ. Sulphide inhibition could most effectively be combatted by circulation of digester gas through a hydrogen sulphide scrubber (e.g. Stannous chloride in HCl @ pH = 0,5). Their methods may be criticized in that the anaerobic population was not allowed to acclimate to the compounds introduced. These compounds were added batchwise to samples of stabilizied anaerobic sludge and cumulative gas readings were taken over a period of only 4 days.

Reasonable reductions of up to 52% BOD_5 were obtained by Ivanof[185] who mixed complete tannery sludge with domestic sewage (1:3). A 2 hour retention time was used. Work by Fendrup et al[170] showed that the anaerobic treatment of tannery sludges improved even further when treated together with animal dung. Königfeld[171] found mixed tannery and sewage effluent readily biodegradable anaerobically with no adverse affect with up to 3 200 mg/ℓ Cr^{3+} present in the tannery effluent.

Research by D G Bailey et al[172,173,186] has looked at anaerobic digestion as a pretreatment for beamhouse waste in an effort to comply with the Environmental Protection Agency's (USA) effluent limitations of 40 mg/ℓ

BOD_5 and 250 mg/ℓ COD for direct discharge. In the groups first approach, an upflow anaerobic filter was used in a roughing treatment of acidified and pre-settled supernatant. COD removals averaging 65% were reported for a HRT of 20 hours (2,25 kg COD/m^3/day). The system proved relatively stable to feed and temperature fluctuations and also to accidental introduction of aerial O_2. The anaerobically treated waste proved amenable to oxidative polishing by conventional means (e.g. rotating biological contactor). Undesirable accumulation of solids (mainly Ca salts) within the reactor was reported however and mooted as a reason for avoiding anaerobic filters in beamhouse waste treatment. Their second approach used an anaerobic contact reactor - essentially a completely mixed activated sludge system - as the roughing treatment which gave similar efficiency to that of the anaerobic filter. Regular COD removals of > 60% were noted with a HRT of 60 hours and 13 days volatile solids retention. The anaerobic organisms proved resistant to environmental upsets and the waste was successfully degraded further by aerobic polishing. High sulphide levels of up to 600 mg/ℓ were tolerated by the system.

Gates and Lin[73] have looked at laboratory scale lagooning of tannery wastes and compared it to the anaerobic contact process. Anaerobic lagooning is inefficient and does not allow optimum contact between biomass and liquid waste. Vertical stratification of deposited sludge over time could conceivably result in stabilized sludge at the bottom with methanogenic intermediary layers and an acidogenic top layer. Washout of volatile fatty acids would then occur and this would minimize the fermentation capacity of the system. The anaerobic contact process used (i.e. completely mixed system), proved efficient and resulted in over 85% reduction in BOD_5 at a HRT of 1,25 days.

Eye and Graef[187] separated beamhouse wastes into lime-bearing and non-lime-bearing streams. The former was treated with an anionic polyelectrolyte, it was then settled and the clarified supernatant added to the non-lime-bearing waste. The whole was then acidified to pH 9 and introduced to an anaerobic digester at a loading rate of 0,24 kg COD/m^3/day. The HRT was approximately 10 days and COD removal efficiencies of 50% were obtained. The anaerobic effluent proved amenable to aerobic polishing and a total COD reduction of 90 - 95% was noted.

1.6.2 Anaerobic Digestion of Vegetable Tannins

Work on the anaerobic treatment of vegetable tannins dates back more than 20 years. In the early sixties, Humphreys and Bailey[89] used a flooded "bio-filter" for treating strong, spent vegetable tanning wastewater and reported a 33% reduction in PV at a loading rate of 5,7 kg PV/m^3/d. (A conversion factor of PV to COD may be given as 1 PV unit $\sim$ 1,97 COD units[62]. The COD loading rate therefore approximates 11 kg COD/m^3/d). This is a high loading rate for wastes containing compounds such as vegetable tans even though spent wastes are low in tan concentrations and have higher biodegradable potential (See Section 1.3). The resulting sludge did not prove amenable to subsequent aerobic treatment.

At about the same period, Toyoda et al[174,188] did work on the treatability of vegetable tannins both in a synthetic and in an industrial waste. The tannins proved amenable to continuous digestion provided an acclimated sludge was used and total COD reductions of up to 77% were recorded. They listed high loading rates of 0,2 to 1,0 g BOD/ℓ/day. (At a very rough estimate this would approximate to 1,3 - 6,5 kg COD/m^3/d. That is using the Mimosa ME extract ratio BOD_{20}:COD::1:6,5 from Hendry[62]).

A few years later, Gates and Lin[73] built a laboratory scale anaerobic/aerobic lagoon for treating vegetable tanning wastes. The HRT was 2,3 days at a loading rate of 0,62 kg COD/m^3/d. COD reductions averaged 70%. The system was unfortunately only run for 1 month but in that time no sludge build-up was noticed. The effluent from the anaerobic section was highly coloured and malodorous. Subsequent aerobic treatment resulted in the removal of odour but it did not reduce either the COD nor the colour. Without mixing or efficient distribution of feed at the base of the anaerobic reactor, the system would appear to be highly inefficient with regard to biomass to substrate contact. To improve on the reactor design, Gates and Lin tried an anaerobic contact process. This involved a completely mixed anaerobic section, a solids separator followed by an aerobic chamber. The HRT was 1,3 days at a loading rate of 2,4 kg COD/m^3/d. COD reduction averaged 60%. The system was run for less than one month but accumulation of volatile suspended solids in the anaerobic section indicated

the efficiency of the solids separator used. The anaerobic effluent was again malodorous and highly coloured. No indication was given of the amenability of the anaerobic effluent to aerobic treatment. Comparison of the influent and effluent COD:BOD ratios gave values of 3,8 and 12,0 respectively. The preferential degradation of easily degradable organics was thus shown. No indication of tannin removal was given but colour removal was not effected.

Parker[12], in 1968, attempted to study full scale, (i.e. Volume = 3 000 m^3 at a HRT of 4 - 36 days), anaerobic-aerobic lagooning of spent vegetable tannins. Efficient surface aeration severely limited the development of anaerobic zones however and Parker came to the conclusion that the lagoon was facultative - aerobic at best. He questioned the efficacy of a lagoon for mixed biological systems and recommended the investigation of different structural designs which allow adequate separation of the anaerobic and aerobic phases.

Aloy et al[5] carried out a few experiments on the anaerobic digestion of vegetable tanning sludges prior to publication of their book "Pollution and Tannery" in 1976. They came to the conclusion that these sludges are amenable to anaerobic digestion but list pH control and a long retention time, (30 days), as reasons for avoiding this form of treatment.

Papers published in 1975 and 1980 by Arora et al[16,189] described the use of an upflow anaerobic filter for treating vegetable tanning effluents. The filter - a 26ℓ column filled with quartz stones - was run in the first trial at a HRT of 3 days and loadings from 0,2 to 3,3 kg COD/m^3/d. Feed COD:BOD ratios averaged 1,8 which indicates a biologically treatable waste. The main contributors to a high COD:BOD ratio would be the tannins and only 0,32 g/ℓ were measured as tannic acid by the tyrosine-sodium bicarbonate method. COD removal efficiencies ranged from a peak of 90% at a loading rate of $\pm$ 1 kg COD/m^3/d to 20% at the highest loading rate. Tannin removals averaged 50% throughout the run and the effluent was partially decolourized. In the second run, the HRT was lowered from 6 days to 0,5 days to effect organic loadings of between 2 and 23 kg COD/m^3/day. The acclimatized reactor was reported to remove up to 90% COD and tannins at the top loading rate. The 4,5 g/ℓ tannins in the feed was reduced to 0,5 g/ℓ in the effluent -

measured as tannic acid. Although these reports are very encouraging, problems of precipitation and clogging within such a filter are expected and results of field trials are still awaited.

Eye and Ficker[15] presented a paper at the 1981 Annual Meeting of the American Leather Chemists Association in which they showed the extent of inhibition on the anaerobic digestion process by wattle and quebracho tannins. Thermophilic (30°C) reactors of 3 ℓ capacity were run at a HRT of 30 days with domestic sewage feed to which stepwise additions of tans were made. Additions of wattle extract increased from 0,2 to 0,9 g per day over a period of 60 days. No indication was given of the relevant COD influent and effluent levels although removal efficiencies dropped from 60% to 30% at the higher tan concentration. COD levels within the reactor were seen to rise from ± 15 000 mg COD/ℓ to ± 35 000 mg COD/ℓ and the authors suggest that the tannins inhibit the breakdown of sewage organics. They were also seen to decrease the settleability of the sludge. A substantial increase in volatile acid concentrations occurred when wattle additions rose to 0,7 g per day and this was accompanied by a drop in pH. Gas production was simultaneously suppressed and the relative percentage of methane in the gas was lowered. Selective inhibition of methanogenesis thus occurred which is not surprising as these species are known to be very pH and substrate specific. After additions of tans to the feed sludge was stopped, signs of recovery were evident. This indicates that the effect of wattle tannins on methanogenesis is more inhibitory than toxic. A mass balance on tannins indicated that up to 95% were removed or converted, but the analytical method[129] used was questionable as COD measurements indicated no tannin COD removal. On the whole, quebracho tannins displayed greater inhibition and poorer degradation than wattle although complete colour removal was reported for both tannin effluents. Unfortunately, the question of tannin removal by adsorption to sludge, (see Šiška[71] and Gajdusek et al[93]), was not addressed.

The most recent report (1985) on anaerobic digestion of vegetable tanning waste is by Tunick et al[190] where they looked at laboratory scale anaerobic-aerobic treatment. They used a 29 ℓ anaerobic reactor with solids recycle and polished the effluent aerobically. HRT for the system was 2,5

days. The anaerobic system removed over 60% of the COD and the effluent proved amenable to aerobic polishing which effected a total of 90% COD reduction. No indication was given as to the concentration of tannins in the industrial waste feed nor their removal through the system. Neither was comment made with respect to colour removal.

A summary of anaerobic treatments of waste containing vegetable tannins is given in Table 6. It is interesting to note that the only attempted full scale anaerobic treatment system was by Parker[12] whose lagoon was in fact more facultative/aerobic than anaerobic. All the other investigations have been on laboratory scale. Toyoda et al[174,188] looked at tannin degradation in a synthetic waste but found it difficult to achieve. Eye and Ficker[15] added tannin extract to domestic sewage sludge and found the tannins inhibitory. All other investigations have been on spent industrial effluents which are known to be low in tan content relative to fresh extract and are hence more biodegradable[52] and one would therefore expect better reactor performance. Only Arora et al[16,189] and Ficker et al[15] attempted to determine tannin removals but the analytical methods used were not specific enough to measure tannins only. Hendry[69] has only recently addressed this problem and has developed analytical techniques to fingerprint the large diversity of polyphenol components in tanning wastewaters. No attempt has been made to isolate and characterize any tannin degradation products from anaerobic digesters and, furthermore, reports on colour removal are contradictory. This present work was therefore undertaken in an attempt to define tannin degradation in a controlled artificial waste environment with a well acclimated anaerobic population. A tannin mass balance was undertaken and the final effluent was screened for tannin degradation products.

Table 6. Anaerobic treatment of waste containing vegetable tannins

Researcher	Reference	Treatment System	Scale	Feed	Loading Rate	Tan Content	Efficiency	Tan Removal	Colour Removal
Bailey	89	Filter	Laboratory	Spent vegetable tans	5,7 kg PV/m^3/d	?	33% PV reduction	?	?
Toyoda et al	188, 174	?	Laboratory	Spent vegetable tans	1 kg BOD/m^3/d	?	50-77% COD reduction	?	?
				Artificial	?	?	?	Difficult	?
Gates & Lin	73	Lagoon + anaerobic	Laboratory	Spent vegetable tans	2,4 kg COD/m^3/d	?	60% COD	?	No
Parker	12	Lagoon (Facultative/ aerobic)	Full (3 000 m^3)	Spent vegetable tans	4-35 kg COD/m^3/d	?	70-35% COD	?	No
Aloy	5	?	Laboratory	Spent vegetable tans	?	?	?	?	?
Arora et al	16, 189	Upflow filter	Laboratory	Spent vegetable tans	2-23 kg COD/m^3/d	4,5 g/ℓ	90% COD	90%	Partial
Eye & Ficker	15	Completely mixed	Laboratory (3 ℓ)	Domestic sewage + wattle tans	?	0,2-0,9 g/day	60%-30% COD	95%	Yes
Tunick et al	190	Activated sludge	Laboratory	Spent vegetable tans	?	?	60% COD	?	?

PART I : AMENABILITY OF WATTLE TO ANAEROBIC DEGRADATION

2. EXPERIMENTAL

2.1 Materials

The commercial spray-dried aqueous extract of wattle bark (ex Natal Tanning Extract Company) known as Mimosa ME was used in the feed to the anaerobic digesters. This product is known to contain 66% wattle tannin[3,62] as well as gums and sugars[19]. The high temperature leachate (90°C) is likely to contain some oxidised or decomposed product but since this will also be present in industrial tannery waste it was used as supplied. The anaerobic sewage sludge used for digester start up was obtained from the Grahamstown sewage disposal works. The works operates a completely mixed anaerobic digester with a retention time of up to six weeks. The glucose used in the synthetic feed was industrial grade from Holpro Analytics (Pty) Ltd (Glucose BP Monohydrate). All the other mineral salt and trace element additives to the feed were chemically pure (CP) or analytical (AR) grade.

Water purified by reverse osmosis (Millipore Water System) and analytical (AR) grade reagents were used for all analytical work.

2.2 Analytical Methods

2.2.1 pH and Alkalinity

pH measurements were done on a Digital Data Systems pH meter type 300 with a combined Metrohm electrode type 6.0202.000 and standard Metrohm buffers. Routine alkalinity measurements[129,191] were done by direct titration of digester supernatant with 0,2 N HCl at pH = 6 and pH = 4,3. The results

are expressed as mg/ℓ $CaCO_3$ according to the formula:

$$Alk_{CaCO_3} = \frac{T \times N_{HCl} \times 50 \times 1000}{V} \quad \text{... Equation 3}$$

where Alk_{CaCO_3} = Total $CaCO_3$ alkalinity in mg/ℓ

T = Acid titre in mℓ

N_{HCl} = ± 0,2 N HCl

50 = Equivalents $CaCO_3$ in g/mol

1000 = mg per g

Alternately, the bicarbonate alkalinity[125,126,192] gives a better measure of digester stability:

$$BA = Alk_{CaCO_3} - [VFA].(0,85).(0,833) \quad \text{... Equation 4}$$

where BA = Bicarbonate alkalinity in mg/ℓ

[VFA] = Concentration of volatile fatty acids expressed as acetic acid in mg/ℓ

0,85 = Disasssociation of acetic acid at pH 4,3

0,833 = Molar equivalent ratio of $CaCO_3$:CH_3CO_2H,

i.e. $\frac{100 \text{ g/mol}}{2} \times \frac{1}{60 \text{ g/mol}}$

This formula assumes there is no significant concentration of other materials such as phosphates, sulphides or other acid salts which would also contribute towards alkalinity.

2.2.2 Volatile Fatty Acids (VFA)

2.2.2.1 Chromatographic Separation[128,129,193]

The centrifuged (3 000 rpm for 10 min) supernatant was acidified to pH 1 with conc H_2SO_4. A sample (5 mℓ) was run into a silicic acid column in a sintered glass crucible under suction. The sample was then immediately eluted with 70 mℓ of acidified $CHCl_3$ - butanol (3:1) reagent and titrated with standard 0,02 N NaOH (in MeOH) using phenolphthalein as indicator. Total organic acids are expressed as acetic acid in mg/ℓ.

2.2.2.2 Distillation Method[128,129]

The direct distillation method was used whereby a centrifuged (3 000 rpm for 5 min), diluted (100 mℓ + 100 mℓ H_2O) and acidified sample (HCl) was distilled at a constant rate (± 5 mℓ/min). The distillate (150 mℓ) was purged with N_2 and titrated with standard NaOH (0,01 N) using phenolphthalein as indicator. Total organic acids were expressed as acetic acid in mg/ℓ.

2.2.2.3 Colorimetric Assay[194]

To an aliquot (0,5 mℓ) of the supernatant of a centrifuged sample (10 min at 3000 rpm) was added 1,2-ethanediol (1,5 mℓ) and sulphuric acid (0,2 mℓ, 50%). The sample was then heated on a boiling water bath (3 min) and cooled. Hydroxylamine hydrochloride solution (0,5 mℓ of 100 g/ℓ) and NaOH (2 mℓ of 4,5 N) were then added followed by 10 mℓ of acidic $FeCl_3$ solution (20 g/ℓ). After 10 min, the green-yellow complex was read at 500 nm on a Beckman Model 35 Spectrophotometer against a distilled H_2O control. The VFA concentration, as acetic acid, was read from a standard curve previously prepared with known additions of acetic acid.

2.2.2.4 Gas Chromatography

A Hewlett Packard (HP 5830A) instrument with automatic recording terminal (18850A) was used. It was fitted with a 2 m glass column packed with 10% SP 1000/1% H_3PO_4 on Chromosorb W-AW 100/120[195] (for column preparation and efficiency see Appendix 1). Samples were prepared by centrifugation (5 min at 3000 rpm) and acidification (50% HCl) to pH 1,0 to 1,2. Oven temperature was 150°C with injection and detector temperatures 170°C and 180°C respectively. N_2 was used as carrier gas at a flow rate of 28 mℓ/min. Sample size was 0,6 µℓ and field ionization detection (FID) was used. Individual fatty acids were identified by comparison of relative retention times (R_T) against a cocktail of known standards (0,1% in H_2O). Standards used and retention times are given below:

VFA	Supply House	R_T (min)	Relative R_T
Acetic acid	Merck	3,2	1,0
Propionic acid	Merck	4,5	1,4
i - Butyric	Carlo-Erba	5,0	1,5
n - Butyric	Carlo-Erba	6,3	1,9
i - Valeric	Carlo-Erba	7,5	2,3
n - Valeric	BDH	9,9	3,0
n - Capronic	Carlo-Erba	14,6	4,6

Concentrations of VFA were determined as follows:

$$\frac{A_{sample} \times V_{sample} \times SG \times 1000}{A_{standard} \times V_{standard}} = [VFA]\ mg/\ell \quad \text{... Equation 5}$$

where A = area of sample or standard

V = volume of sample or standard

SG = specific gravity of relevant VFA

Total VFA expressed as acetic acid were calculated as follows:

$$\sum_{i=1}^{n} \left[[VFA_i] \times \frac{MW_{acetic\ acid}}{MW_i} \right] \quad (mg/\ell) \quad \text{... Equation 6}$$

where i refers to each VFA detected in sample.

2.2.3 Solids[196]

Total solids (TS) were determined by evaporating an aliquot in a pre-weighed crucible to dryness on a hot water bath followed by 2 hours at 104°C in a drying oven and reweighing. Volatile solids (VS) were determined by ashing the sample in a furnace at 800°C overnight. Dissolved solids (DS) were determined by centrifuging a sample (5 min at 3 000 rpm) and analysing the supernatant. Suspended solids (SS) were determined by resuspending and drying the centrifugate.

2.2.4 Oxygen Demand

2.2.4.1 Permanganate Value (PV)[196]

The samples and a blank were acidified with sulphuric acid (10 mℓ of 25% v/v) and added to 20 mℓ of standard potassium permanganate (0,0125 N). The whole was then incubated at 27°C for 4 hours in a constant-temperature water bath. Potassium iodide (0,5 g) was then added and the excess oxidant titrated with standardized sodium thiosulphate (0,0125 N) using starch indicator. Calculation of PV is as follows:

$$PV\ (mg/\ell) = \frac{(Tsample - Tblank) \times N \times 8000}{Vsample} \quad \text{... Equation 7}$$

where, T = Titre of sample or blank in mℓ

N = Normality of sodium thiosulphate in molar equivalents/ℓ

V = Sample volume in mℓ

2.2.4.2 Carbonaceous Oxygen Demand (COD)[196-198]

To the samples and a blank were added mercuric sulphate (0,2 g) and a digestion mixture (40 mℓ). This latter mixture contained standard potassium dichromate (0,250 N), sulphuric acid (conc) and silver sulphate (10 mℓ:30 mℓ:0,3 g respectively). The whole was refluxed for 2 hours, cooled and diluted with distilled water. The excess oxidant was then titrated with standard ferrous ammonium sulphate (0,250 N) using ferroin indicator. Calculation of COD is as follows:

$$COD\ (mg/\ell) = \frac{(Tsample - Tblank) \times N \times 8000}{Vsample} \quad \text{... Equation 8}$$

where, T = Titre of sample or blank in mℓ

N = Normality of ferrous ammonium sulphate in molar equiv/ℓ

V = Sample volume in mℓ

2.2.5 Biogas Analyses

A Hewlett Packard instrument (see Section 2.2.2.4) was fitted with a 2 m glass column which was packed with Porapak Type N packing material, (for column preparation and efficiency see Appendix 1). Oven temperature was

60°C with injection and detector ports both at 80°C. Helium was used as carrier gas at a flow rate of 22 mℓ/min. Sample size was 0,2 mℓ and thermal conductivity detection (TCD) was used. Only methane and carbon dioxide gases were measured with an air peak being present as a contamination. Retention times are given below:

Gas	Supplier	R_T (min)	Relative R_T
Air	-	0,5	0,7
CH_4	Afrox	0,7	1,0
CO_2	Afrox	1,8	2,6

On the assumption that biogas consists entirely of CH_4 and CO_2, the relative percentages of each gas were calculated as follows:

$$\frac{(A_{CH_4} \times 1{,}17) \times 100}{(A_{CH_4} \times 1{,}17) + A_{CO_2}} = \%\ CH_4 \qquad \text{... Equation 9}$$

$$\text{and } \%\ CO_2 = 100 - \%\ CH_4 \qquad \text{... Equation 10}$$

where, A = integrated areas of CO_2 and CH_4 peaks

$$1{,}17 = \frac{1 - RTC\ (CO_2)}{1 - RTC\ (CH_4)}$$

$$RTC(CO_2) = {}^{*}\frac{\text{Thermal Conductivity } CO_2 \text{ @ 80°C}}{\text{" " } He \text{ @ 80°C}}$$

$$= \frac{49{,}5\ cal/s.cm^2.(°C/cm) \times 10^6}{398\ cal/s.cm^2.(°C/cm) \times 10^6} = 0{,}124$$

$$RTC(CH_4) = {}^{*}\frac{\text{Thermal Conductivity } CH_4 \text{ @ 80°C}}{\text{" " } He \text{ @ 80°C}}$$

$$= \frac{101\ cal/s.cm^2.(°C/cm) \times 10^6}{398\ cal/s.cm^2.(°C/cm) \times 10^6} = 0{,}254$$

* Conductivities obtained by interpolation of published values[199]

2.2.6 Tannin Assay

2.2.6.1 Direct UV Method[91]

Diluted samples (1:10 with water) of centrifuged (3 000 rpm for 5 minutes) digest were read at 280 nm on a Beckman Model 35 double beam spectrophotometer against appropriate reference solutions. Concentrations of wattle tannins were interpolated from standard curves made up by adding wattle powder of known tan concentration to samples of centrifuged digest from the control digester.

2.2.6.2 Molybdate Ion Complex[200]

A 10 mℓ centrifuged sample was diluted to 20 mℓ with acetate buffer (sodium acetate 0,5 M and acetic acid 0,5 M in a 1:1 ratio) at pH 4,6. A second sample was diluted to 20 mℓ with acetate buffer which included 0,02 M sodium molybdate. The yellow-orange complex formed by wattle complexed to molybdenum ions was read after 2 hours at 450 nm against the first sample as reference. Wattle concentrations were read off directly from a standardized curve made up with standard additions of wattle powder extract of known tan concentration.

2.2.6.3 Folin-Ciocalteu (F-C) Assay[62,201,202]

Water (75 mℓ) was added to the sample (5 mℓ) in a 100 mℓ volumetric flask. F-C reagent (5 mℓ) was then added followed by sodium bicarbonate (15 mℓ of a 15% m/v solution) after 2 minutes. The volume in the flask was then made up to 100 mℓ and absorbance was read after 2 h at 765 nm on a Beckman Model 35 double beam spectrophotometer against an appropriate reference. Tannin concentrations were interpolated from a standard analytical calibration curve.

2.2.7 Inorganic Compounds

Analyses of nitrogen were done by standard Kjeldahl methods[129,203]. Phosphorous was determined spectrophotometrically as the yellow vanadomolybdophosphoric acid[129] and is reported as P_2O_5. Chlorides and

sulphates were determined gravimetrically as the silver chloride and barium sulphate respectively[129,203]. A varian atomic absorption spectrophotometer Series 1275 was used for determining concentrations of K, Na, Ca, Mg, Fe, Cu and Al.

2.3 Anaerobic Digestion Systems

2.3.1 "Pickle Jar" Digester

A 3 ℓ batch fed "pickle jar" digester was set up as shown in Figure 8. It was initially filled with partially digested anaerobic sewage sludge diluted with 25% tap water. The sludge was dark brown, almost black in colour, and had a fairly strong odour. Parameters for the diluted sludge oxygen demand, solids content and pH were: PV = 470 mg/ℓ, COD = 5 330 mg/ℓ, SS = 6 610 mg/ℓ, TDS = 1 500 mg/ℓ and pH = 6,7. The system was purged with inert nitrogen and sealed. It was allowed to stabilize for one week before feeding commenced. Raw sewage was sieved and diluted 50% before it was added in 2 x 100 mℓ batches daily except weekends when it was not fed. One litre of settled sludge supernatant was wasted weekly from the digester by displacement with nitrogen. Complete mixing was effected by a Gallenkamp magnetic stirrer which was activated for 5 minutes every hour by an Electromatic S185 timer switch. Gas was collected in an inverted cyclinder by displacement of an acidic saline solution (5% HCl (v/v) and 10% NaCl (m/v)). Digest was collected periodically by displacement of the liquor with nitrogen gas. Monitoring of digester performance included periodic measurements of gas volume and composition, pH, VFA, oxygen demand and solids.

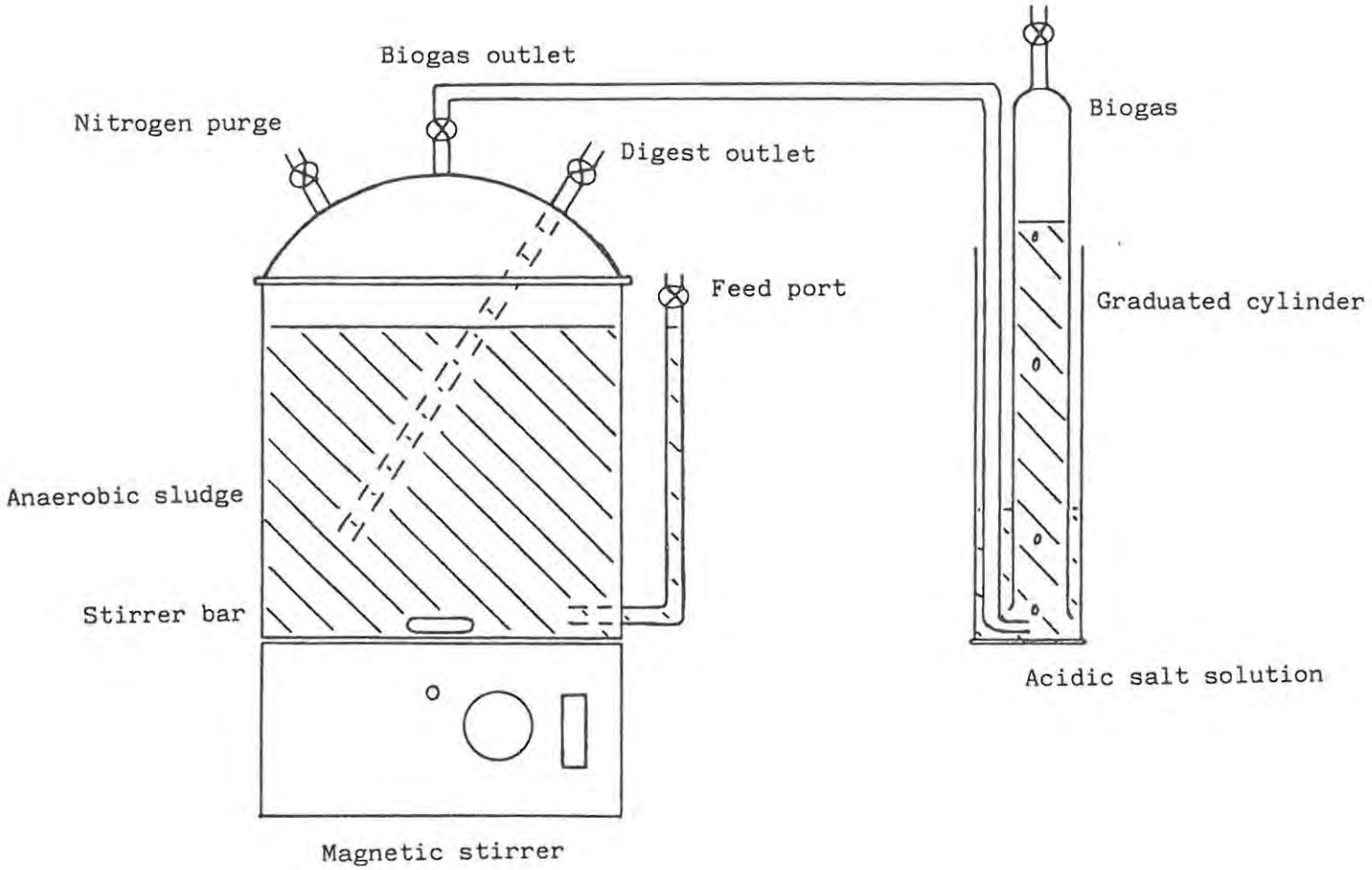


Figure 8. Diagram of anaerobic "pickle jar" digester

2.3.2 Test Tube Amenability Study

In each test, small test tubes (5 mℓ) were filled anaerobically with 3 mℓ of sewage digest innoculum from the "pickle jar" digester. A further 1 mℓ of varying concentrations of glucose feed and wattle extract were then added. The tubes were sealed with Suba-seal septa and purged with nitrogen gas. They were fitted with a hypodermic needle attached to a length of plastic tubing which in turn was filled with water to act as a simple manometric device for measuring gas production. They were incubated at 35°C in a B and T constant temperature waterbath for up to 3 days.

2.3.3 Upflow Anaerobic Sludge Blanket Digesters (UASB)

Diagrammatic representation of one of the UASB digesters used is given in Figure 9. The perspex cylinder had an id of 14,2 cm, height of 60 cm and effective volume of 9 ℓ. The feed inlet was 2,7 cm from the base and digest outlet was 6,5 cm from the top. Sampling points were located at 15,5 cm, 28,5 cm and 41,5 cm from the base. The gas-solids separator consisted of a circular ring of triangular cross-section positioned below a dual inverted funnel. The temperature inside the digester was maintained at 35 $\pm$ ½°C by pumping water continuously from a constant temperature water bath through a heating coil. The digester was wrapped in tin foil to assist in thermoregulation and this also excluded light which prevented the growth of photosynthetic flora. Biogas was vented at the top of the digester and fed through a sampling U-tube into an aqueous displacement measuring system. Biogas bubbles displaced water from an aspirator bottle into a graduated beaker and the gas volume was inferred directly from the volume of water displaced. A Watson-Marlow 501 peristaltic pump was used to feed the digesters through silica rubber tubing of 1,6 mm id. The stock feed was stirred with a T + C 010 magnetic stirrer during feed additions. The bottom of the sludge bed was also lightly stirred during feed additions to effect good feed distribution at the base of the digester. A Janke and Kunkel Ika-Werk stirrer was used with the stirrer shaft protruding through a water tight double seal. Feed additions were made for 60 seconds every 11 minutes (i.e. 5 min/hour) by an Electromatic SC 185 timer system which also controlled the stirring. The peristaltic pump feeding rate was

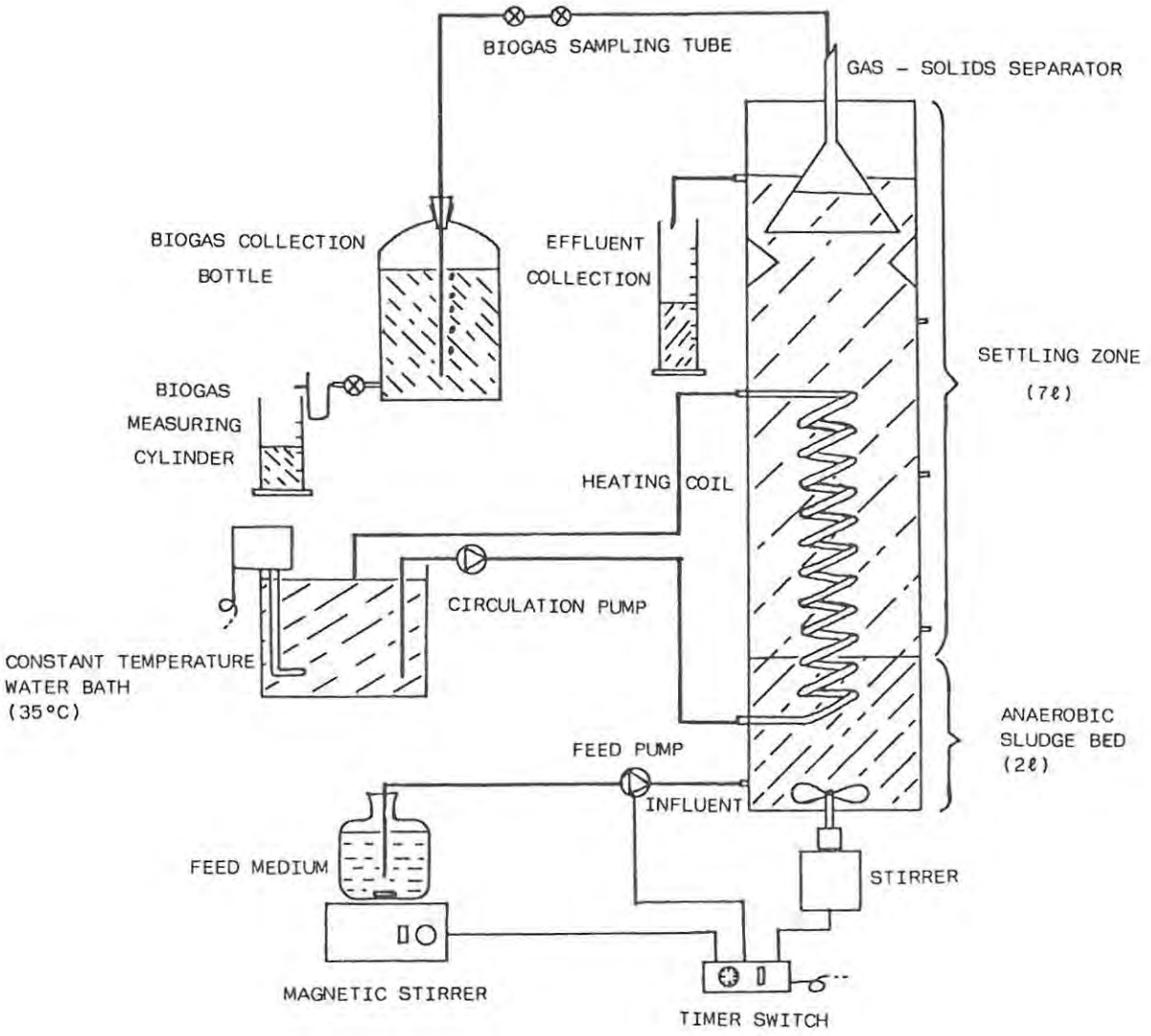


Figure 9. Schematic layout of the upflow anaerobic sludge blanket digester

adjusted to feed 1 ℓ in approximately 24 hours.

In the experimental run, two identical digesters were set up with the one being used as a control. Innoculum for start up was obtained from the Grahamstown Municipal Sewage Works and was supplemented with sludge from the "pickle jar" digester. Table 7 gives the phases of digester functioning over the test period. Both digesters were initially fed diluted sewage sludge until a 2 ℓ sludge bed was built up in the bottom of the digesters. The height of the sludge bed was approximately 13 cm and the rest of the digester volume (± 47 cm) therefore acted as the settling zone. Initial problems were encountered in stabilizing the digesters, but they were finally acclimated to a standard synthetic feed mix during phase 4. The characteristics and composition of this feed is given in Table 8. (The feed was prepared weekly and autoclaved before use). Wattle additions to the test digester increased through phase 6 and it was then allowed to stabilize at additions of 2 g/ℓ/d for a period of 2 weeks. The alternate carbon source (glucose) was then slowly removed from the test digester during phase 8. Only wattle was fed during phase 9 but this was stopped after 28 days. Diluted glucose feed was used during phase 10 after which both digesters were shutdown. During phase 12, both digesters were restarted stepwise on synthetic feed medium over a period of 5 days whereafter they were run for a further 3 weeks at full strength feed.

Table 7. Phases of functioning - anaerobic test digester

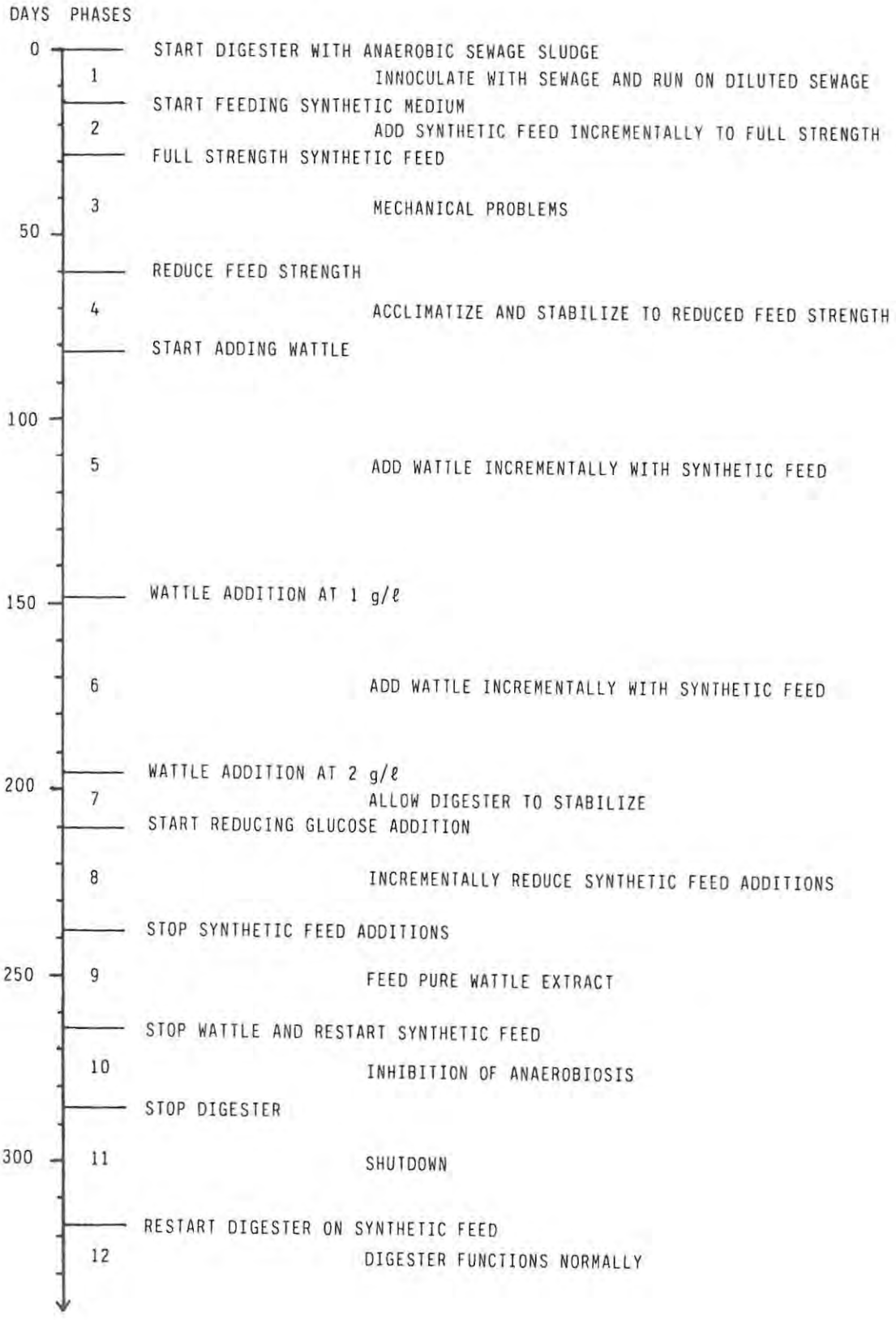


Table 8. Composition and characteristics of synthetic feed medium

Item	Concentration (mg/ℓ)
Glucose	4 125
Urea	3 750
K_2HPO_4	3 750
Yeast Extract	750
$MgSO_4.7\ H_2O$	750
$FeCl_3.6\ H_2O$	3
$MnSO_4.4\ H_2O$	1
$Zn.SO_4.7\ H_2O$	0,5
$CaCl_2.2\ H_2O$	100
$CoCl_2.6\ H_2O$	0,5
$Na_2MoO_4.2\ H_2O$	0,2
NaCl	100
$CuSO_4.5\ H_2O$	0,5
$NiSO_4.6\ H_2O$	0,5
Total	13 331 mg/ℓ

COD = 5 000 mg O_2/ℓ

PV = 650 mg O_2/ℓ

(Wattle: COD = 1 320 mgO_2/g Wattle)

(PV = 720 mgO_2/g Wattle)

3. RESULTS AND DISCUSSION

Investigation into the amenability of wattle to anaerobic digestion took place in 3 parts. Initially, a 3 ℓ "pickle jar" digester was set up to acquaint the author with anaerobic systems and to provide a stock of anaerobic/facultative organisms for other experiments. This digester was used to refine the analytical techniques necessary for monitoring and control of anaerobic systems. Aliquots of stabilized sludge were then used in the second part for small scale batch tests to which various wattle additions were made. The final part involved an acclimatization study where wattle was added to a UASB digester running on a synthetic feed medium.

3.1 Development of Analytical Methods

Different analytical methods were available for determining many of the parameters which required monitoring in this study. Where applicable, the different methods were screened and the most appropriate one was selected and adjusted to supply the data required.

3.1.1 Alkalinity and pH

Total alkalinity represents a samples quantitative capacity to react with a strong acid[129]. Since anaerobic systems have a narrow range of optimal pH function, a high alkalinity measure is desirable. The easiest way to measure alkalinity is by direct titration of a suitable aliquot to a predetermined pH. As mentioned in section 1.5.2.2 it is bicarbonate alkalinity which is of major interest in anaerobic systems as other forms of buffering capacity are negligible at physiological pH's. To a first approximation, total alkalinity is made up of the bicarbonate alkalinity necessary for CO_2 equilibrium and the alkalinity necessary for VFA neutralization[192] as shown below:

$$CO_2\ (g)\uparrow + H_2O \rightleftharpoons H_2CO_3 \quad \text{... Equation 11}$$

$$HCO_3^- + HAc \rightleftharpoons H_2O + CO_2\ (g)\uparrow + Ac^- \quad \text{... Equation 12}$$

In a titration to pH 4,3 approximately 85% of VFA's are dissociated which allows equation 4 (section 2.2.1) to be derived. This gives an approximate bicarbonate alkalinity, corrected for the concentration of VFA present, and is a sensitive measure of digester stability. The level of alkalinity is furthermore very dependant on the type of waste being treated and the digesters equilibrium pH[126,192].

Figure 10 gives an example of a typical titration of an anaerobic sample with 0,2 N HCl. Total alkalinity was determined using Equation 3.

A method for determining bicarbonate alkalinity directly is given by Rozzi and Labellarte[125]. In principle the method involves the saturation of a given sample with CO_2 gas followed by acidification to pH 4. The volume of gas produced is used to determine the bicarbonate alkalinity taking the non-ideal behaviour of gases into account. There is little interference from organic or inorganic compounds commonly found in anaerobic liquors. In this study, an accurate measure of absolute bicarbonate alkalinity was not deemed necessary as the titration method gives reproducible results and will indicate changes in the alkalinity equilibrium which is all that is necessary for digester control.

pH measurement is a relatively insensitive but easy method for digester monitoring and so was done continuously. pH control on the other hand is necessary only when the digester is subjected to shock loadings or other stresses resulting in an alkalinity or pH drop. Li and Sutton[192] reviewed 7 of the chemicals commonly used for pH control and discussed their advantages and disadvantages. In this work, sodium bicarbonate was used as it is highly soluble and will not raise the pH above $\pm$ 8,5. It also does not react with CO_2 in the digester, (cf NaOH), and the cost of the chemical is not prohibitive on laboratory scale[126].

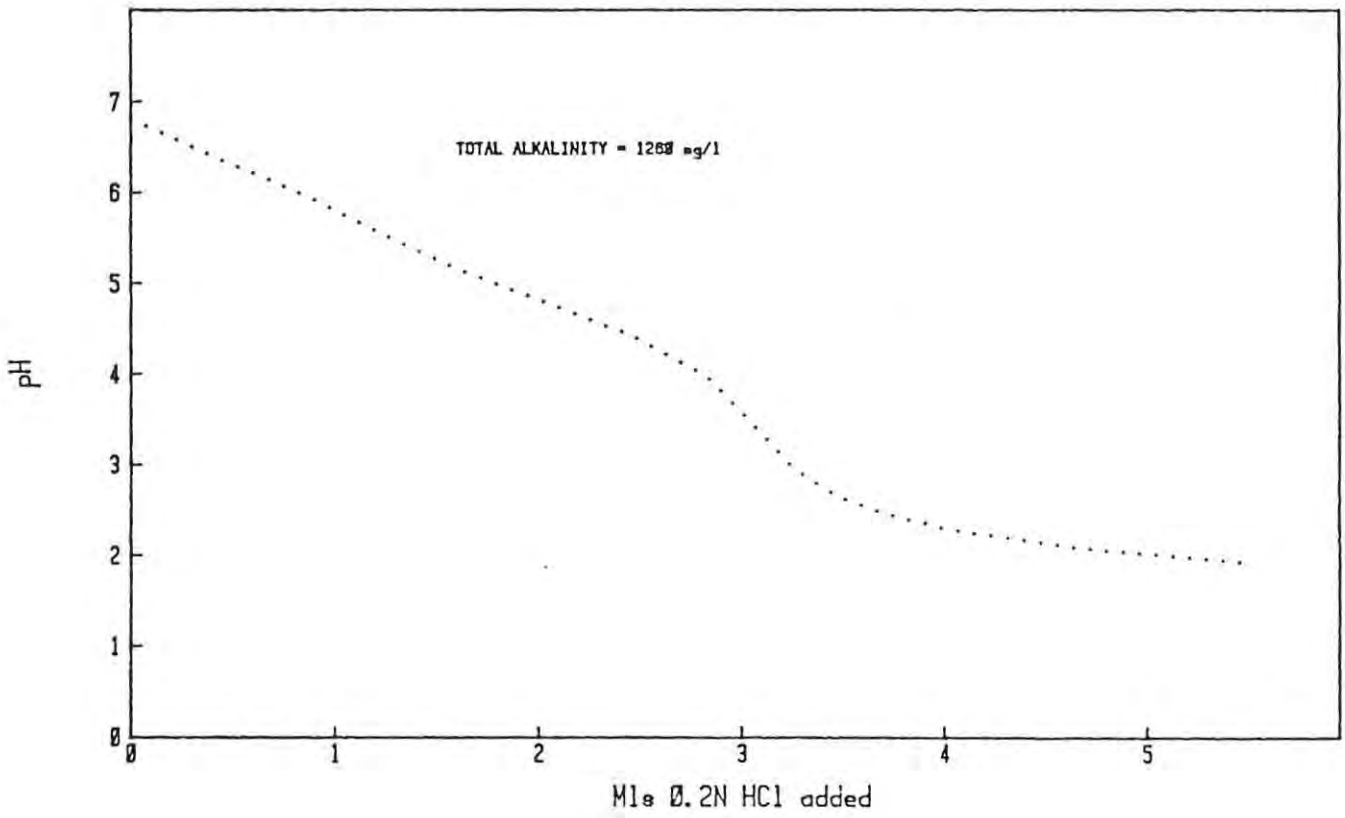


Figure 10. Titration of anaerobic digest (20 mℓ) with standard acid

3.1.2 VFA

Measurement of total VFA concentration and acetic acid concentrations in particular give an indirect measure of the level of activity of the methanogenic population present while concentrations of higher VFA allows speculation on the biodegradation pathways operating.

Three methods were compared for measuring total VFA concentrations and averaged results are given in Table 9. The gas chromatographic method was taken as the control.

The chromatographic method using silicic acid is fairly simple to perform and proved reproducible for a given sample. Organic acids other than volatile acids interfere however[129] giving rise to inflated results. The direct distillation method proved less repeatable and gave results that indicated only ± 70% of the VFA appeared in the distillate. Volatile fatty acids form a green-yellow complex with hydroxyl ammonium chloride solution in the presence of ferric ions and this was the basis of the spectrophotometric method. It required the preparation of a suitable range of standards from which VFA concentrations of prepared samples were read off directly. A standard curve was obtained having a linear correlation coefficient of $r = 0,9997$ but the VFA concentrations measured by this method were high. It was assumed that other components in the digest or feed interfered.

Many references to the use of GC for the direct separation, identification and quantification of VFA are found in the literature[204-209]. Initially, in this study, samples were centrifuged, acidified and extracted into ether before being injected onto the column. Better repeatability was however achieved by direct injection of a centrifuged and acidified aqueous sample. A typical spectrum of such an anaerobic sample is given in Figure 11. The acids eluted fairly rapidly without overlapping of adjacent peaks at the operating parameters used. The phenomenon of ghosting[206], which refers to the release of temporarily bound VFA upon injection of a succeeding

Table 9. Total VFA Concentrations Expressed as Acetic Acid

Sample	Analytical Method			
	Gas Chromatography	Chromatographic Separation	Direct Distillation	Colourimetric Assay
Digest	64 mg/ℓ	70 mg/ℓ	46 mg/ℓ	85 mg/ℓ
Feed	908 "	-	-	969 "

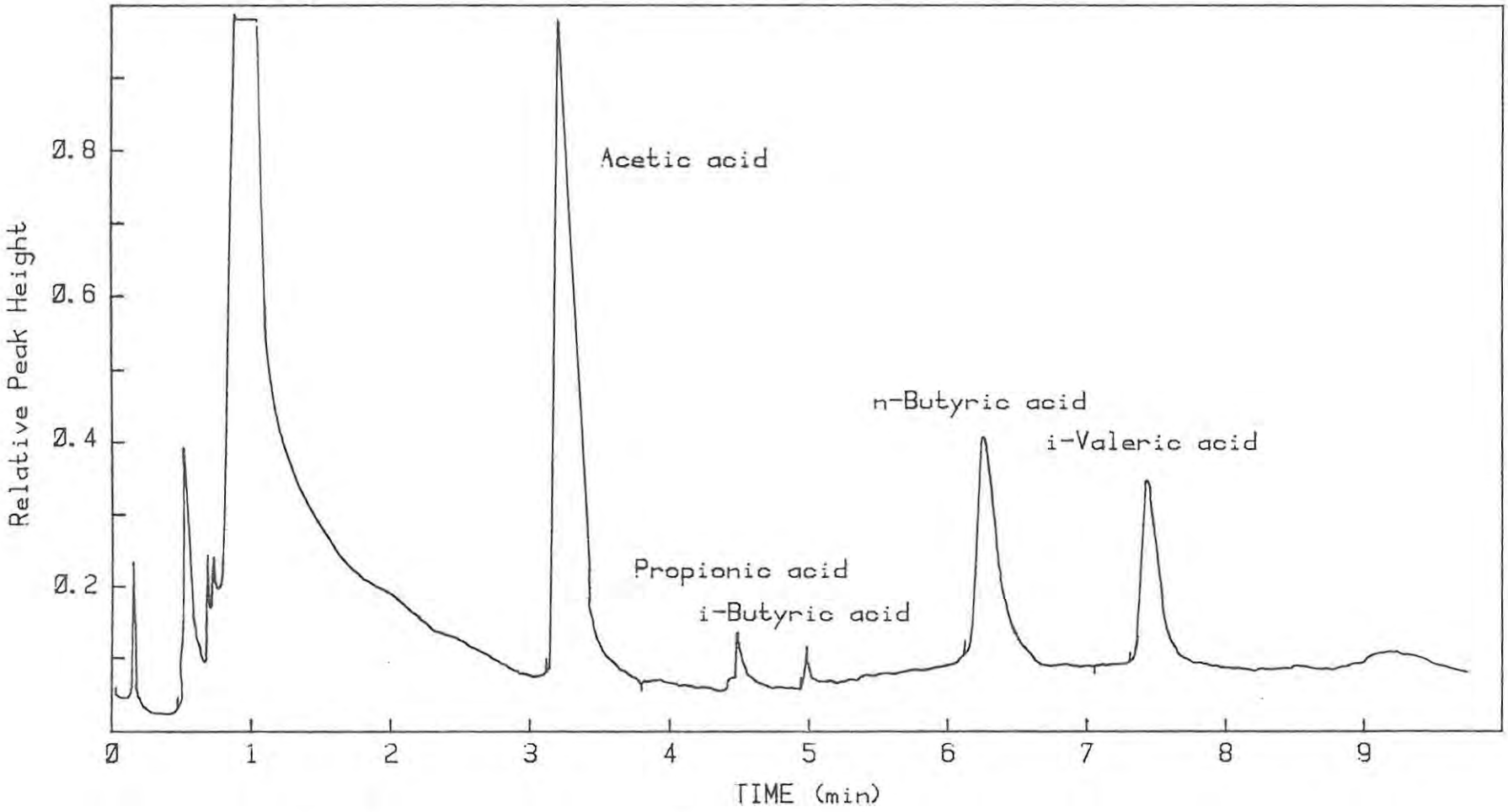


Figure 11. Typical spectrum of VFA from the anearobic digester

sample, was overcome by washing the column peridocially with 2 μℓ of 20% aqueous formic acid. The column was used for 2 years and efficiency decreased from 1 730 plates/m to 1 590 plates/m which is not considerable.

3.1.3 Solids

The standard method[196] for solids was used. Repeatability on a given sample was reasonable taking into account the non-homogenous nature of the anaerobic supernatant.

3.1.4 Oxygen Demand

COD and PV methods are both measures of the pollution load of effluents. Their results indicate the oxygen equivalence of the sample which is oxidised by a stronger and weaker oxidising agent respectively. In the COD method, boiling chromic and sulphuric acids, with silver as a catalyst, quantitatively reduces most organic compounds, with straight-chain aliphatics, (e.g. acetic acid), benzene and pyridine being notable exceptions[102]. Inorganic halides and reduced species such as sulphides are known to interfere[129] but the halide intereference may be removed by complexing with mercury[198]. The milder acidic potassium permanganate oxidation partially oxidises most organics, including phenolics, but it does not oxidise glucose.

Averaged COD and PV values for commercial wattle extract were determined at 1,32 mg O_2/mg wattle extract and 0,72 mg O_2/mg respectively. This is in reasonable agreement with previously reported values of 1,43 and 0,73 mg O_2/mg respectively from Hendry[62]. These values furthermore give a PV:COD ratio of 0,54, (0,51 - Hendry[62]), for the extract which is very much higher than reported values for a typical domestic sewage, (0,10), and mixed industrial waste, (0,13). The reason for this may be found in the composition of the wattle extract which consists of up to 70% polyphenols which are readily degraded by acidic permanganate[62]. The balance of the extract is made up of mainly sugars and gums which are only sparingly oxidised by permanganate. Hendry[62] in fact derived a PV:COD ratio of 0,08 for the non-phenolic constituents of the extract. The PV

method therefore presents itself as a fairly specific measure of polyphenolic removal from an effluent containing a large amount of sugars while the COD method will measure the total reduction in oxygen demand of both the sugars and phenolics.

3.1.5 Biogas

The volume and composition of biogas produced in an anaerobic system is a sensitive measure of microbial activity. Biogas is comprised mainly of methane and carbon dioxide and the relative percentages are dependant on the type of waste being degraded and can theoretically vary from 50% to 75% CH_4 by volume[121,212,213]. The maximum theoretical methane yield may be calculated from COD as follows[121]:

$$CH_4 + 4O \longrightarrow CO_2 + 2H_2O \qquad \text{... Equation 13}$$

therefore 64 g O $\equiv$ 1 mole CH_4 which occupies 22,4 ℓ (@ STP)

So, 1 m^3 CH_4 = 2,86 kg COD)
or 1 kg COD = 0,35 m^3 CH_4) ... Equation 14

Alternately, the total biogas yield can be calculated from the known molecular formula. Take a glucose feed for example:

$$C_6H_{12}O_6 \longrightarrow 6 \text{ moles gas } (CH_4 \text{ and } CO_2) \qquad \text{... Equation 15}$$

If we take x grams of $C_6H_{12}O_6$ we get, ... (where MW glucose = 180 g/mol)

$$\frac{x}{180} \times (6 \times 22,4)\ \ell \text{ of biogas} \qquad \text{... Equation 16}$$

The closer the actual gas yields approach theoretical yields, the greater the anaerobic biodegradability of the waste. In practise, higher percentages of CH_4 are often reported as CO_2 dissolves in the effluent. The solubility of CO_2 is a function of dilution and pH[121,213]. High CO_2 partial pressures are however known to inhibit especially acetate and propionate degradation while PCH_4 has no effect[214].

Other compounds present in trace quantities have also been reported[213,215]. Hydrogen sulfide for instance is indicative of methanogenic suppression[13] as discussed in section 1.5.2.3. Nitrogen arises from the breakdown of nitrogenous compounds such as protein. The presence of carbon monoxide indicates an incomplete stage of degradation for organisms such as Methanobacterium formicicum which utilize CO as substrate[213]. Volatile organics and water vapour escape with the biogas as a function of their relative vapour pressures. The presence of hydrogen is of interest as the partial pressure of H_2 is important in the feedback control of the acetogenic bacteria[108,115,159].

The total volume of the gas is of course related to the general activity of the anaerobes and as such, all the factors which affect anaerobic viability are reflected in the total biogas production. The gas has a net calorific value of 17 - 28 MJ/m^3 and much research has been done on the generation of power from it[213,215].

The Gas Chromatographic method for identification and quantitation of biogas components is widely used[120,129,144,148,156,158,165,207-209]. Accurate analysis of the biogas in this study showed that it consisted of $>$ 95% CH_4 and CO_2. For convenience therefore, it was assumed that the biogas consisted entirely of CH_4 and CO_2. The relative percentages of each gas were then calculated from peak areas after correcting for differences in their thermal conductivities relative to He as shown in section 2.2.5. The validity of this method was checked against standard samples of CH_4 and CO_2 with good correlation. A typical analysis is given in Figure 12. The air impurity which occurs during injection of the sample was kept below 20% as higher levels resulted in a noticable increase in the relative CO_2 concentration. Reproducibility of results was usually within $\pm$ 0,5%.

3.1.6 Tannin Analysis

The official "Society of Leather Trades Chemists" method of analysis[216] measures tannins indirectly by subtracting non-tannins from total solubles after mixing with hide powder. This method is laborious, not highly repeatable[217] and does not measure very small quantities of tan accurately. As a result it was decided to look at the three other methods

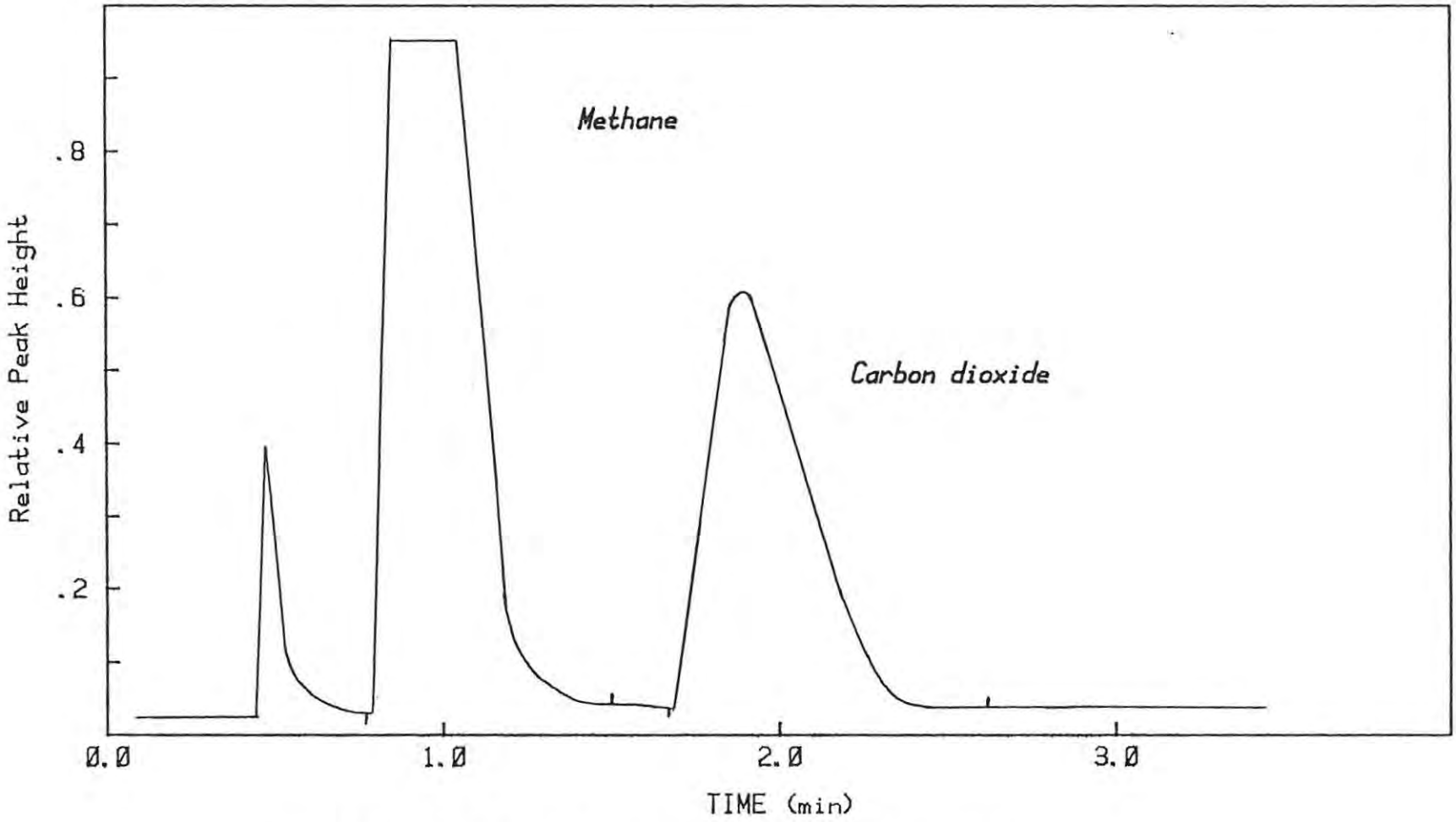


Figure 12. Typical spectrum of biogas from the anaerobic digester

which are described in section 2.2.6.

Wattle phenolics have a 280 nm chromophore which forms the basis of the UV method[91]. Although non-tan phenolics also absorb at this wavelength, the removal of all phenolics from the synthetic wastewater was of interest as they all contribute to the pollution load. Deviations from the Beer-Lambert law were noticed in undiluted standard samples and this was attributed to cloudiness in the solution. A 1:10 dilution of samples gave an approximate straight line with a correlation coefficient of r = 0,986.

Molybdate ions form a 5-membered chelate with O-dihydroxy groups such as that found in the B-ring of wattle phenolics[200]. The yellow-orange colour formed is attribed to a charge transfer process from ligand to metal and its intensity is pH dependant[200]. The complex takes approximately 2 hours to form, but once formed is reasonably stable, (see Figure 13). Standard curves showed correlation coefficients of better than 0,998 over the concentration range 0 to 1 g/ℓ wattle.

The Folin-Ciocalteu (F-C) method is subject to interference from reducing agents but is very sensitive to phenolics. The constituents of wattle extract other than phenolics are reported not to interfere[62]. The method relies on the partial reduction of molybdo- and tungsto- phosphoric complexes by phenolate ions and its application to aqueous tannin solutions is fully discussed elsewhere[62,201,202]. Standard solutions were seen to obey the Beer law at concentrations less than 1 g/ℓ (correlation coefficient $>$ 0,999).

A comparison of these three methods showed the F-C method to be the most sensitive (see Table 10).

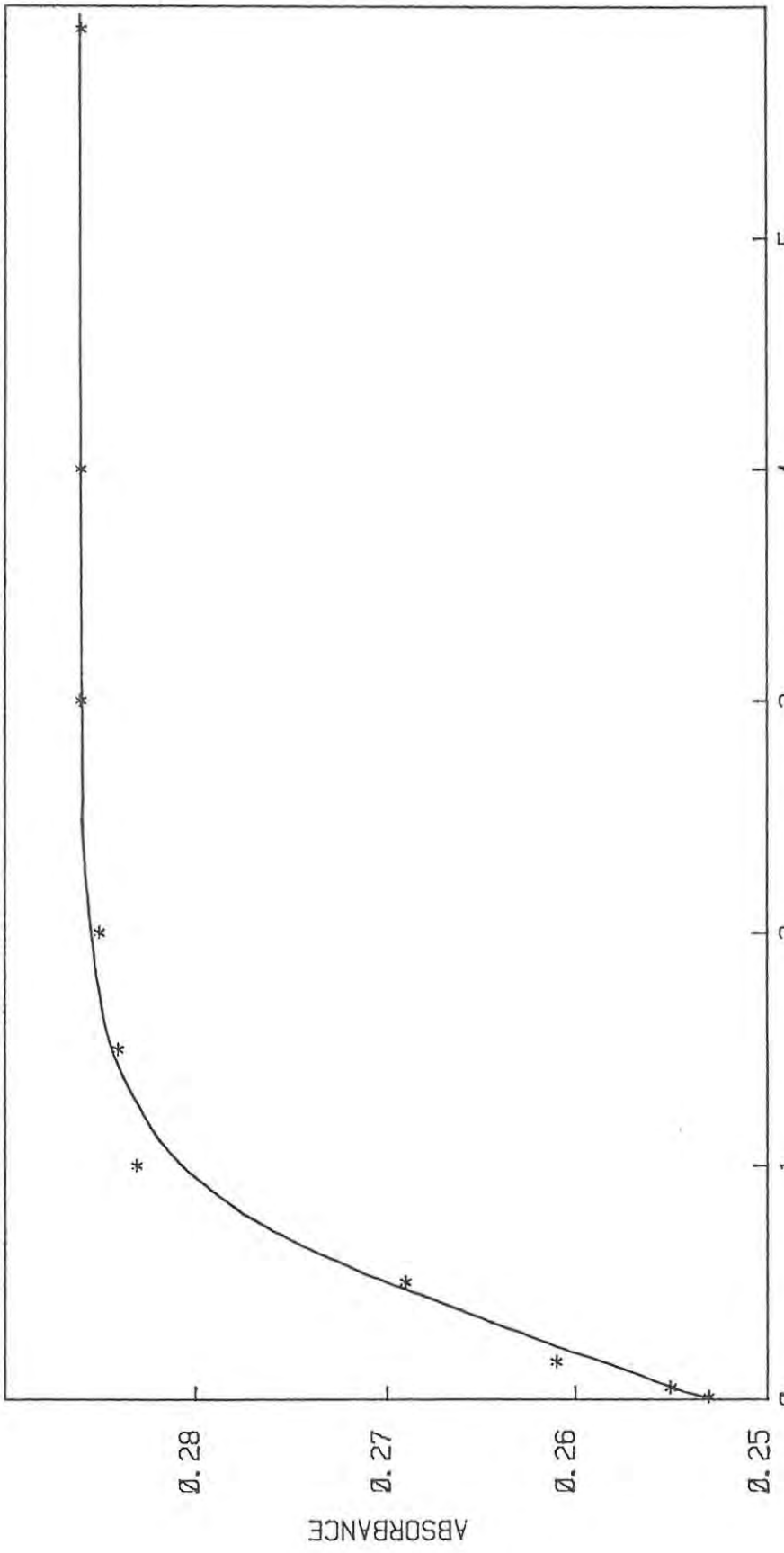


Figure 13. Change in absorbance of Mo complex with time

Table 10. Comparative sensitivities of different tannin analyses

Method	Wattle Addition[a]	Absorbance[b]	Absorbance Ratio
UV	0,5 g/ℓ	4,1	1,0
Mo	0,5 g/ℓ	1,1	0,2
F-C	0,5 g/ℓ	10,4	2,5

(a) Wattle addition of known tan concentration to control digester effluent.

(b) Absorbance corrected for dilution ratios.

Hendry[62] found the UV method to overestimate the oxidised phenolics formed by treatment with an aerobic activated sludge system but attributed this in part to other organic matter absorbing at 280 nm. In this work, the effluent from the control digester was used as blank and so this latter interference was invalidated. Hendry also found the F-C method to underestimate oxidised phenolics. Results from these analytical methods on effluents from anaerobically degraded tannins are discussed further in section 3.4.

3.2 "Pickle Jar" Digester

As this digester was only intended to provide innoculum for other tests and to refine analytical procedures, strict control over feed strengths and monitoring parameters was not kept. Figure 14 shows some of the parameters monitored and indicates trends of the digester's performance.

Biogas production was initially very erratic and throughout the monitoring period ranged widely from the average of approximately 200 mℓ per day. Reasons for this variation may be found in a number of contributing factors. The digester was unheated and subject to both diurnal and annual ambient temperature fluctuation. The feed was not homogenous and feed strength was seen to range widely as measured by both PV and COD methods. Solids concentrations in the feed also showed wide variation (see Table 12). Biogas production stopped from the 27th day till the 37th day.

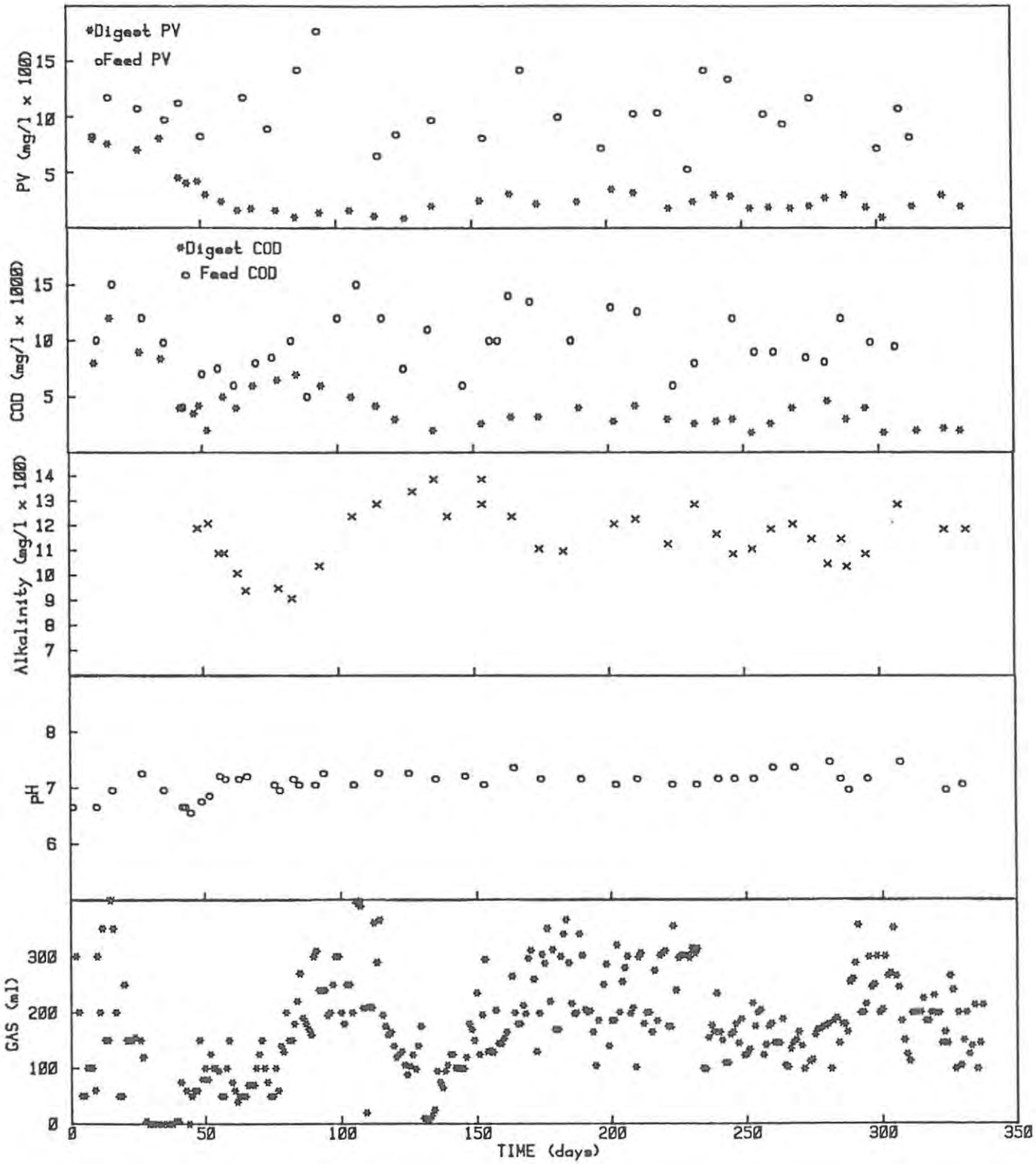


Figure 14. Performance characteristics of Pickle Jar digester

Reasons for this stoppage are not known but it is possible that the accidental introduction of a biocidal detergent took place. Souring of the digester was discounted as pH remained reasonably stable. Alkalinity measurements were unfortunately only started after the digester resumed functioning. The problem was not investigated in depth as reinnoculation of the digester with fresh, digested sewage sludge resulted in renewed biogas production. A second stoppage in biogas production occurred from day 130 to 132 but this was due to the digester not being stirred or fed for 5 days. The digester resumed gassing immediately feeding restarted. Measurements of biogas composition were fairly consistent around 80 $\pm$ 3% methane and 20 $\pm$ 3% CO_2.

pH and alkalinity measurements were seen to be fairly stable over the test period. pH averaged 7,2 after the 50th day. Alkalinity averaged 1 170 mg/ℓ. VFA concentrations were only measured insofar as different analytical methods were tested and was not performed routinely. It is interesting however to compare the feed and digest concentrations as shown in Table 11. The samples analysed by both the distillation and the GC method give values which are of the same order of magnitude with feed and digest averaging 800 mg/ℓ and 27 mg/ℓ respectively (expressed as acetic acid). This represents an approximate 97% reduction in VFA. The major acid present in both the feed and digest was acetic acid with the majority of acids in the feed being completely degraded.

The variation of feed strength to the digester as measured by PV and COD measurements reflected the non-homogeneity of the raw sewage feed. Initially, the residual digest oxygen demands were high but were seen to decrease and stabilize after approximately 100 days of operation. Thereafter, the values were relatively constant with COD averaging 3 260 mg/ℓ and PV averaging 240 mg/ℓ. Feed oxygen demands averaged 9 800 mg/ℓ for COD and 1 040 mg/ℓ for PV. Removal efficiencies were therefore of the order 67% and 77% for COD and PV respectively. The higher removal efficiency for the permanganate oxidation substantiates the assumption that PV reflects the more readily biodegradable portion of the feed.

Solids removal efficiencies are given in Table 12. The total solids had a

Table 11. Pickle Jar VFA and alkalinity measurments (in mg/ℓ)

No.	Sample	Alkalinity	[VFA] as acetic	[Acetic]	[Propionic]	[n-Butyric]	[i-Butyric]	[m-Valeric]	[i-Valeric]	[n-Capioic]	Bicarbonate Alkalinity	Comments
1	Feed	–	680	–	–	–	–	–	–	–	–	Distillation Method
2	Digest	1 480	28	–	–	–	–	–	–	–	1 460	" "
3	"	1 300	24	–	–	–	–	–	–	–	1 283	" "
4	Feed	–	919	431	392	147	26	41	47	0	–	GC Method
5	Digest	941	26	19	9	0	0	0	0	0	923	" "
6	"	1 137	43	32	8	0	0	0	7	0	1 107	" "
7	"	1 254	21	7	0	5	0	0	19	0	1 239	" "
8	"	963	9	8	0	0	0	2	0	0	957	" "
9	"	1 009	47	47	0	0	0	0	0	0	976	" "
10	"	1 015	14	13	0	2	0	0	0	0	1 005	" "

Note: [VFA] Feed $\tilde{x}$ = 800 mg/ℓ, s = 169
Digest $\tilde{x}$ = 27 mg/ℓ, s = 13

Table 12. Pickle Jar digester - solids removal

		Feed (mg/ℓ)	Digest (mg/ℓ)	Solids Removal Efficiency
Total Solids	$\tilde{x}$	8 160	2 333	71%
	highest	14 160	3 895	
	lowest	3 000	1 555	
	s	4 114	678	
	n	8	32	
Total Volatile Solids	$\tilde{x}$	6 088	1 388	77%
	highest	12 440	2 520	
	lowest	2 170	605	
	s	3 395	513	
	n	8	31	
DS	$\tilde{x}$	1 762	1 589	10%
	highest	2 730	2 898	
	lowest	895	818	
	s	527	442	
	n	13	36	
VDS	$\tilde{x}$	1 032	789	24%
	highest	1 655	1 950	
	lowest	368	393	
	s	395	326	
	n	13	33	
SS	$\tilde{x}$	4 400	960	78%
	highest	7 628	3 470	
	lowest	603	278	
	s	2 573	672	
	n	9	33	
VSS	$\tilde{x}$	3 399	721	79%
	highest	6 323	1 905	
	lowest	440	100	
	s	2 017	475	
	n	9	30	

very high volatile component and were fairly low in dissolved solids. Suspended solids were the major component of the total solids and were comprised mainly of volatiles. Little of the dissolved solids were removed and part of the suspended solids removal may be ascribed to precipitation as sludge accumulation within the digester was noticed.

Feed to the digester was stopped after 336 days. After a shutdown period of 26 days feeding was resumed at full strength. Biogas was noted after 1 day and full biogas production was achieved after 3 days running. The system was run for a further 6 months and monitoring parameters were very similar to those in the latter part of Fig.14. In general, the system proved stable to large feed strength variations, temperature fluctuations as well as accidental introduction of large quantities of atmospheric oxygen.

3.3 Test-tube Amenability Study

Generalized methods for determining the anaerobic biodegradation potential of organic chemicals have been described in the literature[147,218]. These methods consist essentially of anaerobic incubation of digested sewage sludge together with the organic matter being tested and then using the net gas produced as a direct indicator of the anaerobic biodegradability of the test material. The major drawback of this strategy is the lack of time provided for the bacterial population to acclimitize to the new substrate. It is also not known if bacteria, which may be specific for the substrate presented, are present in the test bottle or not. It does however give a limited indication of the biodegradability of the organic substrate presented, by the bacterial population used. The aim of the amenability tests with wattle extract was to determine the concentration range which could be fed to an unacclimitzied sewage bacterial population without deleterious effects on the viability of the population.

The contents of the test-tubes for the first two tests is given in Table 13 and the results of the tests are given in Figures 15 and 16. As only qualitative answers were required from this test, gas production is shown as mm distance along the manometric tube, (10 mm $\sim$ $7,4 \times 10^{-2}$). It is

Table 13. Test-tube amenability studies of anaerobic wattle extract degradation

T-tube No.	Digest (mℓ)(1)	Glucose (mℓ)(2)	Wattle Extract (mℓ)(3)	Water (mℓ)
TEST 1				
Control	3,0	0,0	0,0	1,0
1	3,0	0,2	0,0	0,8
2	3,0	0,2	0,2	0,6
3	3,0	0,2	0,4	0,4
4	3,0	0,2	0,6	0,2
5	3,0	0,2	0,8	0,0
6	3,0	0,0	1,0	0,0
7	3,0	0,0	0,5	0,5
8	3,0	0,2	0,0	0,8
TEST 2				
Control	3,0	0,0	0,0	1,0
1	3,0	0,2	0,0	0,8
2	3,0	0,2	0,2	0,6
3	3,0	0,2	0,4	0,4
4	3,0	0,2	0,6	0,2
5	3,0	0,2	0,8	0,0
6	3,0	0,0	1,0	0,0
7	3,0	0,0	0,5	0,5
8	3,0	0,2	0,0	0,8

(1) Digested sludge was obtained from the 2 ℓ "pickle jar" digester.

(2) A 0,69% (m/v) glucose solution was used. It was not fortified with trace elements and had a COD of ± 6 200 mg/ℓ and a PV of ± 500 mg/ℓ.

(3) A 0,75% (m/v) solution of wattle extract was used with a COD of 9 600 mg/ℓ and a PV of ± 3 700 mg/ℓ.

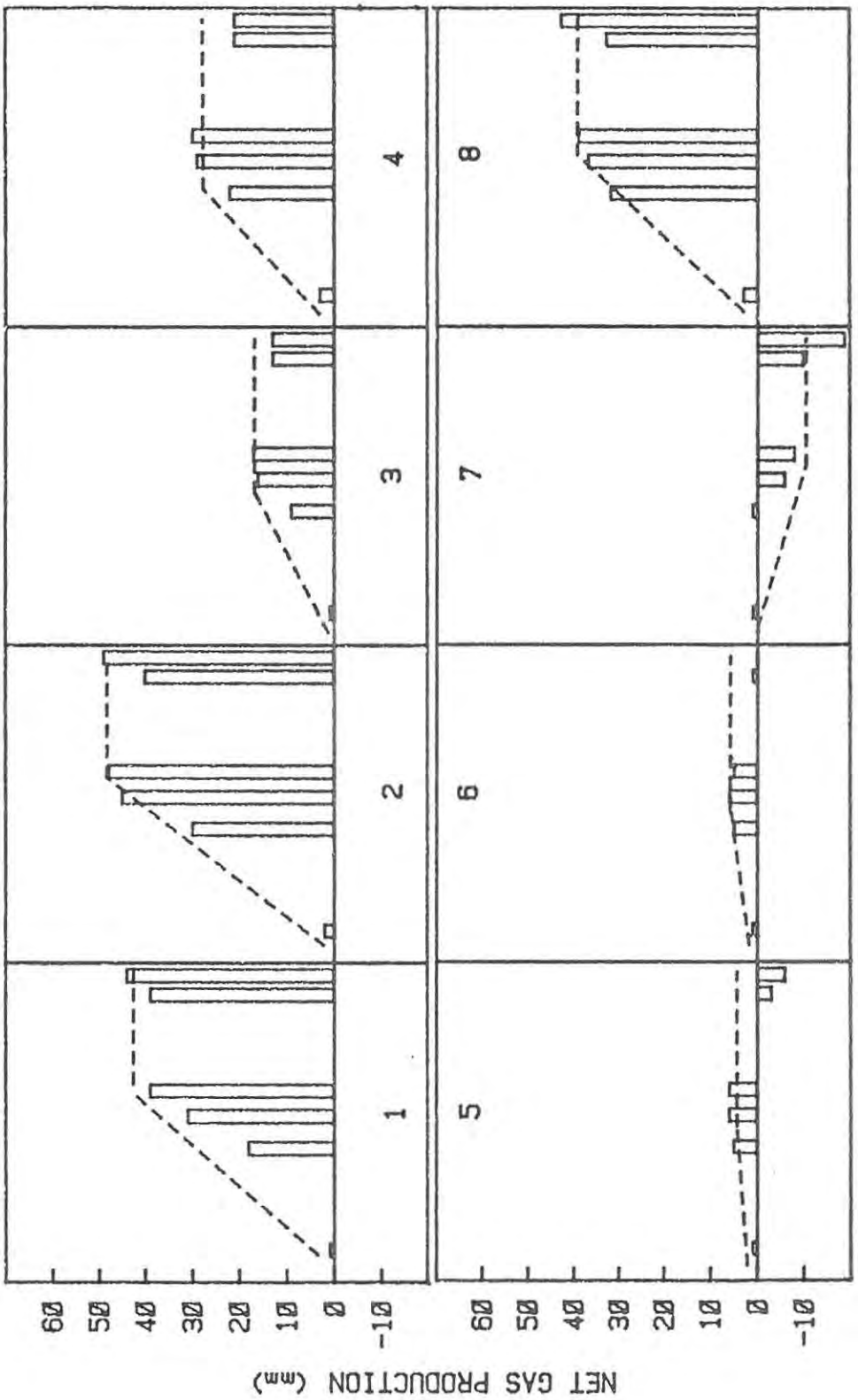


Figure 15. Results of anaerobic test-tube amenability tests - Test 1

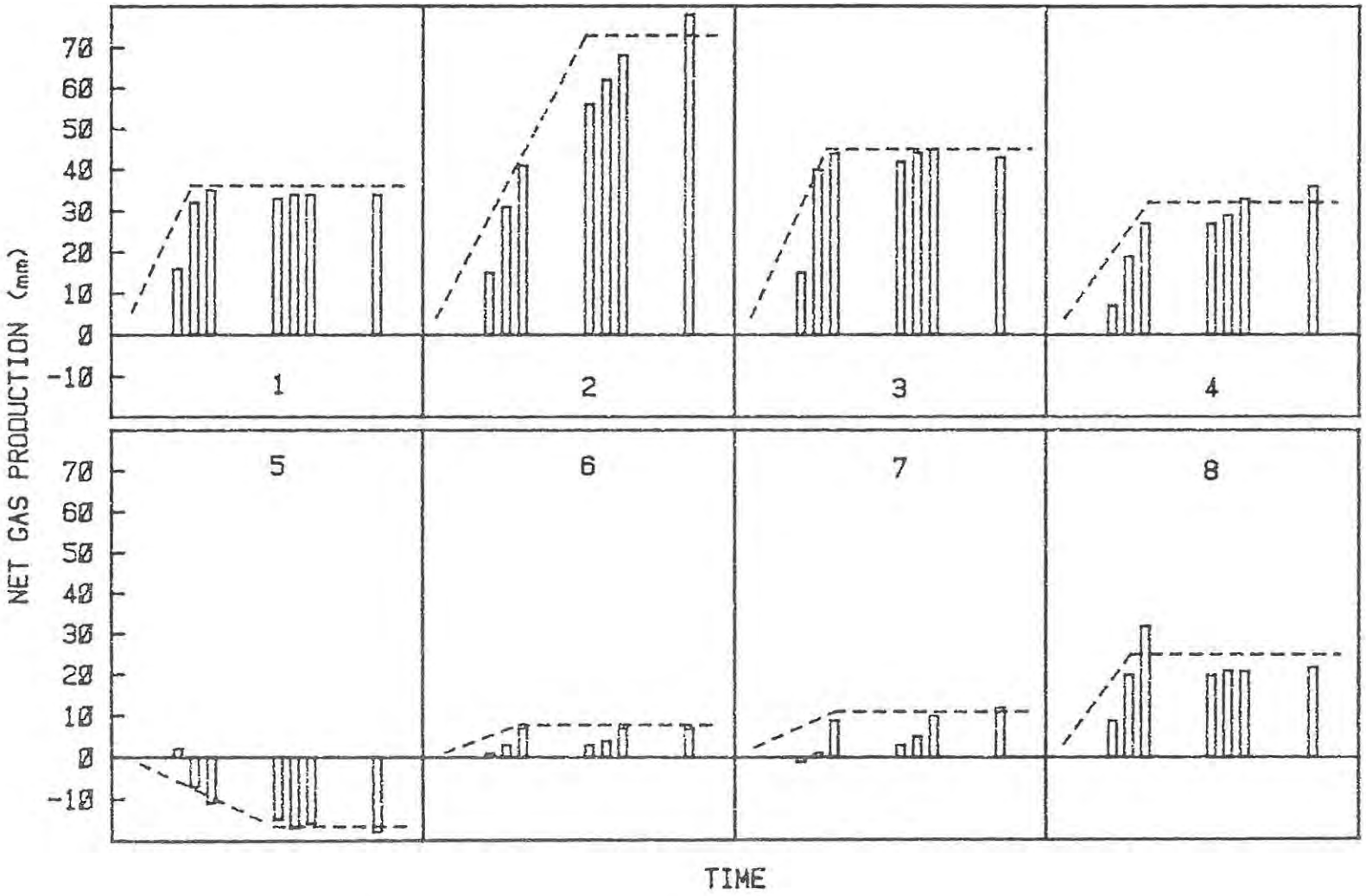


Figure 16. Results of anaerobic test-tube amenability tests - Test 2

also given as net gas production which represents actual gas production minus that of the control. Test 1 showed only test-tube 2 giving a higher gas production than test-tubes 1 and 8 which were used as the glucose control, (i.e. no wattle addition). There was therefore enhanced gas production where 0,2 mℓ of the wattle extract was added. Test-tube 3 was out of line with the expected inhibition due to the wattle concentration gradient and it was seen that anaerobiosis was severely inhibited above a wattle addition of 0,6 mℓ wattle (i.e. Tube 4). This latter addition equates to a level of 1,1 g/ℓ wattle extract. Where wattle was added without an alternate carbon source (i.e. Tubes 6 and 7), anaerobiosis was similarly severely inhibited. Test 2 which was a repeat of Test 1 gives similar results with enhanced gas production at the lower levels of wattle addition over the straight glucose feed and severe inhibition of anaerobiosis above a wattle addition of 0,6 mℓ (i.e. Tube 4 at 1,1 g/ℓ wattle concentration). A gradient of inhibition proportional to wattle addition was again obvious.

The work of Eye and Ficker[15] using batch fed reactors showed enhanced gas production at lower levels of tan addition until a total of 3,3 g/ℓ of wattle was added. The daily wattle addition however was only 0,45 g/day (or 0,13 g/ℓ). It is difficult to draw similarities between the two studies as this study measured gas production to steady state and the latter was a semi-continuous system. The order of magnitude at which inhibition of methanogenesis occurred was the same however and the phenomenon of gas enhancement at low levels of wattle addition was consistent.

3.4 Upflow Anaerobic Sludge Blanket Digester

Figures 17, 18 and 19 summarize the performance characteristics of both the test and control digesters during the first 10 phases of operation and Table 14 gives the performance level at the end of phase 4 to 9 for the test digester.

Phases 1 to 4 show the start up and stabilization of the digesters. During the first 2 weeks a sludge bed was established following which incremental

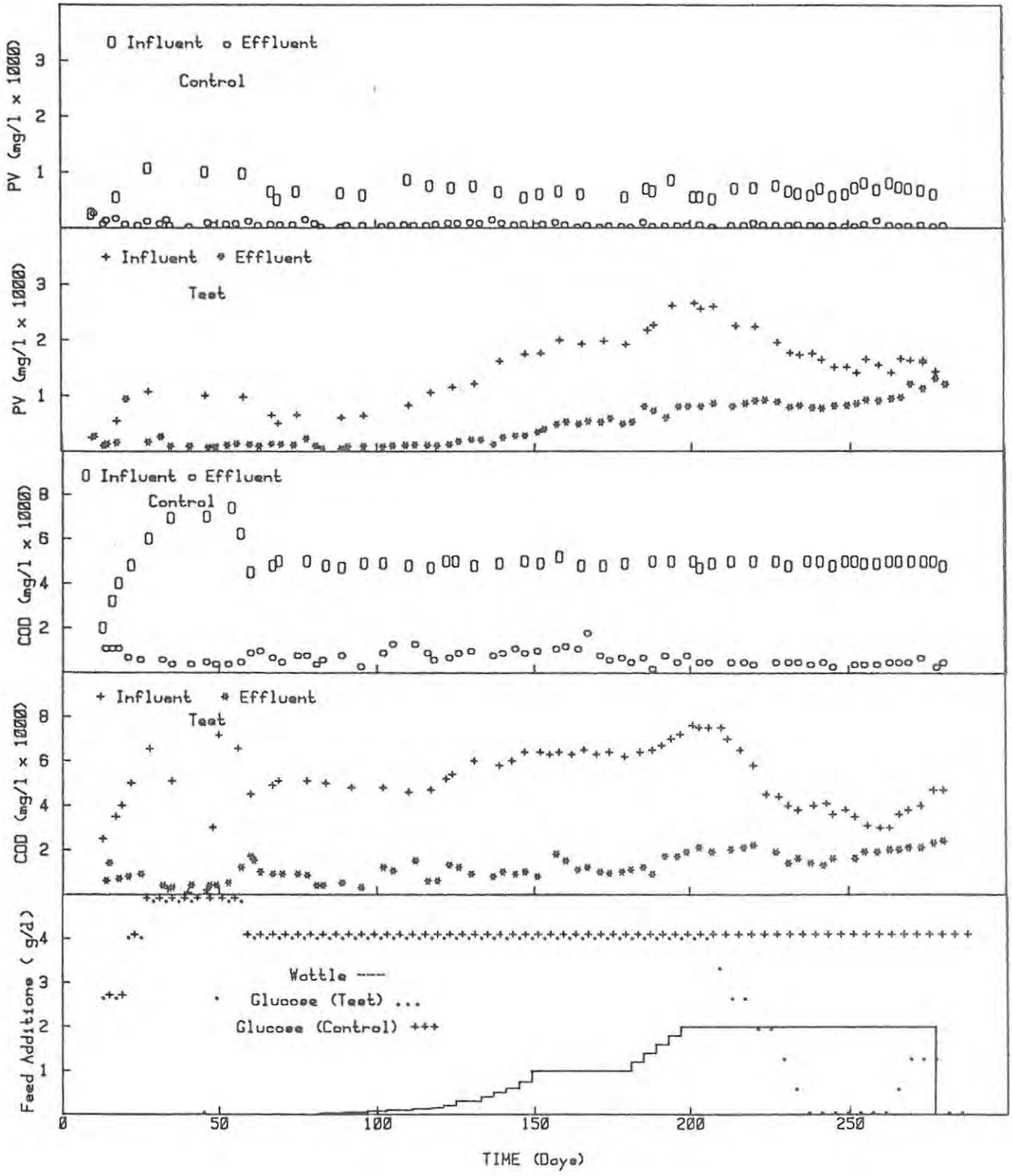


Figure 17. Performance characteristics of Test and Control Digesters

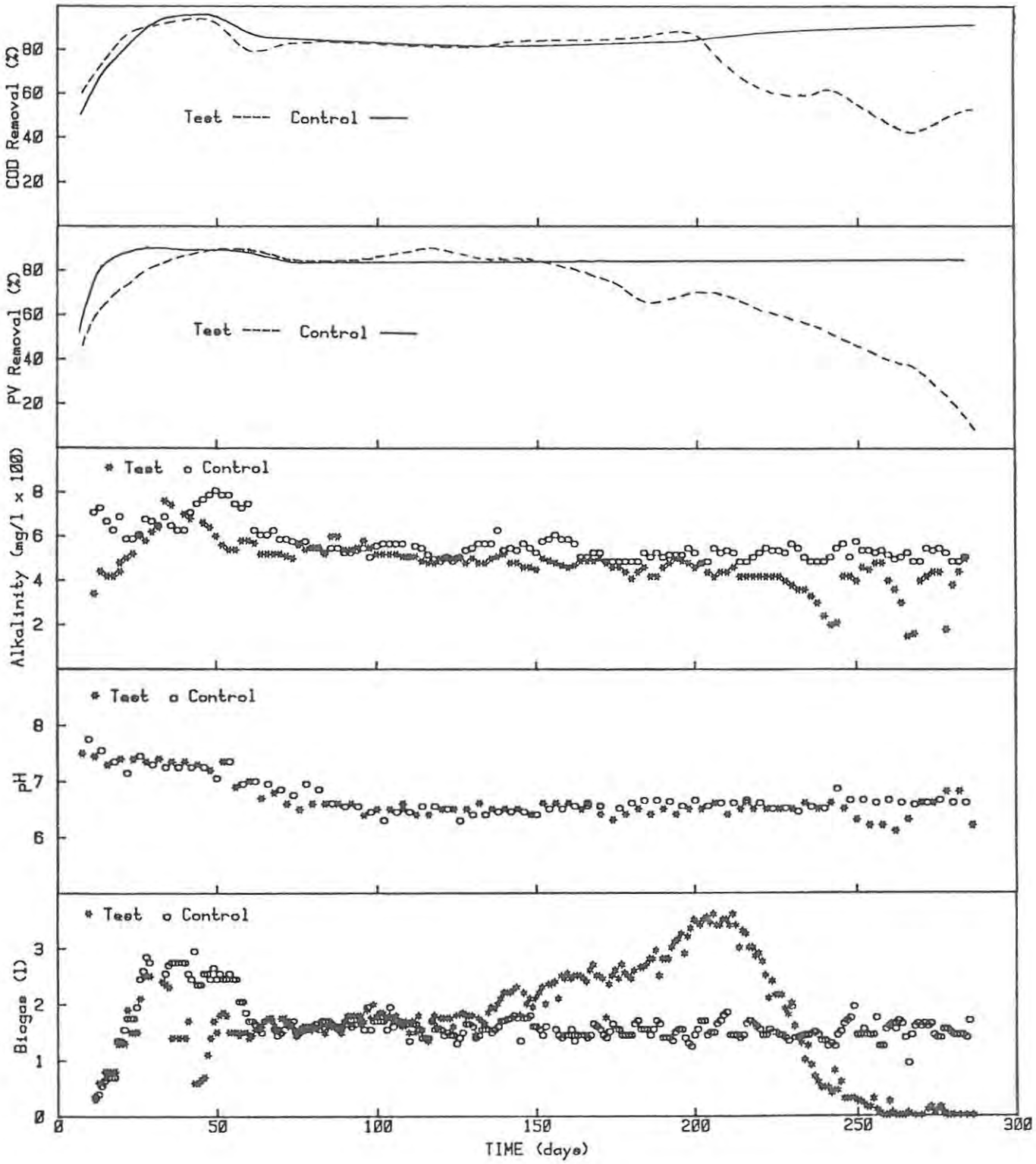


Figure 18. Performance characteristics of Test and Control Digesters

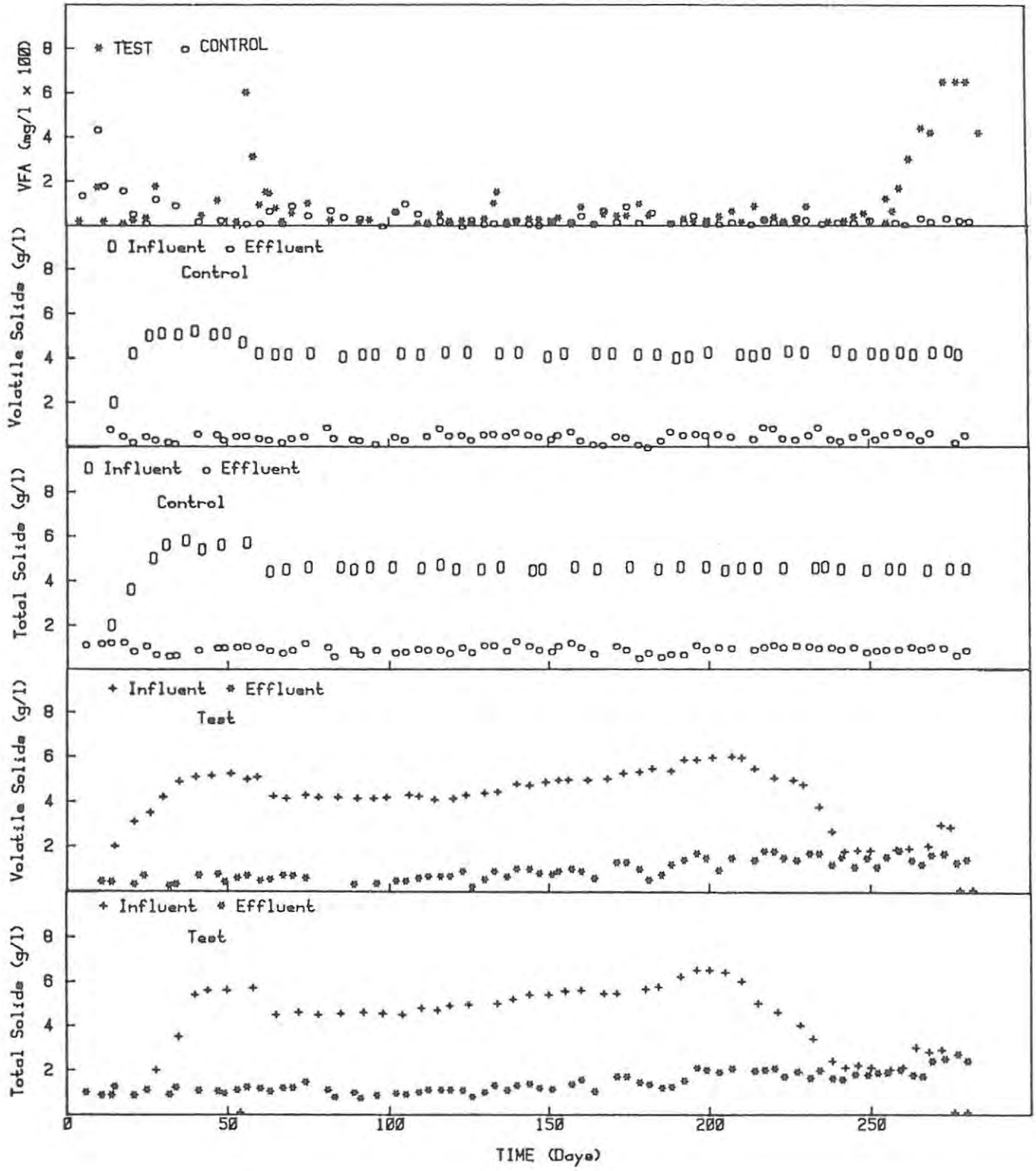


Figure 19. Performance characteristics of Test and Control Digesters

Table 14. Performance levels of test digester for different phases of operation

Parameter	Phase 4			Phase 5			Phase 6			Phase 7			Phase 8			Phase 9		
	Glucose (4,1 g)			Glucose (4,1 g) + Wattle (1 g)			Glucose (4,1 g) + Wattle (2 g)			Glucose (4,1 g) + Wattle (2 g)			Wattle (2 g)			Wattle (2 g)		
pH	6,5			6,5			6,5			6,5			6,5			Unstable		
Alkalinity	550 mg/ℓ			450 mg/ℓ			450 mg/ℓ			450 mg/ℓ			230 mg/ℓ			Unstable		
VFA	100 mg/ℓ			100 mg/ℓ			100 mg/ℓ			100 mg/ℓ			100 mg/ℓ			450 mg/ℓ		
Biogas	1,6 ℓ (60% CH_4)			2m5 ℓ (55% CH_4)			3,4 ℓ (55% CH_4)			3,5 ℓ (57% CH_4)			0,5 ℓ (71% CH_4)			0,1 ℓ (80% CH_4)		
	Inf.	Eff.	% Redn	Inf.	Eff.	% Redn	Inf.	Eff.	% Redn	Inf.	Eff.	% Redn	Inf.	Eff.	% Redn	Inf.	Eff.	% Redn
COD (mg/ℓ)	5 010	750	85	6 300	1 100	83	7 600	1 800	76	7 600	2 010	74	3 980	1 660	58	2 650	2 100	21
PV (mg/ℓ)	880	100	88	1 650	250	85	2 450	750	69	2 500	800	68	1 700	750	56	1 450	950	34
PV:COD	0,18	0,13	-	0,26	0,23	-	0,32	0,42	-	0,33	0,40	-	0,43	0,45	-	0,55	0,45	-
Total Solids (mg/ℓ)	4 510	990	78	5 480	1 100	80	6 500	2 100	68	6 500	2 100	68	2 090	1 980	5	2 000	2 000	0
Volatile Solids (mg/ℓ)	4 220	590	86	5 030	820	84	5 990	1 850	69	5 990	1 980	67	1 980	1 750	12	1 600	1 600	0
Tannin:UV	-	-	-	0,7	0,48	31	1,4	1,0	29	1,4	1,0	29	1,4	1,30	7	1,4	1,25	11
(g/ℓ) FC	-	-	-	0,7	0,38	46	1,4	0,66	53	1,4	0,70	50	1,4	0,72	49	1,4	0,84	40
Mo	-	-	-	0,7	0,38	46	1,4	0,66	53	1,4	0,70	50	1,4	0,72	49	1,4	0,82	41

additions of synthetic feed were made for a further two weeks. During the third phase a series of mechanical problems were encountered and it was decided at the same time to reduce the feed strength for reasons of convenience. Phase 4 represent three weeks of stable functioning with identical conditions being experienced in both digesters.

In this latter phase, the pH dropped from a previous level of 7,3 and ranged between 6,5 and 7,0 with alkalinities stabilized at 550 mg/ℓ. Influent COD and PV was 5 010 mg/ℓ and 880 mg/ℓ respectively with removal efficiencies of the order of 85%. The low PV value was due to the inability of permanganate to oxidise glucose under the PV test conditions as discussed in Section 3.1.4. Total solids in the influents were at 4 510 mg/ℓ and removal efficiencies of 78% were realized. A removal efficiency of 86% was found for the volatile solids with the influents at 4 220 mg/ℓ. The influent feed constituents are given in Table 8. The C:N:P ratio was 23:2:1 which is relatively high in P but chosen as such because wattle extract is deficient in P. The feed was added at a rate of 1 ℓ/day which gave a hydraulic retention time of 9 days. Such a rate may be misleading however as the design of the digester requires the upper portion to be used as a settling zone. The active anaerobic operating zone is the sludge bed which occupied 2 ℓ of digester space and as such reduced the anaerobic retention time to 2 days. The loading rate at this juncture was 0,6 kg COD/m^3/day or 2,5 kg COD/m^3/day taking only the active zone into account. Biogas levels were in slight excess of 1,5 ℓ per day and contained approximately 50% CH_4. Theoretical biogas yields can be calculated to a first approximation from equations 16 and 14. That is,

A) Expected biogas yield from glucose added:

$$\frac{4{,}1 \text{ g glucose}}{180 \text{ g/mole}} \times (6 \times 22{,}4) \text{ ℓ/mol} = \underline{3{,}1 \text{ ℓ}} \rightarrow \text{ biogas}$$

B) Expected biogas yield from COD removed:
1 g COD = 2 x 0,35 ℓ biogas at STP[121] ... (assume 50% CH_4 and 50% CO_2)

Therefore (5,0 g/ℓ x $\frac{85}{100}$) COD removed x 0,7 ℓ/g COD = $\underline{3{,}0 \text{ ℓ}} \rightarrow$ biogas

A 50% yield of biogas was therefore achieved at this stage with the balance presumably being lost to soluble CO_2 (alkalinity) and an increase in biomass. VFA's in the effluent remained below 100 mg/ℓ (i.e. as acetate). The major component of the VFA's was acetic with some propionic acid and traces of iso-butyric, n-butyric and iso-valeric acids also being present in some analyses.

The control digester was seen to perform consitently throughout the experiment at the levels achieved at the end of phase 4. This therefore represented a stable state and all changes in parameters in the test digester in succeeding phases were attributed directly to the addition of wattle extract.

During phase 5, wattle was added stepwise up to 1 g/ℓ/day in the feed to the test digester. Influent COD increased to 6 300 mg/ℓ (up 1 300 mg/ℓ) while PV increased by 770 mg/ℓ to approximately 1 650 mg/ℓ. This latter increase was relatively greater for PV than for COD as explained previously and increased the PV:COD ratio from 0,18 to 0,26 for the whole waste. PV removal rates were seen to increase a little initially but later returned to 85%. COD removal efficiencies remained constant. Biogas levels increased to 2,5 ℓ/day, (i.e. up 1,0 ℓ), which indicated > 100% theroretical yield from net COD removal rates, (i.e. 1 300 mg/ℓ x 85% x 0,7 ℓ/g = 0,8 ℓ ... see Equation 14). If the wattle extract added is examined it may be assumed that it consisted of 70% tannin and 30% carbohydrate of average molecular formula $C_{15}O_6H_{13}$ (MW = 289 g/mol) and $C_6H_{12}O_6$ (MW = 180 g/mol) respectively. From Equation 16 a biogas yield of 1,0 ℓ for complete conversion, would be expected. That is,

$$\frac{0,7\ g}{289\ g/mol} \times (15 \times 22,4)\ \ell/mol = 0,81\ \ell$$

$$+\ \frac{0,3\ g}{180\ g/mol} \times (6 \times 22,4)\ \ell/mol = 0,22\ \ell$$

$$1,03\ \ell$$

That the tannins were not completely degraded however was shown by the various tannin analyses where around 40% of the tannins were identified in the effluent. Mechanisms whereby this enhanced gas production could occur

are speculative. A co-precipitation of tannins and other organics (e.g. glucose) into the active sludge bed could be rationalized, where they would be more available for bacterial attack. From gross COD removal rates however, 3,0 ℓ of biogas would be expected and hence only an 83% yield of biogas was realized.

There was a slight drop in the alkalinity and the relative methane content of the gas which would indicate a slight decrease in CO_2 solubility and could account, in part, for the increase in biogas production. The pH and VFA levels remained constant however which showed that the system was stable. The addition of wattle could thus improve the biogas yield by physico-chemical means, but biochemical processes should not be discounted. Wattle extract removals measured by net COD and PV reductions were 92% and 85% respectively. The spectrophotometric methods for tannins gave 46% removal for the F-C and Mo methods and 31% for the UV method.

During Phase 6, the wattle additions to the test digester were increased to 2,0 g/ℓ. This represented a loading rate of 3,8 kg COD/m^3/day into the active anaerobic zone. Stratigraphic analysis of VFA levels in the digesters (see Table 15) confirms that most stabilization occurs at the base of the digester, especially in the case of the test digester. The proportionate increase in COD and PV influent levels were similar to those for Phase 5, but removal efficiencies dropped to 76% for COD and 69% for PV. Such a drop would be expected for a feed with a proportionately higher level of recalcitrant material (e.g. tannins) in the feed and the sharper drop in PV reflects this method's sensitivity to phenolics as opposed to glucose. The PV:COD ratio of the influent was seen to increase to 0,32 which can be rationalized on the basis of the feed composition. That is:

The feed contained wattle (2 g) and glucose (4,1 g) in a ratio of approximately 1:2

Now, the PV:COD ratio for wattle = 0,72 ... see Section 3.1.4

and the PV:COD ratio for glucose = 0,18 ... see Table 14

therefore overall $\frac{(1 \times 0,72) + (2 \times 0,18)}{3} = \underline{0,36}$

which in turn was a reflection of the higher relative proportion of phenolics present.

Table 15. Stratigraphic analysis of pH and VFA in test and control digesters

	Control Digester - Day A[1] (Height above base)					Test Digester - Day A[1] (Height above base)				
VFA (mg/ℓ)	2,7 cm	15,5 cm	28,5 cm	41,5 cm	53,5 cm	2,7 cm	15,5 cm	28,5 cm	41,5 cm	53,5 cm
Acetic	143	34	19	23	4	99	17	7	11	1
Propionic	58	6	3	2	-	48	-	-	-	-
n-Butyric	32	4	-	-	-	30	-	-	-	-
i-Butyric	-	-	-	-	-	3	-	-	-	-
n-Valeric	23	-	-	-	-	32	-	-	-	-
i-Valeric	3	7	12	-	-	3	-	-	-	-
n-Caproic	-	-	-	-	-	9	-	-	-	-
Total (as acetic)	225	46	29	25	4	186	17	7	11	1
pH	5,95	6,26	6,24	6,25	6,68	6,03	6,22	6,23	6,27	6,84
	Control Digester - Day B[1]					Test Digester - Day B[1]				
VFA (mg/ℓ)										
Acetic	Not[2]	54	26	Not[2]	11	Not[2]	22	13	Not[2]	7
Propionic	Tested	17	8	Tested	-	Tested	-	-	Tested	-
n-Butyric		3	-		-		12	4		-
i-Butyric		-	-		-		-	-		-
n-Valeric		-	-		-		-	-		-
i-Valeric		-	-		-		2	1		-
n-Caproic		-	-		-		-	-		-
Total (as acetic)	-	70	33	-	11	-	31	17	-	7

Note: 1. Day A and B were arbitrarily taken during phases 5 and 7 respectively.
2. The inlet (2,7 cm) and sample port (41,5 cm) were not tested on Day B.

The effluent PV:COD ratios showed a much steeper increase than that predicted by influent composition which in turn indicated that it contained proportionately higher levels of tannins. This was again as expected. Biogas levels however increased by 0,9 ℓ to 3,4 ℓ per day. The expected level from net COD removal rates was 0,7 ℓ (i.e. 1 300 mg/ℓ x 76% x 0,7 ℓ/g = 0,69 ℓ), and therefore again, a biogas production level was experienced which was higher than expected. It should be noted that this was not more than that expected from gross COD removals where 4,1 ℓ was expected and therefore an 83% biogas yield was realized, (i.e. 5,8 g COD removed x 0,7 ℓ/g = 4,1 ℓ and $\frac{3,4}{4,1} \times \frac{100}{1}$ = 83% biogas yield). The total biogas production however correlates well with expected levels of gas production if we assume that the tannins are 53% degraded (this was shown by spectrophotometric measurement of tannin removal) and that all the organics give an 83% biogas yield. That is:

4,1 g glucose $\longrightarrow$ 3,1 ℓ ... (see previously)

and, $\frac{0,6\ g}{180\ g/mol} \times (6 \times 22,4)\ell/mol = 0,45\ \ell$ ... carbohydrate fraction of wattle extract

and, $\frac{1,4\ g}{289\ g/mol} \times (15 \times 22,4)\ \ell/mol \times 53\% = 0,86\ \ell$ ... tannin fraction of wattle extract

Therefore total gas x 83% = 4,4 ℓ x 0,83 = $\underline{3,6\ \ell}$ Biogas

As there was no change in the methane content of the gas or in the alkalinity, this increase in biogas could not have come about by a decrease in CO_2 solubility which was suggested previously as a possible explanation. The initial conclusion at this stage therefore was that the addition of wattle extract to an actively operating anaerobic system improves the biodegrdation of other organics present and results in the partial degradation of the tannins themselves. The most plausible reason for this phenomenon would be physico-chemical precipitation of the organics into the actively degrading anaerobic zone resulting in better feed to anaerobe contact.

Phase 7 represented two weeks of stable functioning with wattle extract in the influent at a level of 2 g/ℓ. The operating characteristics were stable and almost identical to those found at the end of Phase 6. The loading rate was 3,8 kg COD/m^3/day in the active anaerobic zone. At this stage, samples of the effluent were taken in an attempt to isolate tannin degradation products. (This is discussed in Part II). The effluent was highly coloured by tannins and was a dark browny red colour. Table 16 gives an analysis of the composition of the digester sludges for both the test and control digesters. The solids analyses indicated that the test digester had a more compact sludge, but otherwise the composition of both sludges was very similar. Samples of the test anaerobic sludge were also removed and analysed for precipitated tannin. This was done by first treating the sludge with acetone (50% v/v) to "detan" the bacterial protein[219] and then the tannin concentration was determined by the Mo and F-C methods. (The UV method could not be used as acetone absorbs in the UV region). The values of 1,2 g/ℓ and 1,4 g/ℓ respectively were no higher than influent tannin concentrations and so significant precipitation of tannin in the sludge bed was discounted. At this stage it was decided to reduce the glucose addition in the test digester to see if the acclimated sludge could degrade wattle extract as the only carbon source.

At the end of Phase 8 and during Phase 9 where only wattle and trace minerals were fed it was obvious that the test digester was functioning under severe stress. Biogas production slowed down and virtually ceased during Phase 9. It is interesting to note that the proportion of CH_4 increased during these phases, reaching a maximum of 80%. This was presumably due to CO_2 being consumed for stabilizing alkalinity and VFA levels. The alkalinity started dropping during Phase 8 and sodium bicarbonate had to be added to help maintain both the alkalinity and the pH. VFA levels started increasing during Phase 9. Acetic acid accounted for approximately 85% of these VFA and proportionately high levels of propionic and n-butyric acids were also present. Traces of i-butyric, i-valeric, n-valeric and n-caproic acids were also noted. PV and COD removal efficiencies decreased drastically while solids removal was not effected. Tannin removal rates also dropped sharply. It was concluded that the methanogenic bacterial population was being inhibited by the high concentration of tannins present.

Table 16. Analysis of digester sludges

	Concentration mg/ℓ	
	Control	Test
Nitrogen:		
Kjeldahl-N	205	322
Ammonia-N	158	244
NO_3-N	5	5
Phosphorous:		
Total as P_2O_5	10	18
Metals:		
Cr	1	1
K	25	33
Na	40	50
Ca	40	20
Mg	15	10
Fe	3	2
Cu	1	1
Al	5	5
Other:		
Cl	380	340
SO_4	1	1
Solids:		
TS	15 500	19 000
VS	13 500	16 500
SS	14 000	18 000
DS	1 000	1 000

Supplementary additions of glucose were again made during Phase 10 in an attempt to revive the failing digester. The addition of wattle extract and glucose was stopped after 2 weeks however as no signs of recovery were evident. A week later, the VFA levels had decreased from approximately 700 mg/ℓ to 400 mg/ℓ but the pH and alkalinity remained unstable and $NaHCO_3$ additions had to be made to keep the pH above 6. The test digester had obviously failed at this stage and both it and the control digester were therefore shutdown.

Both digesters were restarted after a period of 1 month complete shutdown, which included all monitoring as well as temperature control. They were heated to 35°C and feeding of synthetic feed medium was resumed in stepwise additions. Biogas production started almost immediately and full functioning was achieved within 10 days without any signs of inhibition or instability on the part of both the control and the test digester. The test digester effluent was still however coloured with tannins or tannin breakdown products and the tannin assays registered approximately 0,6 g/ℓ.

That the test digester started without signs of instability indicates that the period of shutdown was sufficient for it to adjust to the high level of tannins present and to remove any inhibition imposed by the tannins. This confirms previous findings[15] that the tannins are inhibitory to anaerobiosis, or more specifically methanogenesis, but that these phenolics are not toxic to the bacterial populations present.

PART II : MONITORING AND ATTEMPTED ISOLATION OF WATTLE COMPONENTS IN ANAEROBIC EFFLUENT

4. INTRODUCTION

The oxygen demand and spectrophotometric methods described in Part I proved fairly efficient and consistent in their detection of gross polyphenol removal. They gave no indication however as to which part of the total extract was degraded nor any information on the structure of any degradation products. Although it could be assumed that the smaller phenolics would be removed more easily, proof for this postulate was required and more detailed analysis was needed for clues to the breakdown process. To this end, some of the more standard separation and analytical techniques were applied to the anaerobic effluent which from its colour and measurements of gross polyphenol removals was known to contain either some tannins or tannin breakdown products.

Paper chromatography is a well documented technique for separating flavonoids[22,28] and in particular has been used extensively for the separation and partial identification of wattle polyphenolics[220-231,24]. Hendry[62] compiled a list of compounds identified from wattle extract together with their R_f co-ordinates for a two-dimensional chromatogram using water-saturated butan-2-ol as the first phase and 2% aqueous acetic acid (v/v) for the second. Roux[220,224,226] demonstrated that the more astringent polyphenols had low R_f values and this in turn was seen to correlate directly to molecular weight. He showed furthermore[24,226] that the affinity of tannin polyphenols for cellulose could be directly correlated to their solubility in water, shape, size and the number and position of functional groups. During migration in water-saturated butan-2-ol, polyphenols are partitioned according to the number of OH groups as well as the cis/trans isomerism at the C-2 and C-3 positions[227]. Mobility in the water eluant on the other hand separates them according to their adsorptivity to cellulose which is a direct

function of molecular planarity and H_2O solubility. This explains the immobility of the flavonols for instance, which have molecular planarity imparted by an α, β -unsaturated carbonyl group in the C-ring[24]. Acetic acid is added to the aqueous eluant as it prevents the weaker organic acids from trailing by depressing their ionization. It also helps to keep the pH down which reduces the atmospheric oxidation of the light sensitive phenolics[230].

Spray reagents have been used extensively in paper chromatographc (PC) work for the partial identification of flavonoids as well as flavonoid functional groups and many of these reagents are highly selective[230,232]. Ammoniacal silver nitrate[233] for instance is reduced by phenolic hydroxy groups and the shade of black, grey or brown produced depends on the nature and concentration of the reducing group. Pyrogallol units for example give brown to black colourations and catechol groups can give a grey colour as well.

Thin layer chromatographic (TLC) techniques are complementary to PC and have been used extensively in flavonoid research, especially for analytical work and small scale separations[22]. TLC's main attraction lies in the larger selection of media available for separation and also provides the choice of a wider variety of selective spray reagents.

Analytical determinations of polyphenolic compounds in general have benefited greatly with the advent of HPLC techniques. HPLC has the advantage of resolution, speed, reproducibility and quantitation over PC techniques[234,235]. It also requires no derivitization[236] of compounds and there is no risk of thermal degradation as with GC where samples must be volatilized. Examples of the separation, identification and quantitation of flavonoids using HPLC abound in the literature[234-243] and Hendry[62] has done extensive work on the analysis of wattle flavonoids in particular. Separation of phenolics on a reversed-phase column is related to their structure and adsorption qualities[243]. The factors affecting flavonoid elution are more fully discussed in the literature[62,234,241,242], but in brief are governed by the degree of hydrophobic interaction with the stationary phase as well as hydrogen bonding with the mobile phase[242]. This latter interaction is

profoundly affected by intramolecular factors such as the hydroxyl group substitution pattern on the flavonoid nucleus.

Good separations of proanthocyanidins and flavonoids in general have been achieved on Sephadex LH20 gels[62,244,245]. These are hydroxypropylated polydextran resins and have MW exclusion limits of 2 000 to 10 000 depending on the type of solvent used[22]. Although Sephadex gels rely primarily on exclusion mechanisms, separation is also effected by adsorption and, for a particular type of flavonoid, is directly proportional to the number of hydroxyl groups present[245,246]. Planarity and hence solvent solubility are also factors affecting elution[245]. In the ethanolic separation of wattle polyphenols, Hendry[62] found the flavan-3-ols to elute first ($V_R/V_O \sim 7$) and this was followed by the dimeric and then oligomeric fractions.

The preceding separation mechanisms were all used both to monitor and to isolate wattle fractions from the anaerobic digester.

5. EXPERIMENTAL

5.1 Materials

The commercial Mimosa ME extract used was the same as for Part I, (See Section 2.1). The ethanol solvent used for column chromatography was redistilled industrial grade. All other reagents were AR grade except for the methanol used in HPLC separations which was chromatographic grade. The wattle flavonoids used for standardization of chromatographic separations were (+)-catechin and quercitin (both from Koch-Light).

5.2 Methods

5.2.1 Two Dimensional Paper Chromatography

Whatman No. 1 chromatographic paper was used. Water-saturated butan-2-ol was used in the first elution (14 hours) followed, after drying, by a 2% (v/v) aqueous acetic acid phase (5 hours) in the second direction. The dried chromatograms were sprayed with ammoniacal silver nitrate, (e.g. to 8 g $AgNO_3$ in 100 mℓ H_2O add NH_3 dropwise till silver oxide ppt. dissolves), to detect catechol functions. The chromatograms were then washed copiously with water, fixed with 1% $Na_2S_2O_3$ and re-dried. The brown and grey spots were tentatively identified by comparison with previously published data.

5.2.2 High-Performance Liquid Chromatography

Waters Associates HPLC apparatus was used and comprised a Model 6000A solvent delivery system, a Model 440 absorbance detector (280 nm) and an automatic Waters 730 data module. The column used was a μBondapak (Techsil C18, 10 μm) steel column of 30 cm length (4 mm i.d.). The solvent was a

standard methanol-water-acetic acid (15:84:1) mixture - the latter addition to suppress flavonoid ionization. Solvents and samples were filtered through a Millipore teflon filter (0,45 µm) before use. Samples were injected with a 25 µℓ Hamilton syringe. The column was periodically washed with methanol to remove adsorbed or very slow moving material. An eluant flow rate of 2 mℓ/min was used at a pressure of 2 700 p.s.i. HPLC chromatograms were used mainly as comparative fingerprints with tentative peak assigments being made by comparing relative retention times

(i.e. $\frac{\text{R sample}}{\text{R (+)-catechin}} = {}^{r}\text{cat}$) to published data.

5.2.3 Thin Layer Chromatography

Strips (2,5 cm x 10 cm) were cut from 0,25 mm thick Merck TLC plastic sheets, Silica Gel F_{254}. Spotted samples were eluted with benzene-acetone-methanol (6:3:1) and developed by spraying lightly with concentrated H_2SO_4 and heating in an oven at 104°C for 1 minute. The samples showed up as yellow to purple coloured elongated bands.

5.2.4 Column Chromatography

The Pharmacia column (1,6 cm x 90 cm) used was packed with Sephadex LH20 slurries made up of the gel swollen in solvent overnight. The sample (5 mℓ) to be partitioned was placed on top of the column and eluted at approximately 0,5 mℓ/min. The eluant passed through a flow-cell cuvette in a Beckman ACTA CII Spectrophotometer (280 nm) which was attached to a W + W 1100 chart recorder. Fractions of between 2 and 6 mℓ were collected on a LKB Minirac 1700 fraction collector. The void volume Vo (46 mℓ) was determined as 46 mℓ by elution of a sample of polydextran blue. The V_{R/V_o} ratio was used as an indication of compound identity by comparison with published data.

5.2.5 General

Proton NMR spectra were recorded on a Bruker 250 MHz FT spectrometer in MeOH solutions with tetra-methyl-silane (TMS) as internal standard. (See acknowledgements).

Preparation of the procyanidin analogues was achieved by heating 5 mg of the compound in a pressure tube with a mixture of iso-propanol and 3 N HCl (4:1) for one hour on a boiling water bath. The formation of a deep red colour reflected the presence of these analogues. Identification of individual compounds was achieved by separation on PC with 90% formic acid and 3 N HCl (1:1) and comparison of R_f values with standards. (See acknowledgements).

Centrifugation was done on a MSE High Speed 18 centrifuge at 4°C.

Freeze dried samples (150 mℓ) were first coated onto 1 ℓ vacuum bottles with liquid nitrogen and the frozen samples sublimed on a Thermovac Industries Corporation freeze drier overnight.

Hide powder analyses were done according to the method described in "Official Methods of Analysis"[216].

6. RESULTS AND DISCUSSION

6.1 Monitoring of Wattle Components in Anaerobic Effluent by PC

Although commercial wattle extract gives a more diffuse PC spectrum than fresh bark extract[3,18,220,247] and component identification is difficult, it was decided to use PC as a monitoring method as it is inexpensive and fairly simple to use and allows one to visualize which components of the extract have been removed by anaerobic digestion (Fig. 20). The reason for the diffuse commercial spectrum lies in the partial atmospheric and enzymic oxidations which occur during preparation of the extract[18]. Figure 20 shows a typical chromatogram of commercial extract dissolved in MeOH.

Ethyl acetate (EA) has proven to be a valuable partitioning solvent for wattle phenolics[222]. It dissolves the lower molecular weight tannins and precipitates sugars and gums[248]. A 50 mℓ sample of the feed to the anaerobic digester, (at 2 g/ℓ wattle extract), which was extracted into 2 x 50 mℓ EA, dried over anhydrous sodium sulphate, evacuated under reduced pressure to 5 mℓ, spotted on chromatography paper and eluted with the relevant solvents is shown in Figure 21. A comparison of this chromatogram with the total extract, Figure 20, showed less definition of the identifiable spots and the higher molecular weight material was less evident. An additional region was noticed which showed an intense yellow-green colour under UV light, had a R_f centred around 0,6 in the alcohol direction and was immobile in the aqueous phase. These characteristics are common to the chalcones, (-)-Butein and (-)-Robtein, as well as the flavonols, Fisetin and Robinetin, all of which have been identified in wattle extract[19,62,229,230]. This elongated area was therefore tentatively assigned to these compounds. The planarity of these compounds, (e.g. 4-keto group with 2 - 3 unsaturation) require an ordered solvent structure and thus a larger entropy in aqueous solvation which leads to preferential uptake in the EA phase during partitioning[234]. This explains why they were seen in the concentrated EA extract and not in a chromatogram of the whole extract where they would be in proportionately lower concentration.

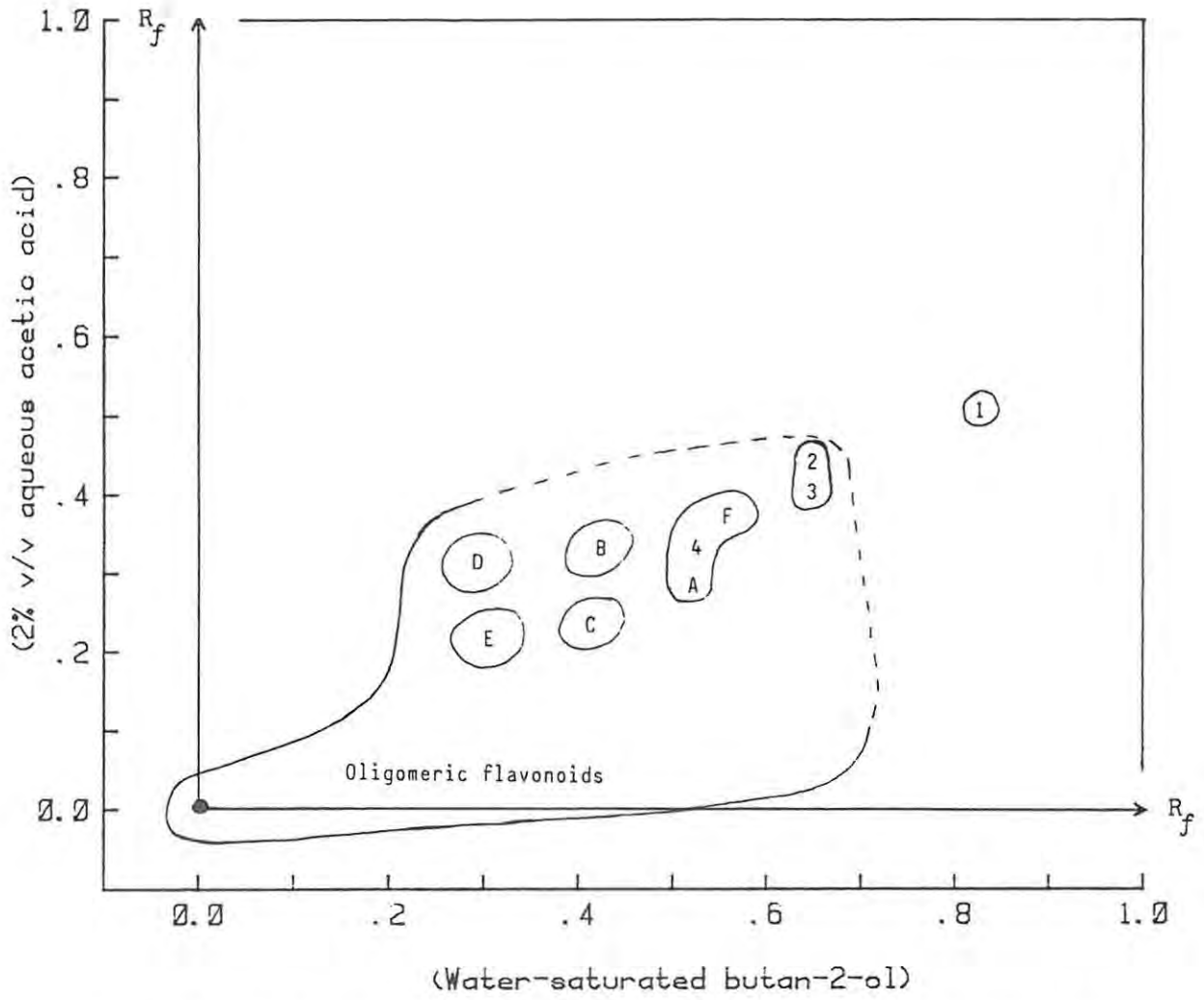


Figure 20. Two dimensional paper chromatogram of commercial wattle extract in MeOH.

Tentative assignment [28], A = Biflavonoid, B = Biflavonoid, C = Triflavonoid, D = Biflavonoid, E = Triflavonoid, F = Biflavonoid, 1 = (-)-Fisetinidol, 2 = (-)-Robinetinidol, 3 = (+)-Catechin, 4 = (+)-Gallocatechin, Area near origin = oligomeric procyanidins

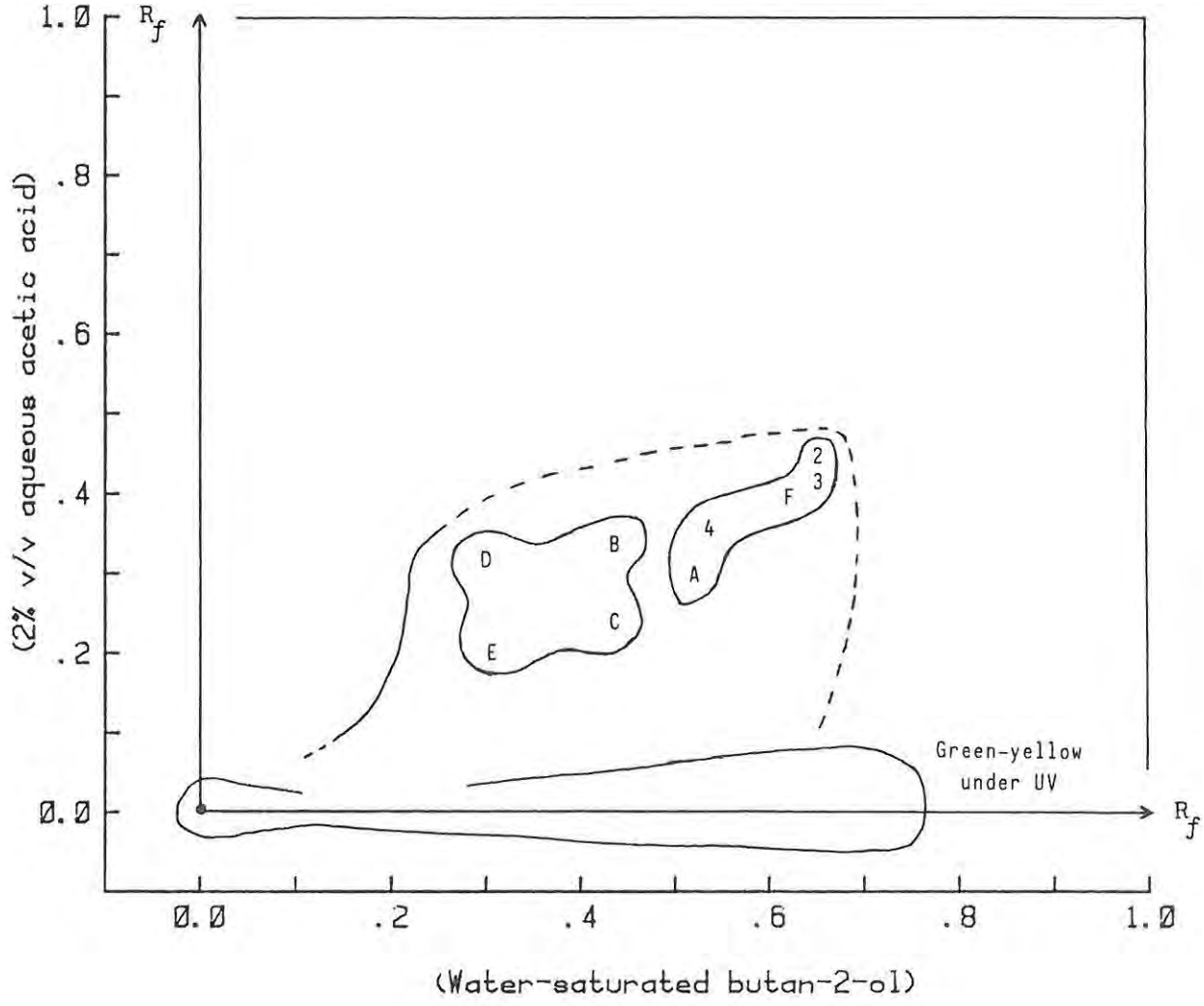


Figure 21. Two dimensional paper chromatogram of EA extract of feed to test digester (2 mg/ℓ wattle). Identification of spots as in Fig. 20.

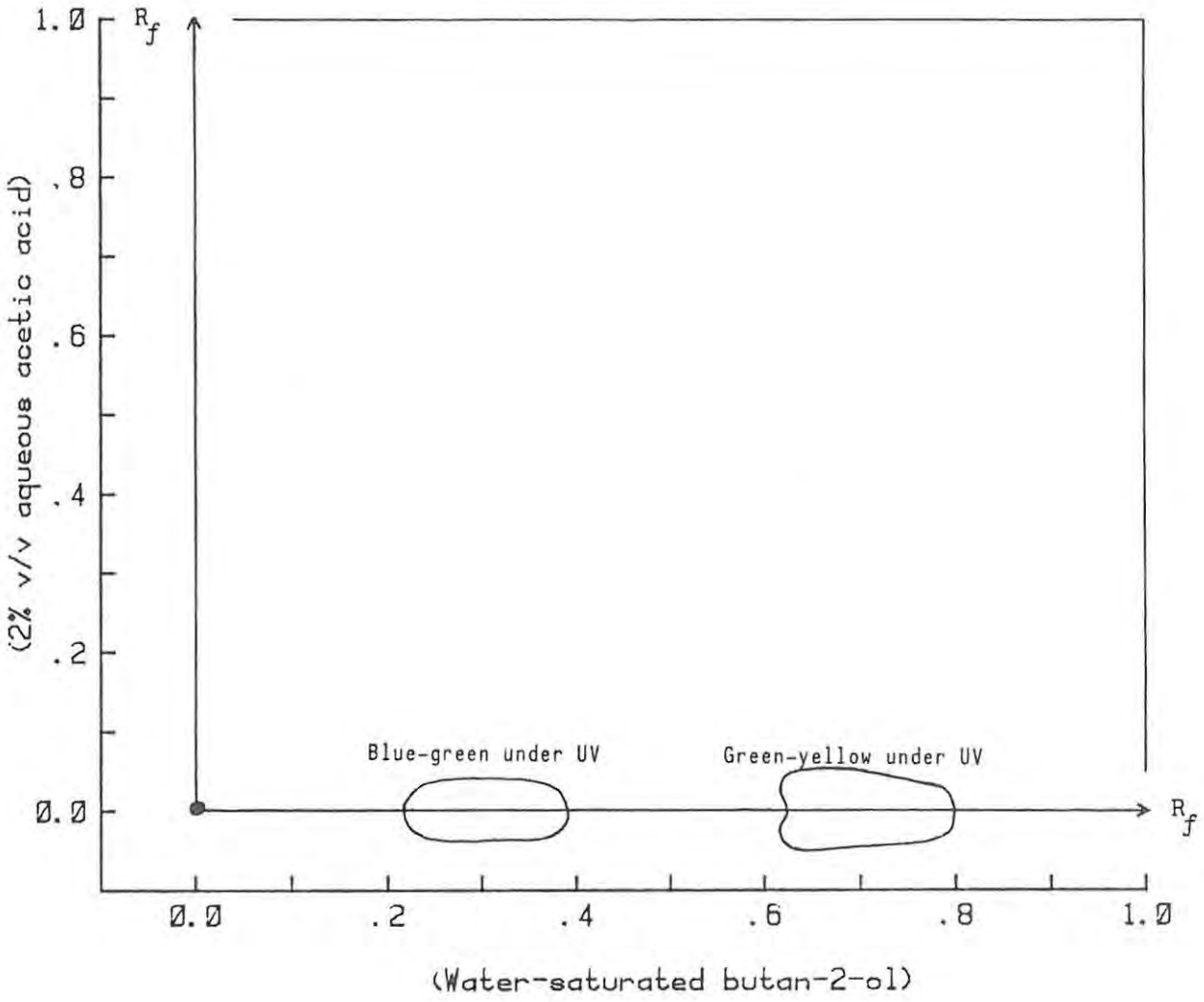


Figure 22. Two dimensional paper chromatogram of EA extract of anaerobic effluents.

This chromatogram was common to both the test and control digesters.

An ether partitioned extract of the feed sample was also tried but not preferred as it extracted much fewer of the more astringent tannins.

As the next step, samples of the anaerobic effluent from the test digester were extracted with EA, dried, evacuated and chromatogramed as explained above. Because of the lower concentrations of wattle in the extract, large volumes of sample were extracted and evacuated. However, even at 50 x concentration, no spots which could be identified as wattle phenolics were seen on the PC's after spraying with silver nitrate, (see Figure 22).

There were two conclusions that could be drawn at this stage. The first was that there were either none or very low levels of phenolics in the effluent. This seemed unlikely as shown by the other methods used for measuring phenolic removals (see section 3.4). The alternate conclusion was that the anaerobic process must have altered the phenolics to a more water soluble form and as such they were not extracted into EA. Support for this proposal was obtained from consideration of the known routes of aromatic degradation by anaerobic fermentation where the first step always involves the extracellular enzymic reduction of the aromatic nucleus[249]. Such a step would decrease the compounds affinity for the EA phase during partitioning. Alternate methods for isolating the compounds were therefore investigated.

6.2 Monitoring of Wattle Tannin Components in Anaerobic Effluent by HPLC

Hendry[62] found that direct injection of a wattle extract sample onto a reversed-phase column with a standard solvent mixture, (i.e. methanol-water-acetic acid, 15:84:1), allowed the resolution of non-tannin and tannin components but the overlapping retention times of the numerous oligomeric flavonoids only allowed fingerprint analyses of the total extract to be achieved. He found furthermore that about 50% of the wattle components eluted in 30 minutes and that the higher oligomers eluted monotonically with no resolution. Figure 23 shows a fingerprint trace of the commercial extract fed to the anaerobic digesters and Table 17 includes identification of some peaks according to Hendry[62]. Poor peak definition was attributed to a deterioration in the column efficiency and a partial oxidation of the extract sample.

The same wattle sample was used throughout the anerobic investigation. It was assumed that a degree of oxidation had taken place as the sample had been partially exposed to the atmosphere for almost 1 year before HPLC analysis. Lea[238,239] confirmed the broadening of peaks and a loss of resolution upon oxidation of flavonoids with his studies on apple extracts. Also noticed in Figure 23 were decreased amounts of components 9a and 10a from those given in Hendry's work, (dotted lines in Figure 23), but in general there was good correlation between the chromatograms.

A sample of the influent to the test anaerobic digester at a wattle concentration of 2 g/ℓ as well as a sample of the effluent were also run on the HPLC and are superimposed on Figure 23. The feed trace mimics the standard wattle chromatogram as would be expected although the definition is poorer due to the lower concentration of wattle present. The effluent sample however showed no peaks except for a poorly defined peak at a t_R of 6,8 minutes.

This investigation confirmed that none of the simpler monomeric or dimeric flavonoids identifiable by HPLC techniques were present in reasonable concentrations in the effluent. It was assumed therefore that they were either completely degraded or otherwise altered so as to be not recognizable in the anaerobic effluent.

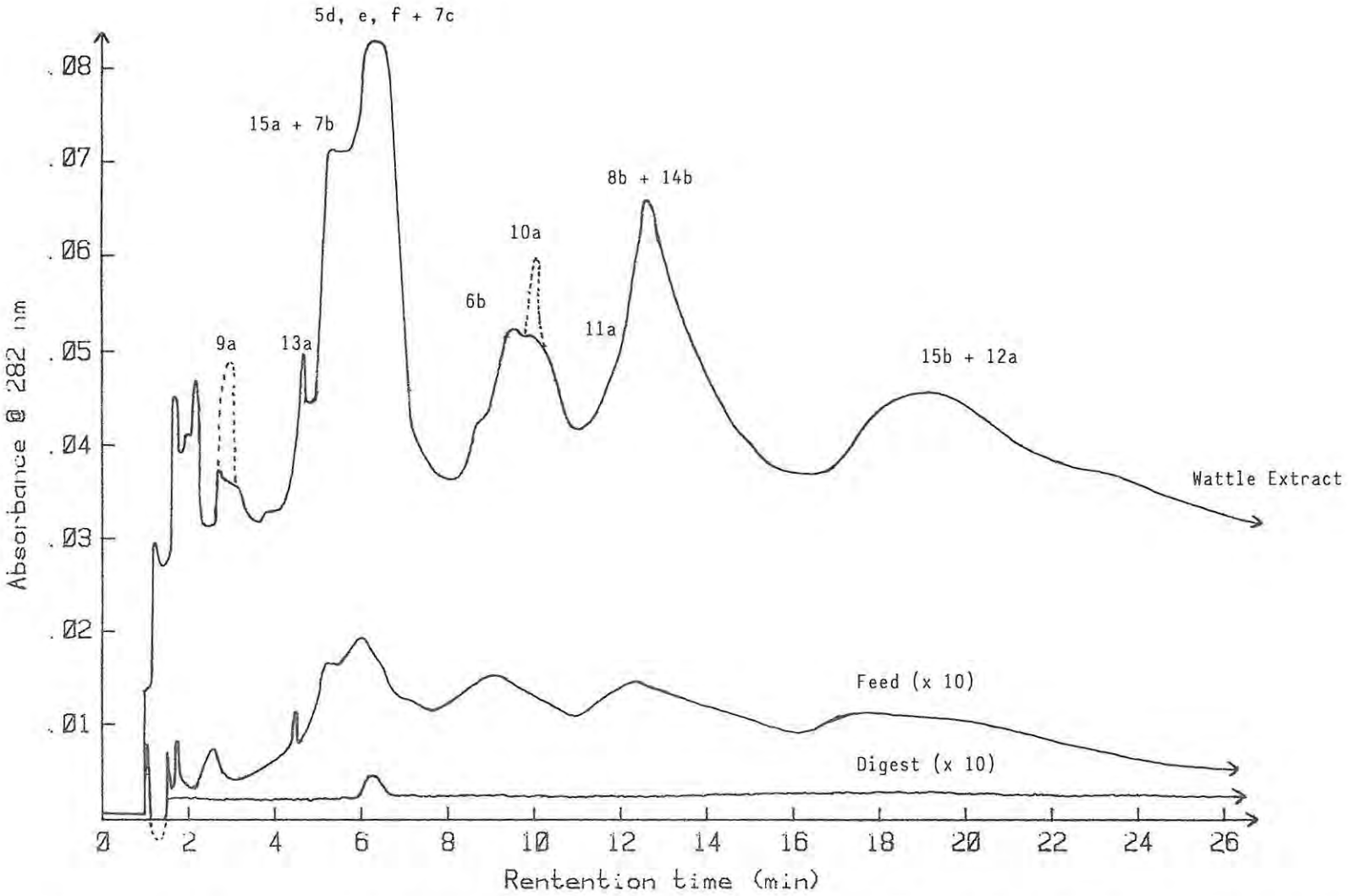


Figure 23. HPLC chromatograms of (A) Wattle Extract Sample, (B) Feed Sample (2 g/ℓ Wattle) and (C) Digest from test digester. Wattle Extract Sample : 15 µℓ of 2,5% aqueous wattle extract. Feed and Digest Sample : 20 µℓ. Mobile phase : MeOH-H_2O-Acetic acid (15:84:1). Flow rate : 2 mℓ/min. UV detection channel : 280 nm. Sensitivity : 0,2 AUFS for (A). 0,02 AUFS for (B) and (C).

Table 17. Identification of peaks in HPLC chromatogram (Fig. 23) according to Hendry[62].

Component[a]	Assignment
9a	(+)-Gallocatechin
13a	Biflavonoid ([4,8]-R-cis-G[b])
15a	Biflavonoid ([4,8]-R-trans-G)
7b	(-)-Robinetinidol
5d	)
5e	) ? unresolved
5f	)
7c	(+)-Catechin
6b	(-)-Fisetinidol
10a	Biflavonoid } ([4,8]-R-cis-C[b])
11a	Biflavonoid }
8b	(+)-Dihydrorobinetin
14b	Biflavonoid ([4,8]-R-trans-C)
15b	Biflavonoid ([4,8]-R-cis-C[b])
12a	Biflavonoid ([4,8]-F-cis-C[b])

(a) Component numbering according to Hendry

(b) Tentative assignment

R = (-)-Robinetinidol

C = (+)-catechin

G = (+)-gallocatechin

F = (-)-Fisetinidol

6.3 Attempted Isolation of Wattle Breakdown Products

Since both the PC and HPLC techniques used for monitoring the digester effluent (see sections 6.1 and 6.2) failed to reveal any identifiable polyphenolic components it was decided to concentrate larger volumes of the effluent and attempt to isolate phenolic compounds from these residues. Figure 24 gives a breakdown of the scheme used.

Direct drying of a sample with heat, even under reduced pressure, is not advised as it can result in oxidation of the light sensitive phenolics. Delcour[244] et al described a method for isolating flavonoids in beer where they pumped large volumes through a column of Sephadex LH20. The beer was adjusted to 10% ethanol content by volume and they recovered the flavonoids by eluting with an acetone - water mixture (60% acetone). Attempts to isolate flavonoids from the supernatant of centrifuged anaerobic effluent in a similar fashion were not successful however as the Sephadex bed became very swollen in water and flow rates of less than 5 mℓ/hour were obtained, even at high pumping pressures. The addition of alcohol to the digest (10% v/v) improved the flow rate somewhat but elution volumes were still inadequate. A second technique which was also abandoned involved the evaporation of the effluent contained in dialysis bags by hanging them in front of a fan overnight. Evaporation on the surface of the bag kept the temperature low which prevented oxidation of the phenolics but the residue left behind was very hard and gummy and proved difficult to redissolve. It was therefore decided to centrifuge the sample first and freeze dry the supernatant. Both the supernatant and the centrifugate were then examined for polyphenolics.

A 2 ℓ refrigerated sample of the effluent was therefore taken and centrifuged for 30 minutes at 8 000 rpm. The sludge was dried on a hotwater bath to produce 0,55 g of grey material which had a Kjeldahl nitrogen content of 8,2% N. Taking the average biological cell composition as $C_5H_9O_3N$[118], 9,3% N would be expected. The major proportion of this sludge was therefore taken as being of bacterial origin. To confirm that there was little wattle phenolic material associated in the sludge,

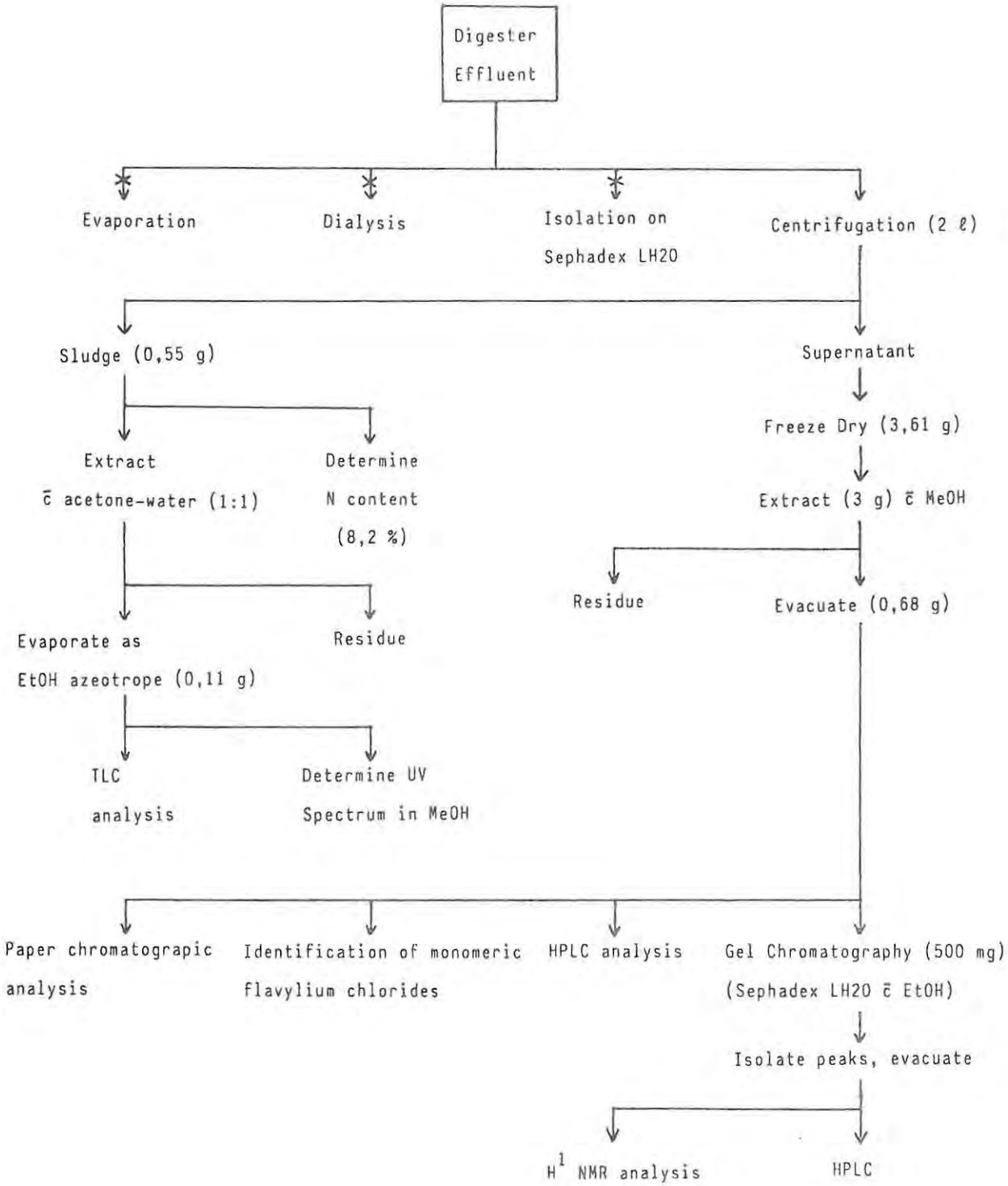


Figure 24. Scheme used for isolating and analysing phenolics from the test digester effluent

a further 2 ℓ of effluent was similarly centrifuged and the sludge treated with acetone-water (1:1) to release any bound tannin[219]. This supernatant was then evacuated to dryness at reduced pressure and temperature (< 60°C) as the EtOH azeotrope. A sample of the grey residue (0,11 g) was redissolved in MeOH (0,2 mg/mℓ) and the UV spectrum gave a very small peak at 280 nm. A spot of the sample on a TLC plate in benzene-acetone-methanol (6:3:1) did not leave the origin which indicates that it was of large molecular weight. Such a small amount of astringent polyphenolic material was considered not worth trying to isolate.

The supernatant from the original 2 ℓ of centrifuged digest effluent was freeze dried to yield 3,61 g of powdery brown material which proved fairly hygroscopic. Three grams of this material was extracted with MeOH (2 x 100 mℓ) at room temperature and filtered through a Gooch No. 4 crucible. the filtrate was evacuated under reduced temperature and pressure to produce 0,68 g of fine, brown-yellow waxy flakes. A sample of this product was separated by PC and gave the spectrum shown in Figure 25. From this separation it was seen that there was little movement of material in the alcohol phase except for one spot which had a R_f value of approximately 0,1. The major portion of the material showed some movement along the aqueous separation phase. The only discrete spot seen was a green-yellow spot under UV on the aqueous axis at R_f 0,6.

A sample of the above product (0,15 g) was also analysed for tannin content according to the standard hide powder method[216] (See Section 3.1.6). The results gave a total of 48% tannins out of 85% total solubles. The non-specificity of this method however does not allow much to be said about the type of material present. A third sample was run on HPLC as a fingerprint against the methanolic extract of wattle (see Figure 26). The chromatograms were seen to be very different. The wattle extract sample gave much more distinct peaks (for comparative identification see Figure 23). The majority of the effluent sample eluted near the solvent front which indicated less planarity and more sample-solvent interaction than the sample of the methanolic extract of commercial wattle extract. The product was also tested for the presence of procyanidins/prorobinetinidins[230] by

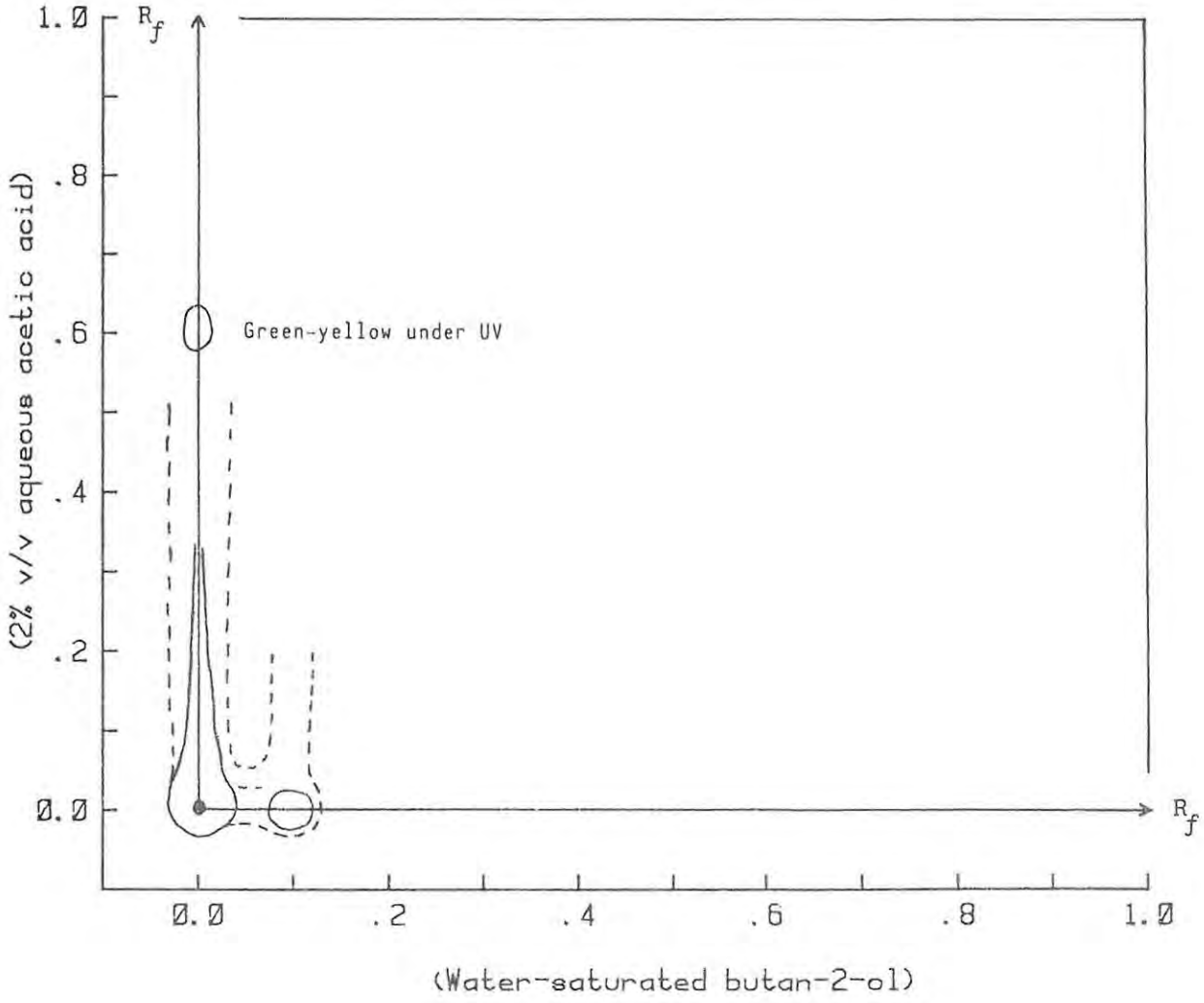


Figure 25. Two dimensional paper chromatogram of MeOH extracted isolate from freeze dried digester supernatant

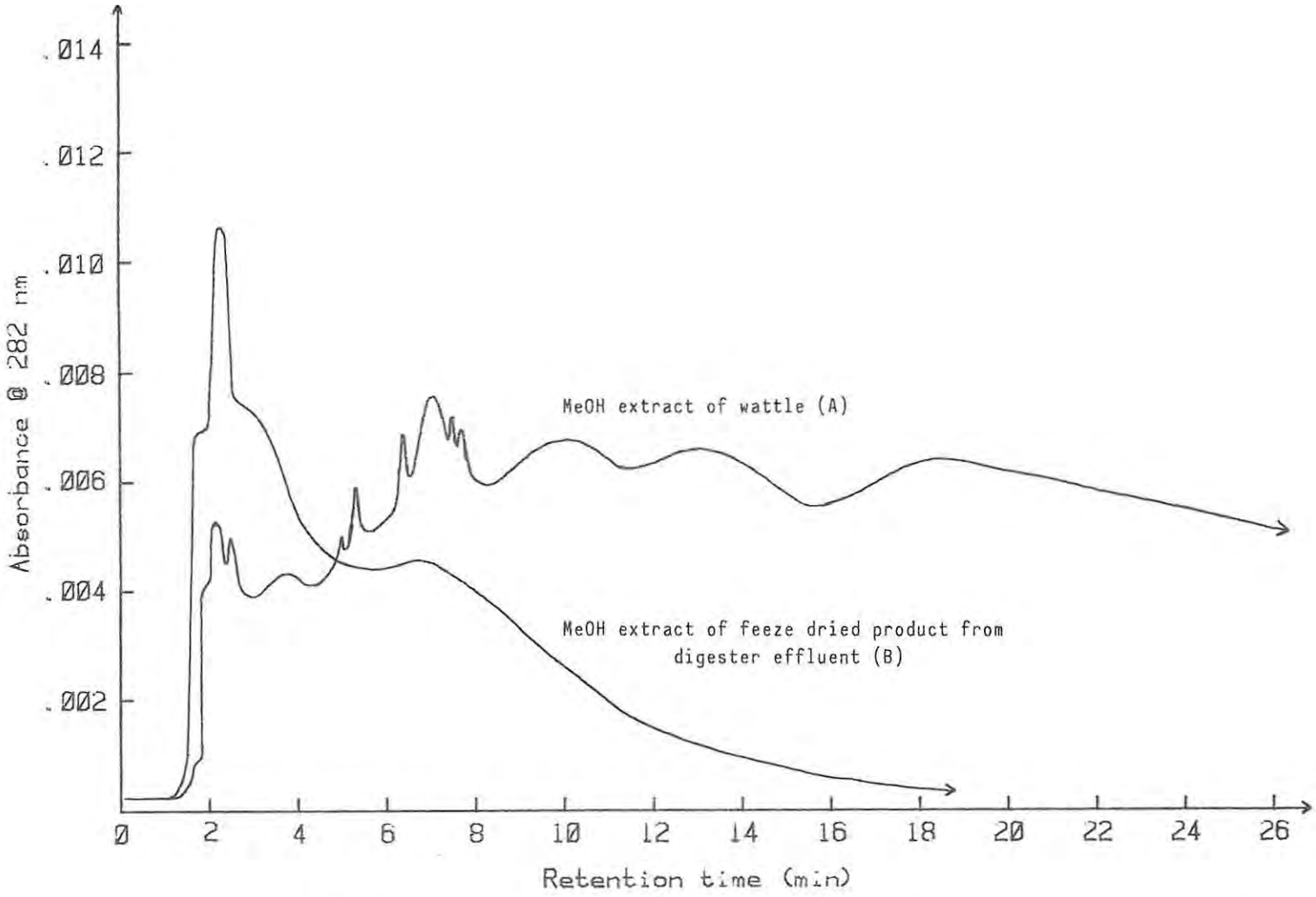


Figure 26. HPLC chromatograms of (A) methanolic extract of commercial Mimosa extract and (B) methanolic extract of freeze dried supernatant

Sample size : 15 µl. Mobile phase : MeOH - H_2O - acetic acid (15:84:1). Flow rate 0,2 ml/min. Pressure : 3000 p.s.i. UV detection channel : 280 nm. Sensitivity : 0,1 AUFS.

the formation of the monomeric chlorides and by comparison of mobilities on PC[250]. Very low yields of the robinetinidin and fisetinidin chlorides were obtained. The behaviour of the product on PC and HPLC as well as the inability to identify known wattle tanning structural units led to certain conclusions regarding the methanolic isolate:

(i) The product was very heterogenous.

(ii) It was hydrophylic.

(iii) It exhibited a certain degree of astringency but also contained a fair proportion of material of lower molecular weight.

(iv) The original tannin material seemed to have undergone a certain degree of oxidative or reductive condensation and rearrangement making it unrecognisable by the above isolation techniques.

As a final separation technique it was decided to use Sephadex LH20 gel chromatography in an attempt to isolate some identifiable phenolics. Figure 27 shows the elution profile of samples of (+)-catechin and quercitin used to standardize the column. The column had a void volume of 46 mℓ and gave a V_{R/V_o} ratio of 5,3 for catechin and 8,0 for quercitin (cf 5,3 and 8,3 - 8,4 respectively for other workers[62,245]). These analagous compounds both have 5 hydroxyl groups in the ring structure but quercetin elutes later because of the planarity imposed by an α, β —unsaturated 4-keto group in the heterocyclic ring as well as 4-carbonyl 5-hydroxyl group interactions. The column parameters used in this work were similar to those used in work previously carried out by Hendry[62] on fresh wattle extract and to whom many direct comparisons were made. Hendry identified monomeric flavonoids with elution ratios between V_{R/V_o} = 4 and 7, (Vo = 50 mℓ). Identifiable biflavonoids eluted after a V_{R/V_o} ratio of 7 and up to a value of 18. The eluted peaks represented 27% of the phenolic material present. The balance of the material in his study eluted after this point in one long unresolved band.

In this study, a sample of 500 mg of the MeOH extract of the digester isolate was placed on the column and eluted with EtOH. After 14 equivalent

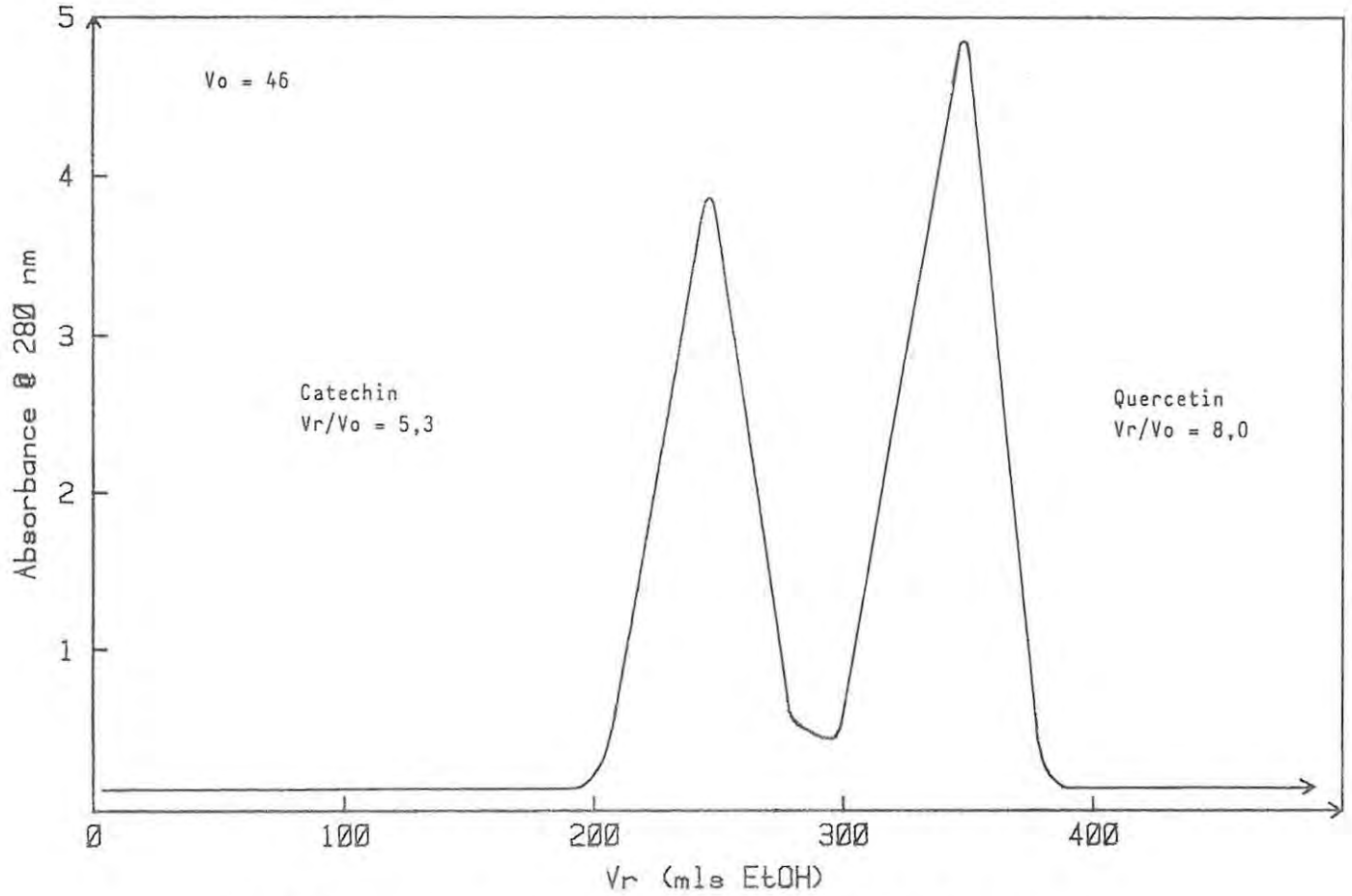


Figure 27. Elution profile of Catechin and Quercitin on Sephadex LH20 using EtOH as eluant and absorbance at 280 nm for detection

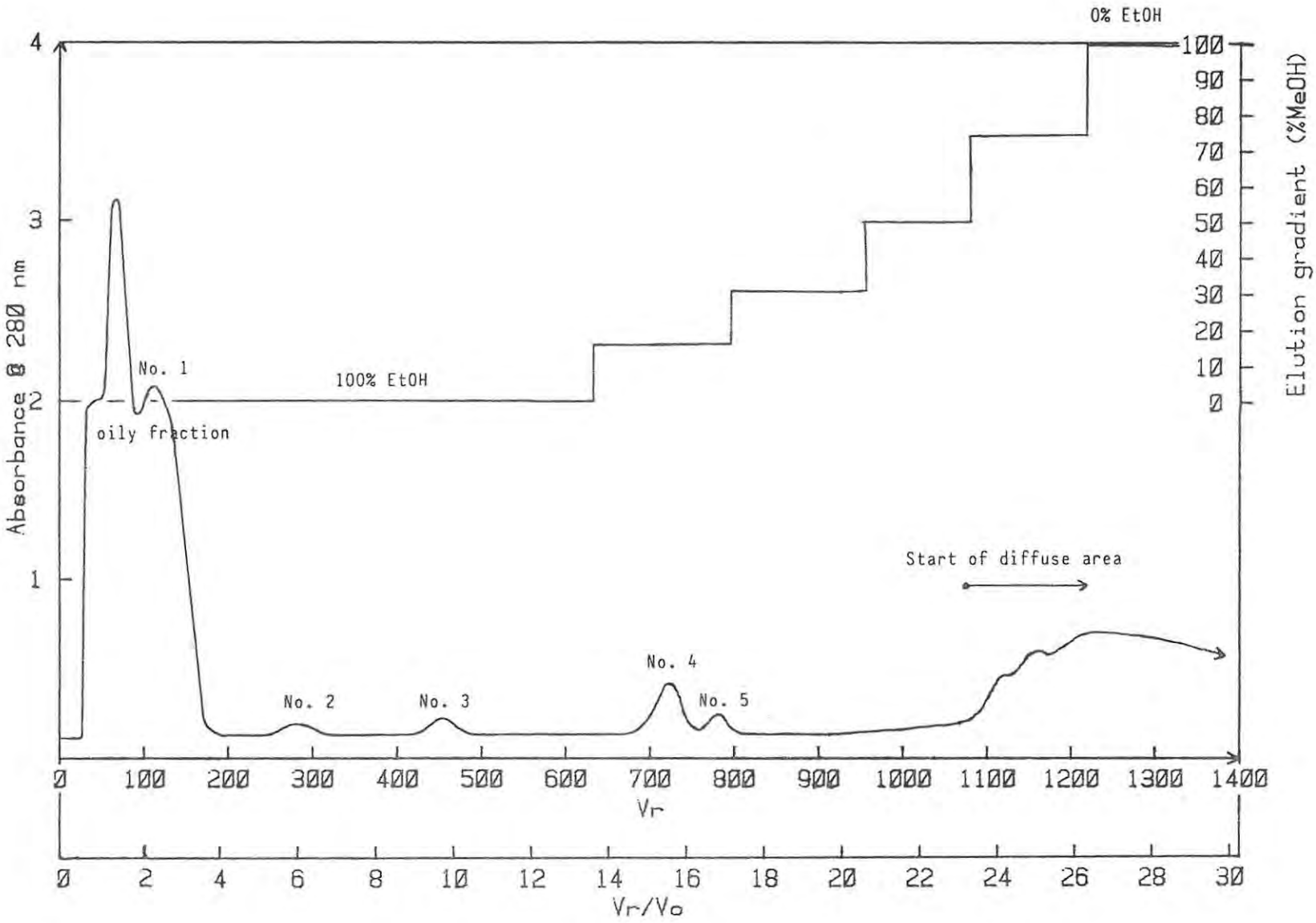


Figure 28. Elution profile on Sephadex LH20 of the sample isolated from anaerobic effluent as well as a profile of the eluant gradient

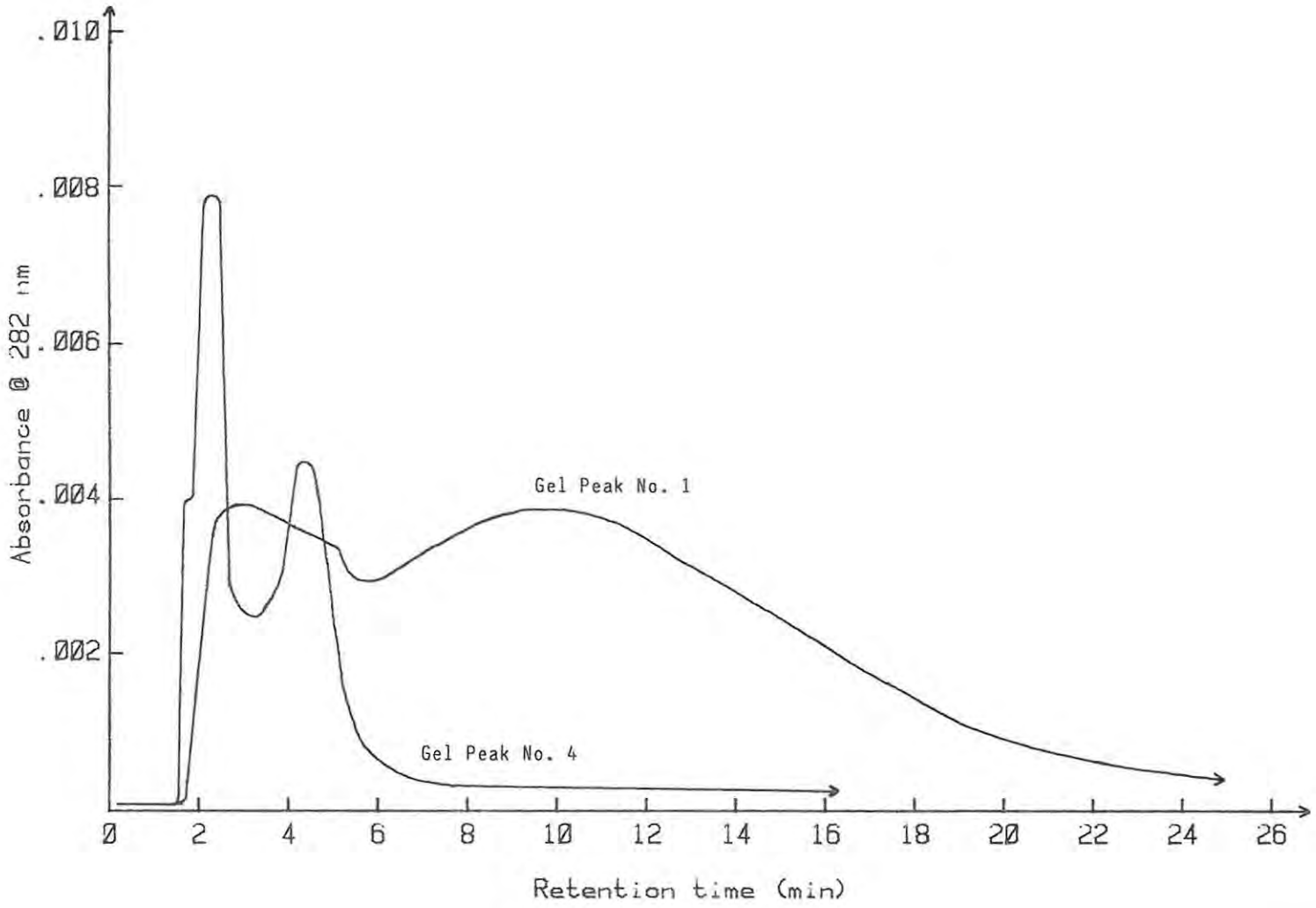


Figure 29. HPLC chromatograms of peaks No. 1 and 4 (Fig. 28) separated by Sephadex LH20 gel chromatography

void volumes of EtOH had eluted, increasing concentrations of MeOH were added in a stepwise gradient. The eluant gradient and elution profiles are given in Figure 28. The first group of fractions that eluted comprised 3 unresolved peaks, ($^{V}R/V_{o} \sim 2$), which were evacuated to an oily residue of $\pm$ 50 mg, (i.e. 10% of the extract). Fractions eluting before $^{V}R/V_{o} = 4$ had not been identified in Hendry's work, but here, further separation of the compounds was attempted on HPLC. The HPLC chromatogram is given in Figure 29 where only two very broad, diffuse peaks were seen. This sample was further investigated by H' NMR but the spectrum of the oil in MeOH was essentially featureless except for an upfield resonance at $\delta \sim 2,2$ and other smaller low frequency peaks around $\delta \sim 1$. As these compounds showed little aromatic structure and reasonable diversity they were not considered worth investigating further.

Peaks 2, 3 and 5 were too small ($<$ 5 mg each) to consider investigating further. From direct comparison of $^{V}R/V_{o}$ ratios in Hendry's work[62], they could possibly be monomeric (Peak No. 2, $^{V}R/V_{o} \sim 6$) and dimeric (Peaks No. 3 and 5, $^{V}R/V_{o} \sim$ 10 and 17 respectively) flavonoids but such an assigment is speculative.

Peak No. 4 ($^{V}R/V_{o} = 15$) yielded 10,5 mg (2% of the extract) of brown flakes which were subsequently further investigated by HPLC and NMR techniques. The HPLC chromatogram is given in Figure 29 where it was seen to consist of two major peaks which eluted near the solvent front. The first peak contained a shoulder which indicated at least 3 components were present. The H' NMR spectrum is given in Figure 30 and shows certain features characteristic of condensed tannin units[251,252]. Starting at the high chemical shift values (δ 7,75 - 6,30) there were aromatic multiplets and lower down (δ 3,8 - 5,8) heterocyclic doublet (e.g. 2-H and 4-H) and multiplet (e.g. 3-H) resonances typical of flavonoid-type structures. The methylene resonances (δ 3,4 - 3,75) were furthermore characteristic of the 4-CH_2function of catechin-type units. This sample contained a number of impurities however and was too small to allow further purification.

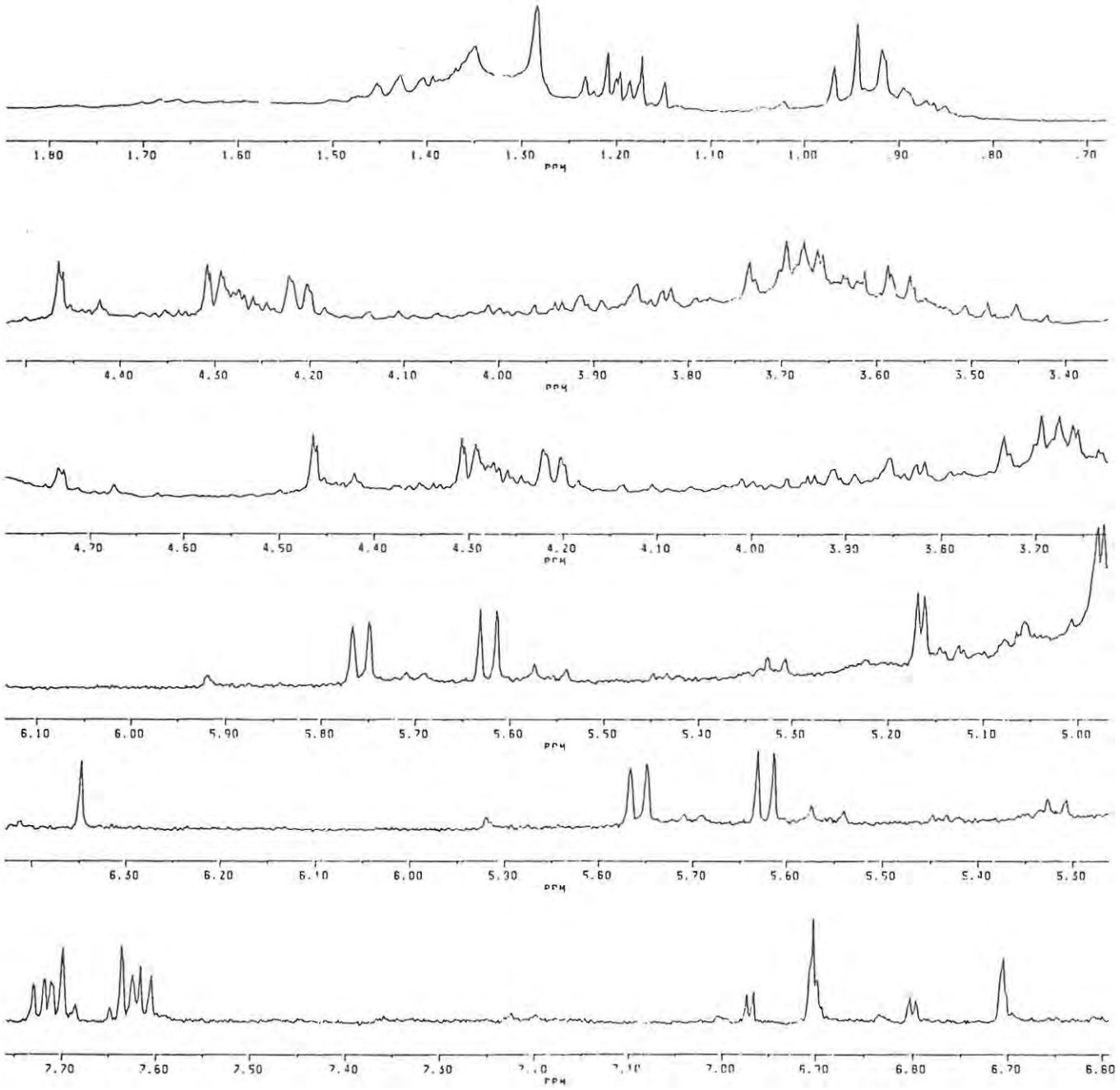


Figure 30. H' NMR spectrum of peak No. 4 (from Sephadex LH20 separation) run in MeOH with TMS as internal standard

These first few peaks eluted from the Sephadex LH20 column represented approximately 15% of the total material placed on the column. The major portion of the sample eluted in one unresolved area after V_{R/V_o} = 18 (i.e. beyond the limits of Hendry's[62] identifications) and when the solvent gradient reached 50% MeOH by volume. This material remained as a brown streak at the top of the column during elution with the EtOH phase but eluted rapidly at a higher MeOH concentration. This indicated that the material present was not necessarily of high molecular weight but that its initial immobility was due to insufficient interaction with the less polar ethanolic eluant. Therefore, an oxidised, reduced or rearranged tannin structure of greater planarity than fresh tannin extract was implied.

It was concluded that although tannin-like material could be found in the anaerobic effluent, it was more hydrophilic than fresh extract and altered so as not to be easily identified. Simple compounds could also not be isolated using methods common to flavonoid research.

7. SUMMARY

Wattle tannins are an effluent component present in vegetable tanning wastewater. They are biologically recalcitrant and have a persistent discolouring effect on receiving waters. The commercial tanning extract (Mimosa) consists essentially of the naturally occurring condensed proanthocyanidins whose breadown in nature is presided over mainly by the soil and sewage microfauna. A wide spectrum of treatment strategies have been proposed for vegetable tanning effluents and these include a number of physico-chemical and biological procedures. Very few accounts of treatment by anaerobic digestion appear in the literature and moreover those that do are contradictory insofar as amenability to treatment and colour removal are concerned. Anaerobic digestion of industrial wastes in general is a reasonably new application of this technology which has come about mainly as a result of a favourable energy balance and low sludge production (cf aerobic digestion). An anaerobic system represents a symbiotic relationship between numerous bacterial species that requires careful control and monitoring in a well designed reactor to ensure optimum functioning. In this study, the amenability of commercial extract to anaerobic digestion was investigated and an attempt was also made to isolate breakdown products.

Methods for monitoring and control were investigated initially and it was decided to use gas chromatography for quantifying biogas yields and volatile fatty acid production rates. Alkalinity was measured by pH titration while influents and effluents were continually monitored for solids and oxygen demand (PV and COD) removals by standard methods. Tannin breakdown was monitored directly by UV measurements (280 nm), by the formation of the coloured molybdate ion complex and by the Folin-Ciocalteu method for phenols. In a small scale amenability test it was shown that an unacclimitized sewage anaerobe population could function with up to 1,1 g/ℓ wattle as a shock load but were severely inhibited above this concentration level.

In the main study, two 9 ℓ upflow anaerobic sludge blanket digesters were started on sewage sludge and fed a synthetic feed medium (glucose as carbon source). Temperature was maintained at 35°C and feeding was semi-continuous at a hydraulic retention time of 9 days. Incremental additions of wattle up

to a level of 2 g/day were then made over a period of 3 months to the one digester with the other used as a control. This represented 35% and 69% of the feed COD and PV respectively. At this level, the loading rate was 3,8 kg COD/m^3/day (sludge bed volume) and the COD removal efficiency 74%. An 83% yield of biogas was achieved which contained 57% methane. The digester was stable and tannin removals were of the order of 50%. Significant precipitation of the tannins into the sludge bed was discounted by analyses of samples of the sludge and theoretical biogas production figures. Colour removal was not effected. In further phases it was shown that the anaerobic system could not function with wattle extract as the sole carbon source although the tannins were shown to be more bacteriostatic than bacteriacidal.

Two dimensional paper chromatography and HPLC methods were used in an attempt to determine which components of the structurally complex tannin extract were removed by anaerobic digestion. The respective chromatograms of the effluent showed no spots or peaks which could be equated with those identified in the influent. Samples of the effluent were then freeze dried, extracted with methanol, separated on Sephadex LH20 and analysed by HPLC and H' NMR methods. It was concluded that although the presence of tannin-like material could be found in the anaerobic effluent it was more hydrophilic than fresh extract and altered so as not to be easily isolated or recognized by methods common to tannin research.

8. APPENDICES

Appendix I

GC Column Preparation and Efficiency

Two gas chromatographic columns, one for VFA and one for biogas, were prepared as follows:

Borosilicate glass of 2 m length and 3 mm i.d. was shaped to standard column size and annealed. These columns were then silylated with a 5% solution of Dimethyldichlorosilane in toluene, washed with MeOH and baked at 250°C overnight. They were packed under vacuum with 10% SP 1000/1% H_3PO_4 on Chromosorb W-AW 100/120 for VFA and Waters Associates Poropak type N for biogas. The packing material was supplied by Langet Laboratories. The open ends were stoppered with phosphoric acid treated glass wool. The columns were then run at elevated temperatures and flow rates for 4 hours to stabilize the packing materials before the operating parameters were optimized for each separation. These optimized parameters are given in sections 2.2.2 and 2.2.5.

Sample retention times were not adjusted for mobile phase hold-up time as peak identification was achieved by direct correlation with appropriate standards.

Column efficiencies reflect the amount of band spreading occurring during passage through a chromatographic system and is a measure of column integrity. Column efficiencies in this work were calculated initially and after 2 years running by determination of theoretical plate numbers. The theoretical plate number may be defined as[253].

$$n = 16(t_R/W_b)^2$$

where, t_R = Retention time (total)

w_b = peak width at base

Efficiencies for the columns prepared were as follows:

Column	n (Initial)	n (after 2 years)
VFA	3 460	3 180
Biogas	1 580	1 540

Appendix II

ABBREVIATIONS

AD	Anaerobic Digestion
a.m.u.	atomic mass units
ASP	Activated Sludge Plant
BA	Bicarbonate Alkalinity
BOD_5	Biochemical Oxygen Demand (5 days)
COD	Carbonaceous Oxygen Demand
DS	Dissolved Solids
GC	Gas Chromatography
HPLC	High- Performance Liquid Chromatography
HRT	Hydraulic Retention Time
i.d.	internal diameter
MW	Molecular Weight
NMR	Nuclear Magnetic Resonance
PC	Paper Chromatography
PV	Permanganate Value
r_{cat}	Retention time relative to (+)- catechin
R_f	Retention time relative to the solvent front
R_T	Retention time absolute
SS	Suspended Solids
TLC	Thin Layer Chromatography
TOC	Total Organic Carbon
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
Ve	Volume of elution
VFA	Volatile Fatty Acids
Vo	Void volume
VS	Volatile Solids
VSS	Volatile Suspended Solids

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