DECOLOURIZATION OF WOOD-ETHANOL STILLAGE USING A GRANULAR ACTIVATED CARBON PACKED ANAEROBIC EXPANDED-BED REACTOR

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF TECHNOLOGY IN BIOTECHNOLOGY AT MASSEY UNIVERSITY

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ABSTRACT

The anaerobic treatment (including decolourization) of wood-ethanol stillage from the Forest Research Institute (FRI) wood-hydrolysis pilot plant at Rotorua has been investigated using granular activated carbon (GAC) packed expanded-bed reactors. Specifically, bioregeneration of the GAC in the reactors in terms of organic and colour removal has been considered.

Two 7.2 l anaerobic expanded bed (AEB) reactors were designed and built. Reactor One (R1) was used for the anaerobic digestion of raw wood-ethanol stillage and Reactor Two (R2) for the decolourization of anaerobic lagoon pretreated wood-ethanol stillage. For R1, a desulphated stillage feed (to 500 $mg.l^{-1}$ sulphate) was used. Depending on the organic loading rate (OLR), the additions of nitrogen (N), phosphorus (P) and alkalinity reagent ranged from 240-350 $mg.l^{-1}$, 80-250 $mg.l^{-1}$ and 2.5-4.5 ml 20% w/v NaOH per litre feed respectively. Only N and P feed supplements were used for R2 at 240 and 80 $mg.l^{-1}$ respectively. The reactors' performance and stability were closely monitored through analyses of volatile fatty acid's, pH, alkalinity, colour, chemical oxygen demand (COD), sulfide, biogas production rate and methane composition, solids concentrations, N and P.

After operating R1 for 227 days, it was demonstrated that this system, is superior to the previous systems reported for the treatment of a similar stillage. A non-maximal OLR of 29.0 kg $tCOD.m^{-3}.d^{-1}$ at 0.85 d hydraulic retention time (HRT) with total and soluble COD (tCOD and sCOD) removals of 74.5 and 83.5% respectively were achieved. Digestion stability was excellent with acetate at 160 $mg.l^{-1}$, propionate at 490 $mg.l^{-1}$ and a gas methane composition of 61.0%. The colour loading rate was 4.7 kg $chloroplatinate.m^{-3}.d^{-1}$ with a 75% colour removal. Higher colour and COD removals may be obtained by operating at a longer HRT (e.g. the percentage colour and sCOD removals were 90.6 and 91.8% respectively at a 2 d HRT). Previously, no significant colour removal for the anaerobic digestion of wood-ethanol stillage has been reported. In this study, only approximately 9% w/v of the chromophoric materials

present in the wood-ethanol stillage are particularly recalcitrant to anaerobic degradation. The methane gas yield was near to that predicted by theory (99.7% at 2 d HRT) with a very low sludge yield (2.8% based on 91.8% sCOD removal). Consequently, the AEB reactor had a very low nutrient requirement for effective treatment. In terms of reactor stability, it can accomodate very high hydraulic loading rates (less than 0.85 d) without problems of cell washout. The use of activated carbon medium also provides a toxicity sequesting potential against biological inhibitors present in the wood-ethanol stillage. Continuous bioregeneration of the GAC in R1 has also been demonstrated using sCOD and colour breakthrough curves for GAC adsorption with and without biological activity. Microbial degradation of the chromophoric species has been confirmed using UV-visible spectrophotometric scans.

Little methanogenic activity was observed in R2 in its 191 days of operation due to the recalcitrant nature of the anaerobic lagoon pre-treated stillage. Only approximately 20% bioregeneration of GAC in terms of colour removal was achieved at a colour loading rate of 1.2 kg chloroplatinate.m^{-3}.d^{-1}.

This study has demonstrated that the GAC packed expanded-bed reactor (R1) provides a very effective treatment of wood-ethanol stillage (including decolourization) while recovering a very significant portion (89%) of the stillage energy. Considerable capital and operating cost savings are possible using the AEB system since effective treatment can be achieved in a single step utilizing a relatively small reactor with minimal nutrient, sludge disposal and GAC regeneration or replacement costs. The only disadvantages of the system are the carbon cost, a long start-up period of 5 months and a recycle energy cost to maintain an expanded-bed. It is believed that they can partly be reduced by using a GAC carrier with a smaller particle size.

Anaerobic digestion, utilizing a GAC packed expanded-bed reactor, thus represents a cost effective and commercially attractive option for the utilization/disposal of wood-ethanol stillage.

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PRELUDE

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PRELUDE

The oil crisis in the 1970's and the current projected shortage in petroleum, a non-renewable fuel, have led to a new surge of interest in the search for alternative sources of energy. Biomass can provide an alternative and renewable energy source and, its conversion to ethanol has been viewed with particular interest as it can then be incorporated directly as fuel into motor vehicles.

The desire to reduce imports of petroleum has led New Zealand (NZ) to an examination of alternative routes to liquid fuels. The production of ethanol from wood is an important liquid fuel option in NZ where a large increase in exotic wood availability will occur early in the next century. As part of its Forest Biomass Program, the Liquid Fuels Trust Board (LFTB) commissioned an extensive review of the status of wood hydrolysis technologies (Arthur D. Little International Inc., 1983). Among the recommendations were to continue the commercialization of dilute acid hydrolysis in NZ using wood or wood waste and, to conduct research in methods to increase the utilization of ligno-cellulosic feedstocks. Interest in energy from biomass is not limited to NZ but has been widely practised overseas as indicated by the recent symposia and publications on 'Energy From Biomass' (Schlegel and Barnea, 1976; Klass and Weatherly, 1980; Cheremmisinoff et al., 1980; Samir and Zaborsky, 1981; White and Plaskett, 1981; Palz et al., 1981; Wise, 1981).

The LFTB has also funded a two-year project for the New Zealand Forest Research Institute (FRI) to characterise effluents from the FRI wood hydrolysis process, and to investigate suitable waste treatment/utilization processes at the laboratory and pilot scales.

The FRI has developed a pilot plant for the production of ethanol from exotic forest trees. This plant involves high temperature dilute acid hydrolysis of the wood cellulose and hemicellulose to simple sugars which

are used as a feed stock for ethanol production by yeast fermentation. However, the process typically produces 30 m^3 of stillage per m^3 of 95% ethanol after distillation. Thus large volumes of stillage will be produced when the FRI process is commercialised and some form of treatment will be required before the wastewater can be discharged to a receiving water.

Anaerobic digestion of the wood-ethanol stillage has proven feasible and offers the benefits of low sludge yield, low nutrient requirements and low operating cost as it is a net energy producer. Previously, anaerobic lagoons, an upflow anaerobic sludge blanket (UASB) reactor, an anaerobic filter and an anaerobic continuous stirred tank reactor (CSTR) have been investigated and, although such treatments demonstrated good removals of chemical oxygen demand (COD) and biological oxygen demand (BOD), no significant reduction of the intense colour has been observed. The chromophoric constituents in the stillage appear to be due to lignin-derived aromatic compounds and sugar condensation products. The recalcitrant nature of the chromophores will give a long lasting discolouration when discharged into a receiving water where they respond very slowly to stream self-purification (Cox, 1981). Although there is still no firm evidence that colour introduced into the receiving water is detrimental to aquatic life (Whittemore and McKeown, 1978), an unnaturally coloured stream is usually considered 'polluted' in the eyes of the general public.

In NZ, a requirement under the Water and Soil Conservation Act (1967) for colour discharge (e.g. Fourth Schedule, Class D water, Section (e)) is that 'the natural colour and clarity of the water shall not be changed to a conspicuous extent'. Due to the vague definition of the receiving water standards set under the Act, the effect of colour is expected to vary according to location (in terms of public acceptance) and the receiving water (in terms of flow characteristics, dilution factor and water ecology).

The effect of effluent colour on a receiving water for wood-ethanol stillage discharge is probably best discussed in the context of pulp and paper mill discharges in NZ. The Commission for the Environment

(NZ) has reported a great reduction in the water qualities of a few NZ waterways particularly the Waikato and the Tarawera Rivers due to pulp and paper mill discharges (Cox, 1981). The main feature is a significant colour change (brown discolouration) from the discharges of the two largest pulp and paper mills in NZ. Treatment of the stillage in terms of organic and colour removal is thus expected to be mandatory for a full scale wood-hydrolysis plant.

Studies have shown that aromatic compounds of lignin origin can be degraded anaerobically to lower molecular weight compounds and to end products of methanogenesis. One reactor design that was considered to have potential in the anaerobic degradation of refractory chromophoric compounds was a high rate anaerobic fixed-film process using granular activated carbon (GAC) as the biomass support. This integrated biological-chemical-physical treatment system has previously been shown to enhance biological activity resulting in the degradation of otherwise recalcitrant compounds. Thus anaerobic decolourization of the stillage was considered conceptually feasible and merited investigation.

This project therefore aimed to assess the feasibility of using the anaerobic expanded-bed reactor packed with GAC for the anaerobic digestion and decolourization of wood-ethanol stillage.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This literature review aims to provide:

(i) An outline of the FRI hydrolysis process and the characteristics of the wastewater and other pollutants produced,

(ii) An assessment of current wastewater treatment and utilization options for wood-ethanol stillage. Emphasis will be given to the anaerobic digestion process and the use of activated carbon.

Wood-ethanol stillage is the watery by-product of distillation (bottom residue) and is the most significant wastestream produced by the wood hydrolysis process. Of all the methods available for stillage treatment, the high rate anaerobic digestion process will be shown to be the most appropriate as it provides an effective treatment of the stillage and generates energy during the process.

However, although anaerobic digestion, in its present designs, is effective in removing organics from wood-ethanol stillage, the effluent is still of unacceptable quality for discharge into a receiving water mainly due to its intense colour. Studies have shown that the chromophores are from lignin derived aromatic compounds and sugar condensation products. The possible methods for the degradation of these compounds are presented with particular reference to the use of granular activated carbon in wastewater treatment.

The anaerobic digestion process for wastewater treatment has greatly advanced since the turn of the century and has received renewed interest since the energy crisis of the 1970's. In this respect and its particular bearing on this research study, a background to the anaerobic digestion process for wastewater treatment is included with emphasis on the 'high rate' processes of the post 1950 period.

2.2 FRI WOOD HYDROLYSIS PROCESS

In the FRI wood hydrolysis process, wood carbohydrates are converted to sugars which are subsequently fermented to ethanol. Figure 2.1 presents a schematic layout of the process. The essential features are (Callander, 1983(b)): -

(i) Batch percolation of dilute acid or recycled hydrolysis liquor through a stationary bed of wood in the form of sawdust and chips.

(ii) Draining and pressing the residual solid lignin to reduce its moisture content before feeding it to a boiler to raise process steam.

(iii) Neutralization of the wood sugar liquor with lime at 140°C to minimize the residual gypsum left in the solution.

(iv) Treatment of the sugar liquor with sulphite to improve its fermentability.

(v) Fermentation of hexose sugars, using yeast, to ethanol, carbon dioxide and minor end products.

(vi) Distillation of the fermentation 'beer' to recover ethanol.

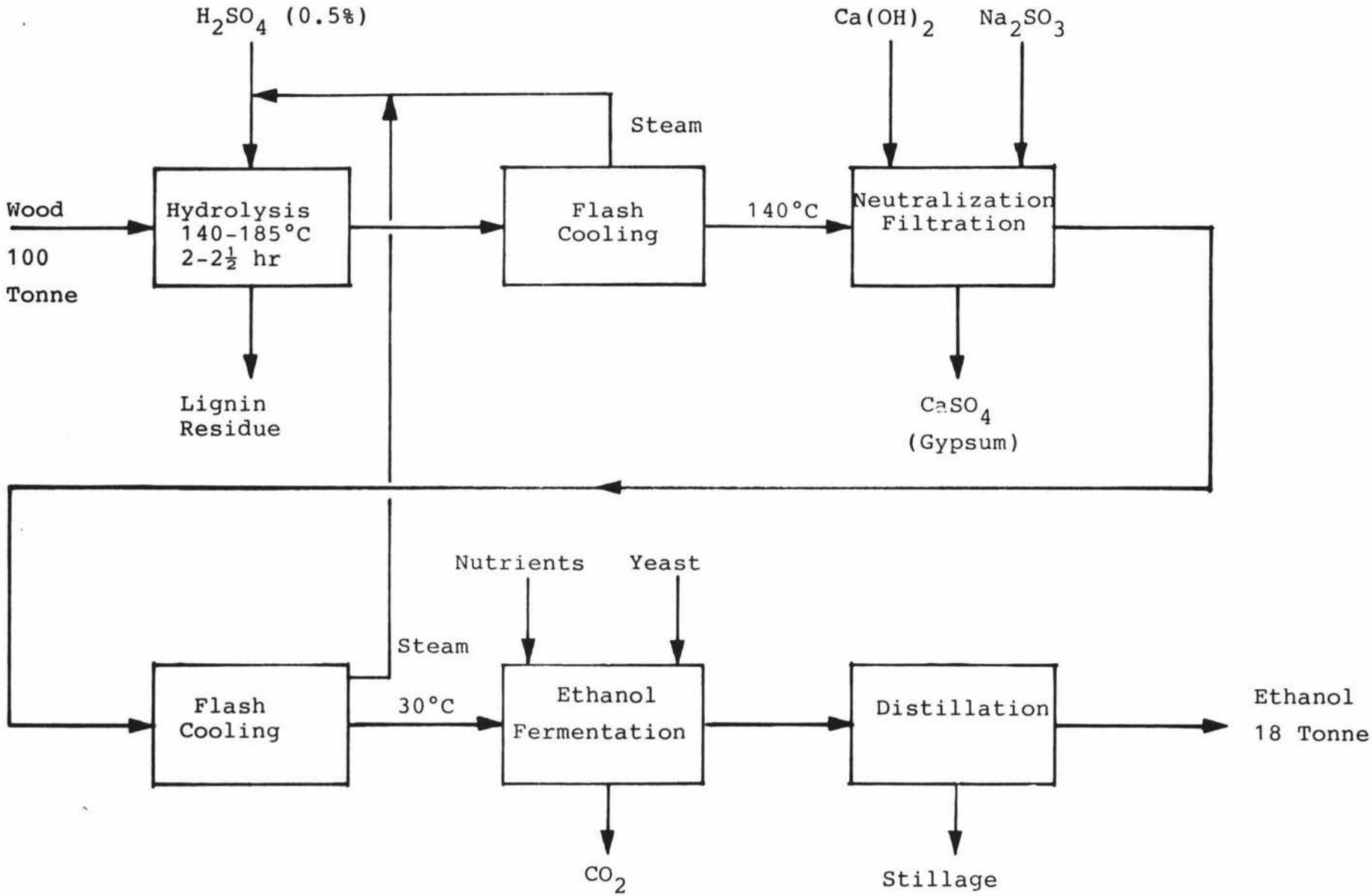
Table 2.1 presents a mass balance of the process based on data obtained from NZFRI for *Pinus radiata*.

TABLE 2.1: MAJOR PRODUCTS FROM DILUTE ACID HYDROLYSIS OF *P radiata*
(Basis: 100 tonne dry wood) (Callander, 1983(b))

Product		Mass (tonnes)	
Lignin		26.8	
Ethanol		20.5	
Carbon dioxide (from fermentation)		21.5	
Stillage:			
Pentose sugars	7.0		
Furfural	.85		
Hydroxymethyl furfural	1.2		
Laevulinic acid	1.8		
Formic acid	0.8		
"Tars" or humic organic matter	25.00	36.7	
TOTAL		105.5*	

*Note: Product mass exceeds wood entering process due to uptake of water during hydrolysis

FIGURE 2.1: A FLOWSHEET OF THE FRI WOOD HYDROLYSIS PROCESS



Since hydrolysis lignin can be burnt to raise steam and the CO_2 harmlessly released into the atmosphere, the two waste streams which require some means of disposal are:

(i) Gypsum filter cake - The consideration of gypsum disposal techniques is beyond the scope of this project.

(ii) Stillage - This constitutes 36.7% of the organic matter entering the wood hydrolysis process and represents 30% of the wood energy, equivalent to that contained in the ethanol product. In view of the high organic content of the stillage, it is proposed that it be utilized to generate a by-product with a market value rather than just being disposed of (Callander, 1983(b)).

Anaerobic digestion is one means of recovering a useful product from a wastewater and in the following section, the characteristics of wood ethanol stillage, especially with regard to anaerobic digestion, will be summarised.

2.3 CHARACTERISTICS OF WOOD-ETHANOL STILLAGE

Typically, ethanol production from the FRI acid hydrolysis pilot plant produces 30 m^3 of stillage per m^3 of 95% ethanol after distillation. As presented in Table 2.1, the stillage constitutes 36.7% of the dry wood organics representing the largest single hydrolysis product compared to lignin (27%), ethanol (20%) and CO_2 (21%).

Mass balances done at FRI show that the origins of the stillage organic matter are about (Callander, 1983(b)): -

- 60% from the degradation of hexose and pentose sugars e.g. acetic, formic and laevulinic acids, furfural, hydroxymethylfurfural, aromatics and condensation polymers.

- 20% from solubilised wood extractives and non-carbohydrate wood components.

- 19% from unfermented pentose sugars (xylose and arabinose).

A detailed analysis of the stillage is presented in Table 2.2.

TABLE 2.2: CHARACTERISTICS OF THE STILLAGE GENERATED BY FRI PILOT PLANT HYDROLYSIS PROCESS (Callander, 1983(b)) (Basis: 18.5 kg stillage/kg dry wood)

	$mg.l^{-1}$ (unless specified)
COD	23,000
BOD_5	14,000
TS*	13.7 ($g.l^{-1}$)
SS*	0.4 ($g.l^{-1}$)
VS*	8.8 ($g.l^{-1}$)
N (total)	18
P (total)	3
S (SO_4^{2-})**	600
Na	175
K	29
Mg	21
Ca	1410
Cr	3.0
Mn	3.0
Fe	73
Co	<.03
Ni	11
Cu	<.05
Zn	.65
Se	<.4
Sr	1.4
Sn	<.05
Pb	<.3
B	.24
phenols	0.5
pH	4.5-5.0
colour	2400 (CPU)

* Data from Archer et al, (1983)

** SO_4^{2-} level is dependent on neutralization temperature

The stillage is essentially a high strength soluble waste of low pH and is highly coloured. It also has quite high levels of calcium and sulphate. The sulphate concentration is dependent upon the neutralization temperature with concentrations of 470 and 1800 $mg.l^{-1}$ being recorded at respective temperatures of 140 and 80°C at equilibrium. However, 500 $mg.l^{-1}$ SO_4^{2-} is a realistically achievable level for a commercial scale operation (Callander, 1983(a)). The concentrations of other metals are low with large proportion of the nitrogen, phosphorus, sodium, potassium, magnesium and other metals in the stillage originating from added nutrients in the fermentation stage. Chromium, nickel and iron are almost certainly primarily derived from reactor corrosion in the hydrolysis process (Callander, 1983(b)).

There are many ways of utilizing or treating stillage. One disposal alternative, anaerobic digestion incorporating colour removal was the object of this study. Much of the COD such as pentoses, residual hexoses and acetic acid will be easily degraded anaerobically. However, there may be treatability problems due to:

(i) The presence of inhibitory compounds produced during the acid hydrolysis process by hydroxymethylfurfural, lavulinic acid and furfural (Jaenchen et al., 1981; Henze and Harremoes, 1982; Callander et al., 1983).

(ii) The high concentration of sulphate ions in the neutralized wood sugar solution. This will cause high soluble sulfide concentration in the digester which will inhibit the methanogenic bacteria;

(iii) Nutrient deficiencies, particularly in nitrogen (N) and phosphorus (P). The N and P requirements for anaerobic digestion of many substrates (e.g. acetate, glucose, starch and nutrient broth) have been estimated by Speece and McCarty (1964) (see Section 2.5.3.1 (f)).

Thus, some form of stillage modification and the use of acclimatized methanogenic bacteria will be required for the anaerobic digestion of wood-ethanol stillage. The metal and nutrient requirements for the anaerobic digestion of FRI stillage have already been studied (Callander,

1983(a); Callander et al., 1983) and will be considered in Section 2.6. The Section to follow will consider the alternative methods of disposing of stillage.

2.4 ALTERNATIVE TREATMENT TECHNIQUES FOR WOOD-ETHANOL STILLAGE

The alternative methods of stillage disposal can be listed as (Jackman, 1977; Ripley, 1979; Costello Branco and Costa Ribeiro, 1980; Sheehan and Greenfield, 1980; Coombs, 1981; Costa Ribeiro and Costello Branco, 1981; Willington and Marten, 1982; Callander, 1983(b); Cabib et al., 1983; Essien and Pyle, 1983; Maiorella et al., 1983).

(i) Direct discharge to an adjacent waterway, land area or marine outfall;

(ii) Evaporation or concentration of the stillage for:

- combustion in process boiler to generate steam;
- incineration for the production of potassic ashes and phosphate to be used as a fertilizer;
- use as animal food supplement;

(iii) Fodder yeast and biochemicals production;

(iv) Wet oxidation using oxygen at high temperatures and pressures;

(v) Pentose fermentation;

(vi) Irrigation;

(vii) Conventional biological oxidation;

(xiii) Anaerobic digestion (detailed in Section 2.6).

These stillage treatment options either dispose of the organics of the stillage or combine the utilization of the organics with the production of a useful or marketable by-products (chiefly stock feed, fodder yeast, fertilizer or biogas).

They vary enormously in their environmental consequences and the cost of treatment. The stillage handling options which require the smallest capital investment usually involve discharge of one sort or another (Option 1). With the possible exception of stillage handling by irrigation, all the other options are generally more expensive though they are environmentally more desirable.

For the commercial production of fertilizer, the stillage potash and phosphate concentrations are low. For irrigation, there is a limit to the amount of stillage that can be added to farm land due to the changes in soil ion balance, water logging and toxicity effects on soil microflora (Coombs, 1981). These effects can be minimized by distributing the stillage over a large land area, as applicable to the New Zealand situation, but this means a more expensive distribution system.

A lot of interest is given to biomass or biochemicals production utilising stillage as the raw material (Sheehan and Greenfield, 1980). This is becoming increasingly more viable as the price of oil-based competing products rise. The most significant of these is fodder yeast production both in the Western World and Russia. However, this option usually requires an expensive product recovery step and the remaining liquor (still high in BOD though all the easily degradable organics were consumed) may itself constitute a waste disposal problem.

Evaporation or concentration of the stillage as a stock feed supplement has been shown to be possible (Sheehan and Greenfield, 1980). Dry stillage is a laxative in cattle but also improves milk yields. Feeding trials would be required to quantify the effects of concentrated wood-ethanol stillage addition as a feed supplement, the optimal dose required and any necessary stillage modifications (e.g. de-ashing, neutralization and mineral addition) if this option is to be considered.

In a preliminary investigation for wood-ethanol stillage, evaporation followed by combustion of the concentrate or wet oxidation of the whole stillage were considered the 'best' alternatives to anaerobic digestion (Callander, 1983(b)). Both methods would effectively dispose of all

the stillage organics (including colour) while recovering a portion of the stillage energy content and process water as steam.

The problems anticipated for these alternatives are the possibilities of evaporator scaling and ash accumulation in the boiler (mainly calcium salts). A high capital and operating cost (particularly with wet oxidation) is also expected.

Conventional aerobic wastewater treatment processes are not favoured since they require excessive waste dilution, do not recover any of the stillage energy content, have high capital and operating costs, require large land areas and generate large volumes of biological sludge (Coombs, 1981; Callander, 1983(b)).

Another stillage utilization option that has received great attention recently is the fermentation of pentoses, chiefly xylose to ethanol by *Pachysolen tannophilus* (Schneider et al., 1981; Maleszka et al., 1981; Wong et al., 1982; Schneider et al., 1983; Kurtzman, 1983; Jeffries, 1983; Debus et al., 1983; Deverell, 1983; Arthur D. Little International Inc., 1983). The ability of this yeast to ferment xylose with a yield in excess of 72% has only recently been identified. Unless the yeast can be incorporated into the hexose fermentation step, it is likely that the pentose fermentation will suffer the problem of expensive product recovery due to the low concentrations of ethanol involved. Also it is not a feasible stillage disposal option as it can only remove a small portion of the organics present in the stillage (less than 19% as shown in Table 2.1).

It is relevant to note that the economics of these stillage handling options depend on the following factors (Costa Ribeiro and Costello Branco, 1981; Willington and Marten, 1982):

(i) scale of production;

(ii) type of stillage;

(iii) market price of substitute conventional products;

(iv) availability and cost of land;

(v) distillery location relative to receiving waters and by-product markets;

(vi) distillery energy requirements;

(vii) the applicable effluent discharge and emission standards.

They are, hence, case specific. However, with the exception of combustion and wet oxidation, these alternatives considered usually stabilize 20-50% of the stillage organics as compared to 90% by anaerobic digestion (Sheehan and Greenfield, 1980; Archer et al., 1980; Good et al., 1980; Callander et al., 1983; Callander, 1983(b)). Also, methane as a relatively insoluble gas and therefore does not require any energy intensive or expensive product recovery/separation step.

The use of anaerobic digestion as the most preferred stillage handling option will be discussed in detail in Section 2.6. But before that, a review of the anaerobic digestion process for wastewater treatment will be presented as a thorough understanding of the anaerobic digestion process is a prerequisite to this study.

2.5 THE ANAEROBIC DIGESTION PROCESS FOR WASTEWATER TREATMENT

2.5.1 Introduction to Anaerobic Digestion

Anaerobic digestion is a process for biological treatment of waste solids or wastewater in the absence of oxygen. During the process, complex organic matter in the waste (e.g. carbohydrates, proteins and fatty acids) is converted to methane, carbon dioxide and new bacterial cells. The process occurs widely in nature at places where organic materials accumulate in an anaerobic environment (e.g. in marsh land, in lake sediments and in the digestive tract of ruminants). The anaerobic digestion process thus plays a very important role in the mineralization of organic matter as part of the carbon cycle.

The most serious limitation is the slow growth of the methanogenic consortium but this can be overcome by the use of high bacterial concentrations and running the reactor under optimal conditions. These are features of the high rate anaerobic processes.

2.5.2 Microbiology and Biochemistry of Anaerobic Digestion

Anaerobic digestion of complex organic matter to methane depends on the coordinated activities of a few groups of obligate and facultative anaerobic bacteria. Due to the symbiotic nature of the process, a well coordinated and balanced activity of the microbial population is essential for stable operation.

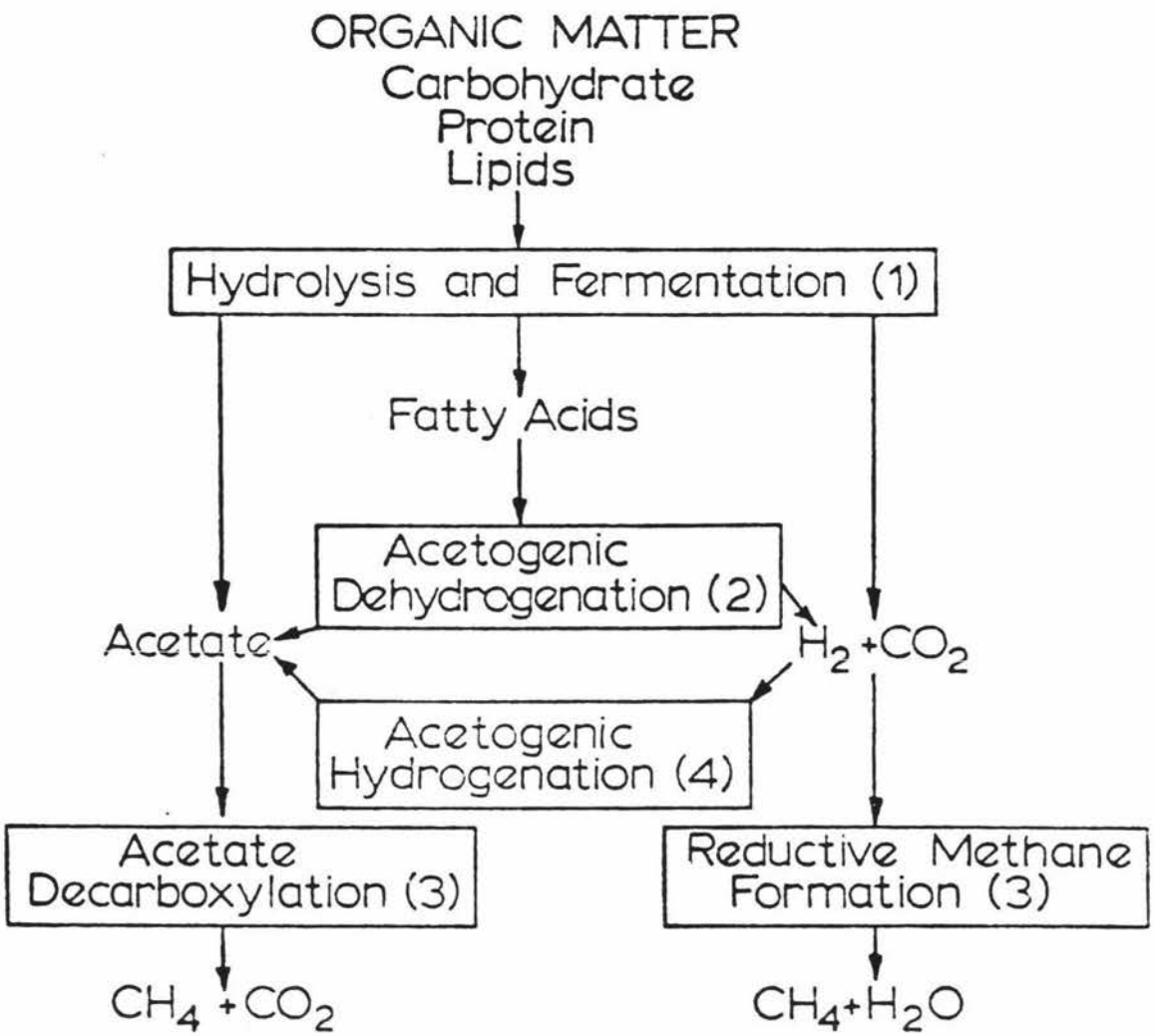
The anaerobic digestion process was originally considered a two stage process involving two major groups of bacteria (McCarty, 1964; Young and McCarty, 1968; Hausler, 1969; Toerien and Hattingh, 1969). The two steps were:

(i) The acid formation step (fermentation step) where polysaccharides, proteins and lipids are hydrolysed and fermented to fatty acids, CO_2, H_2 and alcohols. This step involved the metabolic activities of facultative and anaerobic microorganisms.

(ii) The methane formation step involving a smaller group of methanogenic bacteria (highly specialized strict anaerobes) in the conversion of the organic acids to methane and carbon dioxide.

Currently, anaerobic digestion is taken as a three stage process involving at least four trophic groups of bacteria according to the scheme presented in Figure 2.2 (McInerney et al., 1980). These four tropic groups of bacteria, each with its distinct carbon catabolizing function, are as follows:

Group (1): The hydrolytic bacteria which hydrolyse carbohydrates, proteins and lipids to fatty acids, amino acids, H_2, CO_2 and glycerol.



Key:

(1) Hydrolytic bacteria

(2) H_2 - producing acetogenic bacteria

(3) Methanogenic bacteria

(4) Homo-acetogenic bacteria

FIGURE 2.2: SCHEME FOR 3 STEP ANAEROBIC DIGESTION OF ORGANIC MATTER (McInerney and Bryant, 1981).

Group (2): The obligate H_2-producing acetogenic bacteria which convert the fatty acids and alcohols to acetate, H_2 and CO_2, and in so doing, energy is obtained for growth.

Group (3): The methanogenic bacteria which convert the first and second stage products (i.e. H_2, CO_2 and acetate) ultimately to CH_4 and CO_2.

Group (4): The obligate H_2 consuming or homo-acetogenic bacteria which catabolize H_2 and CO_2 to acetate.

A scheme for the fermentation of a carbohydrate e.g. glucose to CH_4 and and CO_2 can be represented as (Hobson, et al., 1974):

$$C_6H_{12}O_6 \longrightarrow 2\,CH_3CO\,COOH + 2\,H_2$$

$$2\,CH_3\,CO\,COOH + 2\,H_2O \longrightarrow 2\,CH_3\,COOH + 2\,H_2 + 2\,CO_2$$

$$4\,H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O$$

$$2\,CH_3\,COOH \longrightarrow 2\,CH_4 + 2\,CO_2$$

$$C_6H_{12}O_6 \longrightarrow 3\,CH_4 + 3\,CO_2$$

Although the three-stages of fermentation can be separated using such a scheme, the efficient metabolism of each group is dependent on each other symbiotically. Table 2.3 depicts the types and number of anaerobes present in a sewage sludge digestion.

TABLE 2.3 : ENUMERATION AND IDENTIFICATION OF ANAEROBIC BACTERIAL POPULATION IN SEWAGE SLUDGE DIGESTERS (Zeikus, 1980).

Group	Number.ml^{-1}	Generic Identity
Hydrolytic		majority identified
Total	10^8 - 10^9	G-ve rods
Proteolytic	10^7	*Eubacterium*
Cellulolytic	10^5	*Clostridium*
H_2-producing acetogen bacteria	10^6	unidentified G-ve rods
Homo-acetogenic bacteria	10^5 - 10^6	*Clostridium* *Acetobacterium*
Methanogens	10^6 - 10^8	*Methanobacterium* *Methanospirillum* *Methanococcus* *Methanosarcina* *Methanothrix*
Sulfate reducers	10^4	*Desulfovibrio* *Desulfotomaculum*

(a) Group (1): Hydrolytic Bacteria

These are a mixture of obligate or facultative anaerobes such as *Streptococcus* and Enterics (Hobson et al., 1974; Bryant, 1979). The fermentation of carbohydrates, lipids and proteins into volatile fatty acids, organic acids, alcohols, H_2, CO_2, NH_3 and sulfide involves the presence of several extracellular hydrolytic enzymes such as cellobiose, proteases and amylases.

Lipids are hydrolysed very slowly and can result in the overall rate limiting step for waste containing high amounts of lipids

and other slowly hydrolysed compounds (Henze and Harremoes, 1982). Carbohydrate catabolism serves as the major energy source for the metabolism of the fermentative bacteria. Other simple substrates also utilized are the simpler carbohydrate hydrolysis products or end products from the metabolism of other bacteria such as lactate and glycerol (McInerney et al., 1980). The degradation of complex polysaccharides (e.g. liqnified cellulose and hemicellulose) can be a very slow process due to steric hindrance of celluloses to all parts of this type of substrate. Pretreatment of such substrates will be essential for increased biodegradability (Healy et al., 1978; van Velsen and Lettinga, 1979; Colberg et al., 1980).

(b) Group (2): The H_2-producing Acetogenic Bacteria

This group of organisms degrades small organic molecules from stage one to volatile fatty acids and ultimately to acetic acid, H_2 and CO_2. A wide range of ligno-aromatic compounds have also been shown to be degraded to CH_4 and CO_2 by H_2-producing acetogenic bacteria (Healy and Young, 1979). Subsequent research has implicated *Syntrophobacter wolinii* (Boone and Bryant, 1980); and *Syntrophomonas wolfei*, (McInerney et al., 1981) as the organisms.

Their ability to catabolize the products from the first-stage hydrolysis and fermentation processes is essential to the digestion process as it links the fermentative and the methanogenic stages. The formation of H_2 and CH_3COOH is not favourable energetically unless H_2 is used to reduce CO_2 to CH_4 resulting in an extremely low hydrogen partial pressure in the digester. Thus, these organisms can only grow in the presence of hydrogen-utilizing bacteria like the methanogens or sulphate reducing bacteria (Zeikus, 1980; McInerney et al., 1980).

It had been postulated that H_2 partial pressure (or redox potential) regulates the production of the volatile fatty acids by means of a negative feedback control mechanism (Kaspar and Wurhrmann, 1978(a) and (b); Mosey, 1982(a) and (b)). For digesters operating at very short solids retention times, the concentrations of H_2 and

propionic acid increase , especially during unsteady state operation or varying load conditions. This is because the free-energy change for the catabolism of propionate (and butyrate) to acetate and CO_2 is highly positive and hence the reaction is thermodynamically unfavourable. The stoichiometry and the free-energy change can be described as (Bryant, 1979):

(i) Propionate-catabolizing acetogenic bacterium

$$CH_3 CH_2 COO^- + 3 H_2O \quad \rightleftharpoons \quad CH_3COO^- + H CO_3^- + H^+ + 3 H_2$$

$$\Delta G^{\circ\prime} = + 18.2 \text{ Kcal.reaction}^{-1}$$

(ii) H_2 - utilizing methanogenic bacterium

$$H CO_3^- + 4 H_2 + H^+ \quad \rightleftharpoons \quad CH_4 + 3 H_2O$$

$$\Delta G^{\circ\prime} = -32.4 \text{ Kcal.reaction}^{-1}$$

Sum of (i) + (ii). Syntrophic association

$$4 CH_3 CH_2 COO^- + 3 H_2O \quad \rightleftharpoons \quad 4 CH_3COO^- + H CO_3^- + H^+ + 3 CH_4$$

$$\Delta G^{\circ\prime} = -24.4 \text{ Kcal.reaction}^{-1}$$

Thus the maintenance of an extremely low partial pressure of H_2 in the reactor (by balanced syntrophic association) is essential for stable reactor operation (Mah et al., 1977; Bryant, 1979; Mosey, 1982 (a) and (b); Archer, 1983). High levels of propionic acid and H_2 concentrations are indication of load variations or a process operating near its maximum point making it suitable as an instability or stress indicator of the anaerobic system. Unless corrective actions are taken, the accumulation of H_2 in the system can inhibit the metabolism and growth of the H_2-producing acetogenic bacteria which would otherwise convert propionic acid back to acetate. High levels of propionic acid are inhibitory to the methanogenic consortium and can lead to reactor failure.

(c) Group 3: The Methanogenic Bacteria

The methanogenic bacteria are the 'key' organisms in the production of methane, primarily from CH_3COOH (70%) and the rest mainly from H_2 and CO_2 (Kaspar and Wurhrmann, 1978(b)). The methanogenic step is the most crucial of the three steps because of:

(i) The release of gas (particularly CH_4) from solution achieves a high degree of waste stabilization.

(ii) Due to the slow growth of the methanogens with minimum doubling time in the vicinity of 2-3 days (Mosey, 1982(a)), this step is generally the rate-limiting step. The inherent low growth rate of the acetate-fermenting methanogen is because very little free energy is available for growth ($\Delta G°' = -8.6$ Kcal.mol^{-1}) from the metabolism of acetate to CH_4 (Zeikus, 1977).

(iii) Their ability to remove H_2 provides thermodynamically favourable growth conditions for the preceding non-methanogenic stage (group 2) (Mah et al., 1977; Archer, 1983).

The methanogenic bacteria represent a unique group of organisms. They require a strictly anaerobic environment with a redox potential below -300 mV and many species have been found to have simple inorganic nutritional requirements (Zeikus, 1980). While different species have quite different cell shapes and structures (Hausler, 1969; Zeikus, 1977 and 1980); all species studied so far have a similar and peculiar energy metabolism. Few natural groupings of microorganism, such as those designated in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974), are as morphologically diverse as the methanogens (Zeikus, 1977). The methanogenic bacteria have been classified by Balch et al (1979) into three orders as presented in Table 2.4. This classification differs from that approved by the International Committee on Systematic Bacteriology of the International Association of Microbiological Societies. For example, species *ruminantium* is named under genus *Methanobacterium* in the Approved Lists of Bacterial Names (Skerman et al., 1980), but has been classified by Balch

TABLE 2.4: PROPOSED TAXONOMIC SCHEME FOR METHANOGENIC BACTERIA BASED ON COMPARATIVE CATALOGUING OF 16 S rRNA AND SUBSTRATES USED (Balch et al., 1979)

Order	Family	Genus	Species	Substrates for Growth and Methane Production
I	1	*Methanobacterium*	*formicicium*	$H_2 + CO_2$ formate
I	1	*Methanobacterium*	*bryantii*	$H_2 + CO_2$
I	1	*Methanobacterium*	*bryantii*, strain M.o.H.G.	$H_2 + CO_2$
I	1	*Methanobacterium*	*thermoautotrophicum*	$H_2 + CO_2$
I	1	*Methanobrevibacter*	*ruminantum*	$H_2 + CO_2$ formate
I	1	*Methanobrevibacter*	*arboriphilus*	$H_2 + CO_2$
I	1	*Methanobrevibacter*	*arboriphilus strain AZ*	$H_2 + CO_2$
I	1	*Methanobrevibacter*	*arboriphilus strain DC*	$H_2 + CO_2$
I	1	*Methanobrevibacter*	*smithii*	$H_2 + CO_2$
II	1	*Methanococcus vannielii*		$H_2 + CO_2$ formate
II	1	*Methanococcus voltae*		$H_2 + CO_2$ formate
III	1	*Methanomicrobium mobile*		$H_2 + CO_2$ formate
III	1	*Methanogenium cariaci*		$H_2 + CO_2$ formate
III	1	*Methanogenium marisnigri*		$H_2 + CO_2$ formate
III	1	*Methanospirillum hungatii*		$H_2 + CO_2$ formate
III	2	*Methanosarcina barkeri*		$H_2 + CO_2$ methanol acetate
III	2	*Methanosarcina barkeri strain 227*		$H_2 + CO_2$ methanol acetate
III	2	*Methanosarcina barkeri strain W*		$H_2 + CO_2$ methanol acetate

Order I: *Methanobacteriales* Order II: *Methanococcales* Order III: *Methanomicrobiales*

et al., (1979) under the genus *Methanobrevibacter*.

(d) Group (4): The Homo-acetogenic bacteria

The homo-acetogenic bacteria show a mixotrophic metabolism and catabolize mixtures of CO_2/H_2 (to acetate and longer chain volatile fatty acids) and multicarbon compounds. Their exact role in the anaerobic digestion process is not yet clear but the metabolism of these bacteria maintains a low hydrogen partial pressure in the digester thus contributing to process stability (Zeikus, 1980).

Various strains of the homo-acetogenic bacteria have been reported with complete conversion of acetate to CO_2 and CH_4 when co-cultured with acetate-adapted *Methanosarcina barkeri* (Winter and Wolfe, 1979). They have been reported as *Acetobacterium woodii* (Balch et al., 1977; Winter and Wolfe, 1979); *Acetogenium kivni*, (Leigh et al., 1981); *Clostridium thermoautotrophicum*, (Weigel et al., 1981; Martin et al., 1983); *Clostridium aceticum* (Weiringa), (Braun et al., 1981). Complete degradation of carbohydrates to CO_2 and CH_4 has thus been shown possible with an 'artificial consortium' of only one fermentative and one methanogenic organism (Winter and Wolfe, 1979). At least two strains of *Acetobacterium woodii*, have been selectively isolated and enriched using methoxylated aromatic compounds as substrates e.g. vanillate, syringate and trimethoxycinnamate (Bache and Pfennig, 1981).

2.5.3 Effect of Environmental Parameters on Anaerobic Digestion

The control of environmental parameters is of paramount economic importance to optimise the biological reactions within the digester. Optimal operation results in a high degree of waste stabilization and a high methane gas production rate with a lower frequency of reactor instability. Reactor instability should be avoided if possible because the slow growing methanogens will take a long time to recover from any inhibition.

Reactor stability has been one of the major problems of anaerobic digester operation as related to environmental parameters. Due to the sequential nature of the microbial process, any cause of upset to the fine ecological balance of the anaerobic consortium will be amplified in the process. Also important in this respect is that optimum conditions differ for the various groups involved and they have different tolerance to toxicity. This means that the rapidity of environmental parameter changes also has a very significant effect on digester performances. It is thus advisable (especially during reactor start-up) to make environmental parameter changes in a series of small steps rather than using a single large step to effect the change (Andrews, 1968; Andrews and Graef, 1971).

Environmental agents that can affect anaerobic digestion include, but are not limited to, the following:

(i) Temperature;
(ii) pH and alkalinity;
(iii) Organic and hydraulic loading rates;
(iv) Nutrient availability;
(v) Presence of stimulatory and toxic substances.

The effects of some of these environmental parameters on digester performance have been widely studied using kinetics and modelling of the anaerobic digestion process (McCarty, 1966; Lawrence and McCarty, 1969; Lawrence, 1971; Andrews, 1978; Chen and Hashimoto, 1979; Hashimoto et al., 1980; Hill, 1983; Hill et al., 1983; Hobson, 1983). It is outside the scope of this study to discuss the effect of each of the above environmental agents. Instead the criteria necessary for good digestion will be considered.

2.5.3.1 Criteria for good digestion

The effect of environmental parameters can be different for different waste types and different reactor conformations. With this taken into account, the general criteria for good digestion of organic wastes are:

(a) Temperature

The bacterial consortium reacts similarly qualitatively but dissimilarly quantitatively to temperature changes. Thus, maintenance of a uniform temperature within a certain range is considered to be more important than maintaining the temperature which gives the maximum possible rates as different balance of microorganism species will occur for reactor developed at different temperatures (Cowley and Wase, 1981).

There are two temperature optima for high-rate digestion, namely the mesophilic range at around 35°C and the thermophilic range at about 55°C (Kugelman and Jeris, 1981). Generally, unless the wastewater to be treated is in or above the thermophilic temperature range the economics of digestion dictate the reactor be operated at the mesophilic range or lower.

(b) pH

A pH around neutrality is the preferred range for satisfactory operation. Care should be taken such that pH does not fall below 6.5 and below 5.0 a 'stuck' digester will result. The pH of the system can be adjusted by lowering the organic loading rate or by acid-base dosing (Stafford et al., 1980; Speece, 1983(a)).

(c) Alkalinity

A value of 2500 $mg.l^{-1}$ as Ca_2CO_3 is considered normal. A much higher value will provide a better buffering capacity for large variations in volatile fatty acid concentrations but the cost of base addition can be prohibitive (Speece, 1983(a) and (b)). Satisfactory operation may be obtained at lower alkalinities provided good control over the organic and hydraulic loading rates is achieved.

(d) Gas production and methane composition

A methane content of 55-72% in the digester gas indicates satisfactory operation (Hobson et al., 1974). The amount of methane produced can be calculated from the COD reduction. Stoichiometically,

$$CH_4 + 2\ O_2 \longrightarrow CO_2 + 2\ H_2O$$

Thus one gram mole of methane at STP (i.e. 22.4 l) has a COD of 64 g. This means that a theoretical maximum of $(\frac{22.4}{64})$ or 0.35 l of CH_4 can be obtained per g COD removed (at 0°C and 760 mm Hg). A methane gas yield around this figure (after temperature correction according to the Gas Law) indicates optimal reactor operation. Some of the COD will also be used for cell growth.

(e) Volatile fatty acid (VFA) concentrations

200 to 400 $mg.l^{-1}$ VFA's as acetic acid are considered normal in a well balanced digester. A high and rising propionic acid concentration indicates an imbalance in the bacterial consortium (see Section 2.5.2). This is normally due to organic overload or the presence of toxic material in the digester.

(f) Nutrient requirements

Speece and McCarty (1964) have determined quantitatively the nutritional requirements of the anaerobic digestion of a wide range of substrates for nitrogen, phosphorus (and qualitatively for potassium, magnesium and iron). Using a general empirical biological solids formula of $C_5\ H_9\ O_3\ N$, the cell weight/N ratio was found to be 9.4 (or 11.4 for COD/N ratio) implying 1 gm of N is required for every 9.4 grams of cells produced (Speece and McCarty, 1964). As the N/P ratio approximated 7, the phosphorus requirements for all substrates were approximately one-seventh of the nitrogen requirement. Thus, a COD: N : P ratio of 100 : 88 : 1.25 would be required for a nutritionally balanced anaerobic digester in terms of C, N and P requirement. Assuming 10% cell yield in the digestion process, the optimal feed COD : N : P ratio is 100 : 0.88 : 0.125.

Apart from nitrogen and phosphorus, the methanogens have a unique requirement for trace nutrients, particularly iron, nickel, cobalt and sulfide (Diekert et al., 1981; Murray and van den Berg., 1981; Ashley et al., 1982; Speece et al., 1983; Speece, 1983(a)

and (b)). A very high acetate-utilization rate (34 - 36 $g.l^{-1}.d^{-1}$) was obtained using an acetate-enriched methanogenic culture with nickel, iron, cobalt and yeast extract supplemented at the following concentrations: 10 $mg.l^{-1}$ $NiCl_2$; 250 $mg.l^{-1}$ $FeCl_2.4H_2O$; 10 $mg.l^{-1}$ $CoCl_2$ and 200 $mg.l^{-1}$ yeast extract (Speece et al., 1983). Other metals that have been shown to be stimulatory to methanogens are molybdenum, selenium, tungsten and sodium (Jones and Stadtman, 1977; Schonheit et al., 1979; Murray and van den Berg, 1981; Perski et al., 1981; Jones and Stadtman, 1981). Growth of the organism *Methanococcus vannielii* on formate was found to be markedly stimulated by selenium (1 µM selenite) and tungsten (100 µM tungstate) addition to a mineral salts-formate medium (Jones and Stadtman, 1977). These metals are of lesser importance in this study since anaerobic oxidation of formate represents only a small fraction of the conversion of organics to methane and is not a preferred route of methanogenesis from pyruvate (Archer, 1983). At this stage, no other metals have been shown to be required for growth. A dependence of growth on Cu, Mn, Zn, Ca, Al and B could not be demonstrated (Schonheit et al., 1979).

However, there is still undoubtedly much more to be discovered about the nutritional requirements for all phases of anaerobic digestion. This problem is compounded by the fact that complex interactions within the aqueous anaerobic system tends to precipitate mutually essential trace metals with phosphorus or hydrogen sulfide (Speece, 1983(b)). In this situation, it would be more appropriate to measure the soluble nutrient levels which are more easily available for microbial uptake. But, studies on nutrient requirements have been mainly concerned with nutrient supplementation or defined growth media and fail to comment on nutrient availability. Recently, a methodology for the quantitative assessments of individual effects of precipitation and chelation of metal ions (e.g. by PO_4^{3-}, S^{2-} and CO_3^{2-}) in anaerobic digester and hence the availability of metals as nutrients in anaerobic digestion has been developed by Callander and Barford (1983(a) and (b)).

To ensure adequate supply of nutrients for the methanogenic consortium, the levels of these essential nutrients have to be measured regularly. A substantial effluent level can be interpreted as an adequate supply of that element while a low or zero level indicates possible deficiency. This can then be tested by supplementing with that element to the feed and monitoring the effluent level. The recovery of all of the supplement in the effluent means that the supplement had not been necessary (Callander, 1982).

(g) Toxic material

Toxicity is a relative term as the toxic material is often stimulatory at low concentrations or below the threshold level e.g. NH_3-N and sulfide toxicity (McCarty, 1964(b); Lawrence et al., 1964; Kugelman and Chin, 1971; Mosey et al., 1971; van Velsen, 1981). There are many known inhibitors to the anaerobic process and a lot of work has been carried out in this area (Kugelman and McCarty, 1964; Kugelman and Chin, 1971; Mosey and Hughes, 1975; Mosey, 1976; Ahring and Westermann, 1983; Parkin et al., 1983; Speece and Parkin, 1983). Henze and Harremoes (1982) give a summary of the possible toxicity of compounds to the anaerobic processes (for methanogenic bacteria or mixed cultures).

There are a number of ways to overcome these toxic problems and they can be listed as (McCarty, 1964(b); Kugelman and McCarty, 1964; Kugelman and Chin, 1971; Speece, 1983(a)).

(i) Acclimation of the methanogenic consortium to the toxic material in the waste;

(ii) Dilution below the threshold level;

(iii) The formation of an insoluble complex or precipitate;

(iv) Removal of the toxic material from the waste;

(v) Antagonization of the toxic material by supplementation.

In short, the criteria for good digestion require a proper control of the important environmental parameters that affect the anaerobic digestion of organic matter and a nutritionally balanced substrate preferably with absence of toxic material (or below its threshold level).

As for wood-ethanol stillage, the C : N : P ratio is approximately 100 : 0.078 : 0.013. Hence, the raw stillage is deficient in N and P for anaerobic digestion. The stillage is also expected to contain toxic or potentially toxic material:

(i) The SO_4^{2-} level from the pilot plant is in excess of 1500 $mg.l^{-1}$. In an anaerobic digester, this sulphate will become reduced and may result in sulfide toxicity or the precipitation out of essential metals.

(ii) The lignin-derived aromatics from the hydrolysis process e.g. hydroxymethylfurfural, laevulinic acid and furfural are known biological inhibitors (Jaenchen et al., 1981; Henze and Harremoes, 1982; Callander et al., 1983).

Thus some form of stillage feed modification may be necessary and the use of a methanogenic consortium acclimated to the stillage would be advisable for efficient anaerobic degradation.

2.5.4 The High Rate Anaerobic Digestion Process

The conventional, completely mixed and heated high rate digester generally used for sewage sludge and manure digestion is not included in this category. In this study, high rate digestion refers to processes where cell recycle or attachment is used to control the biological solids retention time (SRT).

The first high rate anaerobic digesters were developed in the 1950's after the value of a large bacterial population in the digester for efficient treatment of wastewater was recognised (Stander, 1950; Stander and Snyder, 1950). Initially, the pace of scientific research and development in this area was rather slow, possibly due to misconceptions

of the anaerobic digestion process (slow and unreliable) from a lack of understanding of the relevant microbial biochemistry. These misconceptions were unwarranted and subsequently, the anaerobic digestion process has been developed to the point of reliable process application for many potential biomass feestocks (Speece, 1983(a); Callander and Barford, 1983(c)). The growing interest in the anaerobic digestion process in the past twenty years has resulted in new process developments and reactor designs which have reduced capital costs and increased process stability. Figure 2.3 presents a summary of the major conventional and the high rate anaerobic digesters.

The high rate process compensates for the slow growth rate of the anaerobic bacteria by employing high biomass concentrations within the reactor. This is achieved by independent control of the biological solids retention time (SRT) and the hydraulic retention time (HRT). Like its aerobic counterpart, the high rate anaerobic digestion process can be divided according to how the high ratio of $\frac{SRT}{HRT}$ is achieved. Thus the two major categories of the high rate processes are:

(i) <u>The anaerobic suspended growth reactors</u>

They achieve independent control of SRT and HRT by internal or external settlement or by recycling of biomass. Thus a separation step is required. Examples are: -

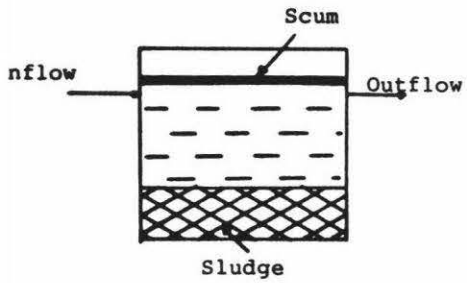
* The 'clarigester' (Fig. 2.3(D));
* The Anaerobic Contact Process (Fig. 2.3(E));
* The UASB reactor (Fig. 2.3(G));
* The 'Baffled' reactor (Fig. 2.3(I)).

(ii) <u>The anaerobic attached-film reactors</u>

These reactors use an inert support medium for the bacteria to grow on. This adherence of biomass to the support prevents the washout of cells at low HRT (i.e. high hydraulic loading rate exceeding the specific growth rate of the bacteria) while achieving a long SRT. The major categories are: -

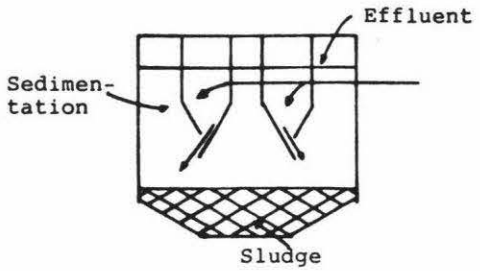
* **The anaerobic filter (Fig. 2.3(F));**
* **The anaerobic Fluidized bed reactor (Fig. 2.3(H));**

(A) SEPTIC TANK



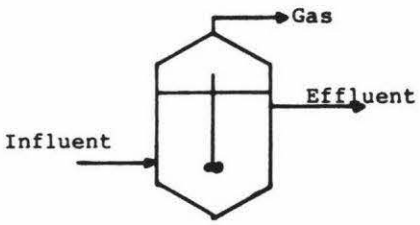
(a) First patented 1895
(b) Problem - Effluent black and offensive

(B) IMHOFF TANK



(a) Modification of 'Travis Tank'
(b) Wastewater flow through sedimentation chambers only
(c) Problems with sedimentation and reactor hard to construct

(C) CONVENTIONAL ANAEROBIC DIGESTER

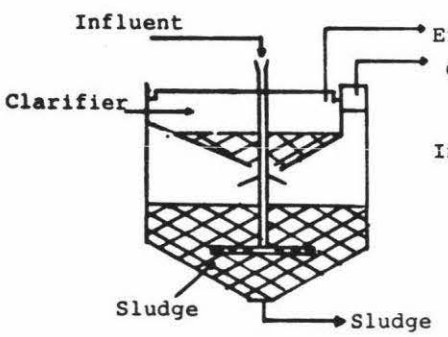


(a) Can be mixed and heated to increase digestion rate
(b) Mainly for municipal sewage sludge and recently, animal manure digestion
(c) Can be single or two stage process
(d) A very well established system.

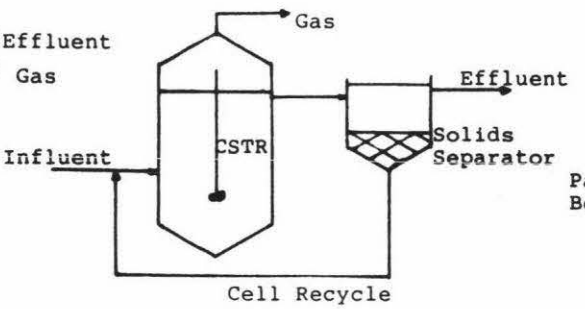
(1) CONVENTIONAL ANAEROBIC DIGESTERS

PRE 1970

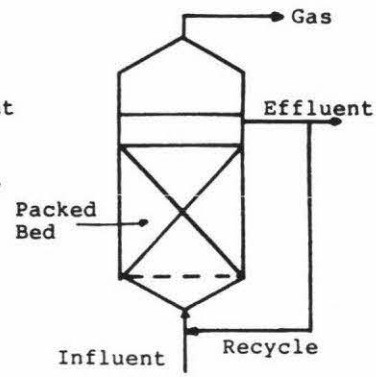
(D) THE ANAEROBIC CLARIGESTER



(E) ANAEROBIC CONTACT PROCESS

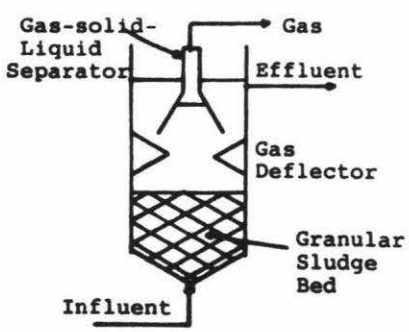


(F) ANAEROBIC FILTER

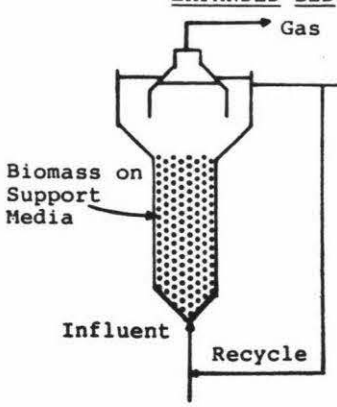


POST 1970

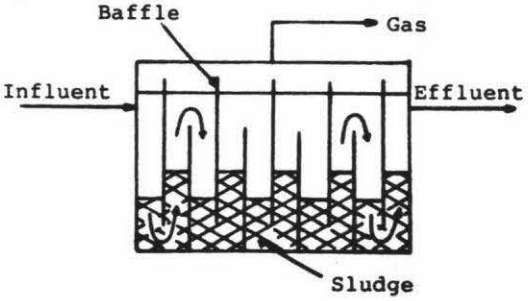
(G) UASB REACTOR



(H) ANAEROBIC FLUIDIZED OR EXPANDED-BED REACTOR



(I) BAFFLED REACTOR



(2) HIGH RATE ANAEROBIC DIGESTERS

FIGURE 2.3: A SUMMARY OF THE CONVENTIONAL AND HIGH RATE ANAEROBIC DIGESTERS

* The anaerobic expanded-bed reactor (Fig. 2.3(H)).

The anaerobic attached-film processes have been recently reviewed by Henze and Harremoes (1982) and Switzenbaum (1983).

The development and performance of each of these reactor designs will now be briefly considered.

2.5.4.1 Design of the anaerobic suspended growth reactors

(a) The Anaerobic Clarigestor

This system was first used for the treatment of wine distillery wastewater. This concentrated waste (COD 22,400 $mg.l^{-1}$) was considered to be uneconomic to treat using conventional aerobic means because it would require excessive dilution apart from its noxious nature. Stander (1967) described this full scale plant commissioned in 1962 in South Africa. The plant was actually a Dorr-Oliver Clarigester modified so that raw waste entered at the base of the digester and flowed upward through a dense bed of flocculant biomass. The maintenance of active cell material and biota was largely achieved by a reverse flow of the clarigester with internally recycled sludge (Fig. 2.3(D)).

With a maximum digester load of 3.2 kg $COD.m^{-3}.d^{-1}$ (HRT of 7.2 d) and an operating temperature of 30°C, COD removals in excess of 95% were achieved. The sludge density was 19 g $VSS.l^{-1}$. This maximum loading rate achievable was limited by the volumetric flow rate attainable without excessive loss of sludge. This indicated the plant performance was limited by sedimentation efficiency and not by the microbial process. This reactor was the first UASB design utilizing an internal sludge recycling system.

(b) The Anaerobic Contact Process

Like the aerobic activated sludge process, this process consists of a completely mixed digester (but enclosed for anaerobic treatment) with an external cell separation device which may be a simple clarifier or a centrifuge for return of sludge to the digester.

For many years, the goals of this design were not realized because of difficulty in preventing the washout of a small quantity of biomass produced. Because of the fine balance of the biomass in the system due to the slow methanogenic growth, separation efficiencies of better than 98% have to be achieved (Rippon, 1983). The problem with the buoyancy of biomass flows caused by adhering gas bubbles in the sedimentation stage may be overcome by vacuum degasification prior to sedimentation (with some success) or, more recently, in the Bioenergy process (Biomechanics Ltd), by the application of thermal shock to temporarily stop further gas production during the settling phase (Anderson et al., 1980; Rippon, 1983).

The Anaerobic contact process has been used for the treatment of a wide variety of wastes including stillage (Roth and Lentz, 1977; Costello and Ribeiro, 1980; van den Berg and Lentz, 1980; Anderson et al., 1980; Langton, 1981; Rippon, 1983). In the mesophilic temperature range, successful operation has been reported with BOD_5 loadings of 1.2 to 11.8 $kg.m^{-3}.d^{-1}$ at HRTs ranging from 0.5 to 3.8 days (Langton, 1981). Mixed liquor suspended solids (MLSS) ranging from 2,500 to 16,000 $mg.l^{-1}$ can be achieved depending on the organic strength of the waste fed.

(c) The Upflow Anaerobic Sludge Blanket (UASB) Process

The UASB process was developed in the Netherlands in the early 1970's by Lettinga and co-workers for the treatment of sugar beet wastewater (Lettinga et al., 1979; Lettinga et al., 1980; Pette et al., 1981; de Zeeuw and Lettinga, 1981). This reactor design overcame the problems associated with the clarigester and the contact process by employing a deep bed of flocculated or granular biomass. This flocculent biomass of good settling properties is produced using a carefully controlled start-up procedure. This has to be done in three stages involving adaptation of the seed sludge (normally from digested sewage sludge) to the new substrate, adaptation to increase sludge activity and finally pelletization of the sludge to a granular form which is superior in settleability (de Zeeuw and Lettinga, 1981).

Like the clarigester design, waste liquid enters the base of the digester via a feed distribution system and flows upward through the densely packed granular sludge which is unmixed other than by turbulence from the rising gas which is produced. The effluent leaves the top of the reactor via an internal baffle system which separates the gas, sludge and liquid (Fig. 2.3(G)). Claims of exceptionally high organic loading rates (i.e. 30-40 kg $COD.m^{-3}.d^{-1}$) have been made (de Zeeuw and Lettinga, 1981). With a very short retention time of 3 h - 1 d as applied to medium and high strength wastewater, 80 - 98% reduction in sCOD load have been achieved at 15 kg $COD.m^{-3}.d^{-1}$ loading rate (Pette et al., 1981). Due to its partial plug flow characteristics, the UASB process achieves a higher COD removal for soluble wastewaters compared to the expanded or fluidized bed system which are essentially completely mixed systems (Forster and Wase, 1983).

Although these UASB plants can possibly handle much higher loading rates, most of them have been designed for an organic loading rate of 10 - 15 kg $COD.m^{-3}.d^{-1}$ at 30°C (Lettinga et al., 1982(a) and (b)). Hydraulic loading rates of as high as 8 $m^3.m^{-3}.d^{-1}$ have successfully been applied with gas loading of 3-8 $m^3.m^{-3}.h^{-1}$ being used to avoid excessive turbulence which can lead to floc break-up and washout. Biomass densities ranging from 50-100 kg $SS.m^{-3}$ at the digester base to 5-50 kg $SS.m^{-3}$ near the settling region have been reported (Pette et al., 1981).

Although the process has the potential advantages of simplicity and low cost, the success or failure of the system depends on the ability to develop and maintain a suitably flocculent sludge. The process is sensitive to substantial shock loading which may cause the loss of the flocculating and sedimenting capability of the biomass. If this occurs, the biomass will take a long time to redevelop the necessary sludge concentration. At present, full scale UASB plants are operating in Europe and America on sugar beet wastewater, potato processing wastes, potato starch waste, brewery, alcohol and maize starch waste (Lettinga et al., 1980; Switzenbaum, 1983). In New Zealand, one such plant of

4,200 m^3 has just been commissioned at the Alliance Freezing Works, Invercargill for the treatment of meat processing wastewater and energy production (AF News, August 1983). This UASB plant is reputed to be the second largest in the world.

(d) The Baffled Reactor

This reactor is a modification of the Anaerobic Rotating Biological reactor and has not been tried yet in a large scale. In this system the horizontal movement of sludge is minimized by the baffles (Fig. 2.3(I)). Some vertical movement of the biomass is possible due to the feed flow of the system but the flocculant bacteria tend to stay in the reactor (McCarty, 1982). This reactor is just one example of the many process modifications now being examined at Stanford University, California (McCarty, 1983 personal communication).

2.5.4.2 Design of the anaerobic attached-film reactors

(a) The Anaerobic Filter

This is the stationary-medium type, anaerobic fixed-film reactor developed by McCarty and co-workers in the 1960's (Young and McCarty, 1967; Young and McCarty, 1968). It is normally operated in an upflow mode and the reactor can be filled with 24-40 mm stones or with plastic media of high porosity (in excess of 95% having a unit area ranging from 90-138 $m^2.m^{-3}$) (Young and Dahab, 1982). With stone packing (porosity 0.42), biomass densities of 10-25 g $VSS.l^{-1}$ have been obtained with protein-carbohydrate wastes at a loading of 3.4 kg $COD.m^{-3}.d^{-1}$ (Young and McCarty, 1967).

The anaerobic filters have been shown to successfully treat several wastewaters including stillage (Mueller and Mancini, 1975; anonymous, 1982; Kennedy and van den Berg, 1982; Anderson et al, 1982; Good et al., 1982). HRT's in the range of 3 hours to 1 day have been used with sCOD loadings ranging from 3.2 - 27.2 $kg.m^{-3}.d^{-1}$ and achieving 90% - 50% sCOD removals respectively

(Mueller and Mancini, 1975).

To date, a few full scale plants have been built for the treatment of wheat starch waste, distillery waste and a chemical factory waste effluent (Taylor and Burn, 1973; Witt et al., 1980; Anonymous (1982). Using rum distillery slops, a COD removal of 75% can be achieved at a COD loading rate of 9 $kg.m^{-3}.d^{-1}$ (Switzenbaum, 1983). A 'vinyl core' synthetic media (B.F. Goodrich, Environmental Products Unit) made from PVC has been used as the biomass support material (Switzenbaum, 1983).

Studies using chemical industry wastes show that the anaerobic filter can withstand short period of overloading e.g. at eight times their normal loading rate for a 24 hour period (Kennedy and van den Berg, 1982). For all cases, recovery occured within 12-48 hours after overloading stopped and while being loaded normally. This stability to hydraulic overloading is one of the characteristics of fixed film processes because the immobilized cells on the support surface are less susceptible to washout (particularly the slow growing methanogens) than those in dispersed growth reactors.

The anaerobic filter itself is a misnomer both as a 'filter' or as a 'fixed-film reactor'. The reactor itself performs no filtering function and it has repeatedly been observed that anaerobic biota that results in waste degradation is not attached to the packing material but lay loosely as flocs in the media interstitial spaces (Young and McCarty, 1967; Young and Dahab, 1982). However, this observation will be affected by the packing medium used. Hysman et al., (1983) have studied the factors affecting the colonization of porous and non porous packing materials in upflow methane reactors and concluded that, for porous packing, colonization depends primarily on the size of the pores and the degree of porosity. The methanogenic biomass appears to be mechanically retained within the porous material. This is unlike the case using a non-porous media where the bacteria is attached to the outside of the matrix. Thus depending on the media porosity

and the diffusional path between the cells and the free interspace solution, the bacteria retained inside a niche may face diffusion problems. Cubes of 140 pieces with 1.25 cm sides (reticulated polyurethane foam) have been shown to be not rate limited by diffusion (Hysman et al., 1983).

(b) The Anaerobic Fluidised Bed Reactor

This process was originally developed from work on denitrification of sewage effluent and has now been acclaimed as the most significant achievement in the wastewater treatment field in the last 50 years (Cooper and Atkinson, 1981). The reactor is normally filled with a fine granular support material (usually sand of 0.5 mmØ) to which biomass adheres in a thin film. The support material is highly motile when it is fully fluidized (taken as 50% bed expansion) by an upward flow of feed and recycled reactor effluent (Fig. 2.3(H)). Atkinson et al. (1981) give a full account of the types of support particles available.

The development of the anaerobic fluidized bed reactor has been described by Cooper and Wheeldon (1980), and Mosey (1982(b)). Studies have shown that the fluidized (and expanded) beds are able to operate at a lower HRT and higher loading rates than the anaerobic filters because of higher surface area to volume ratios (Switzenbaum, 1982). This process conformation is now being marketed by the Dorr-Oliver Co. as the 'Anitron' process (Sutton and Li, 1982; Sutton et al., 1983). This type of reactor is reportedly capable of removing 85 - 95% of the COD from wholly soluble wastewater with COD loading rates ranging from 8.5 - 20 $kg.m^{-3}.d^{-1}$ (Mosey, 1982(b)). Studies have also been performed on molasses wastewater, and pulp and paper industry wastewater (a highly toxic waste) (Frostell, 1982; Hakulinen and Salkinoja-Salonan, 1982; Barnes et al., 1983). With external solids recycling, the fluidized bed system has been reported to operate stably at 20 - 25 $kg.COD.m^{-3}.d^{-1}$ on molasses wastewater achieving 43% COD conversion to methane at 30°C. The maximal biomass concentration was 22 kg $VS.m^{-3}$ of which 13 kg $VS.m^{-3}$ was attached (Frostell,

1982). Switzenbaum (1983) reported two full-scale anaerobic fluidized bed installations for soft-drink bottling waste and soy process waste. At 9.6 kg COD.m^{-3}.d^{-1} (6 h HRT) loading of the bottling waste, a COD removal of 77% was achieved using 0.6 mm sand media.

The main disadvantage of this system is the long start-up period (Frostell, 1982; Bull et al., 1983) and the energy cost to maintain the bed in a fluidized state. The problem can be partly overcome with a lighter media requiring a much lower recycle rate as in the anaerobic expanded-bed process. However, media other than sand can also represent a disadvantage with regard to cost.

(c) Anaerobic Expanded-Bed Reactor (AEB)

This reactor design is the same as a fluidised bed, but with a lighter support medium and it is normally operated at 10-20% bed expansion. The AEB reactor was first developed at Cornell University for the anaerobic treatment of very dilute industrial wastewater (COD < 1000 mg.l^{-1}) (Mosey, 1982(b)). The optimum support media is claimed to be a bed of small light weight particles of 20 - 30 µm diameter and 1.05 - 1.2 g.cm^{-3} in density. The basic idea is to provide a large surface area for microbial growth and a mechanism for entrapment of fine particles with no clogging. A biomass concentration of 30 kg.m^{-3} and sometimes reaching 100 kg.m^{-3} reactor volume has been obtained (Jewell, 1981).

Various studies have been made using this reactor mainly involved the treatment of domestic sewage (Switzenbaum and Jewell, 1980; Jewell et al., 1981; Rockey and Forster, 1982). Greater than 80% COD removal has been achieved at an HRT of about 4 hours and an organic loading rate of 8 kg COD.m^{-3}.d^{-1} regardless of the influent substrate concentration or temperature range studied (Switzenbaum and Jewell, 1980). There is little adverse temperature effect in the process since, using low strength wastewater, a SRT value in excess of a year can be achieved even at a low HRT indicating a high biomass concentration in the reactor.

The ability of the fixed-film processes to retain biomass even at very high liquid velocities enables them to operate at extremely short residence times, a matter of minutes. Thus they possibly have an advantage over the suspended growth processes (e.g. UASB) for low strength waste digestion by overcoming the problem of biomass floc washout at very low HRT's. However it remains to be seen whether this advantage over the suspended growth processes justifies the additional cost relating to bed fluidization/ expansion and purchase of the support medium.

2.5.5 Future Potential

The water pollution control industry has made good advances since the end of the last century. At this stage, the anaerobic digestion process has been developed to a point of reliable process application for many potential biomass feedstocks. During the coming decade, the water pollution control industry will face a transformation in technology as anaerobic rather than aerobic processes become more widely used for treatment of high and low strength wastewaters because: -

(i) The new processes are more economically feasible or more cost effective to implement. They possibly contribute to providing net profits while overcoming the industrial or municipal major waste disposal problems.

(ii) These new procedures are environmentally more acceptable in a world of increasing populational growth since they can be contained and occupy a smaller land area.

(iii) They are more versatile and hence a wider application including the treatment of many wastes which currently have to be solved by great expenses using physical or chemical means.

The development of these novel wastewater treatment processes (especially the 'upflow' types), a part of the growing application of Biotechnology will trigger a substantial increase in interest and awareness of environmental pollution control.

2.6 ANAEROBIC TREATMENT OF WOOD-ETHANOL STILLAGE

Successful anaerobic treatment of stillage has been widely reported for wine distillery wastes (Stander, 1967), for rum stillage (Roth and Lentz, 1977; Anonymous, 1982), for molasses, cane-juice and mandioca stillage (Costello Branco and Costa Ribeiro, 1980), for molasses, grain and wine distillery stillage (Sheehan and Greenfield, 1980) and for molasses stillage (Basu and Leclerc, 1972; Skogman, 1979). The application of the high rate anaerobic digestion processes for stillage treatment has been reviewed by McFarlane (1982). However, the treatment of wood hydrolysate stillage has not been reported until recently, probably because of the unavailability of the stillage in the Western World.

Good et al. (1982) have successfully treated diluted eucalyptus wood hydrolysis stillage with a COD concentration similar to that of stillage from the FRI. Two types of reactor were investigated: -

(i) A fixed film anaerobic filter using stainless steel wire mesh as the support structure (specific area of 4 $cm^2.cm^{-3}$ reactor volume);

(ii) A continuous stirred tank reactor (CSTR) utilizing a 2 l glass vessel equipped with a stirrer (180 rpm).

The fixed film reactor was found to be far superior in performance to the CSTR and at an organic loading rate of 10.7 kg $COD.m^{-3}.d^{-1}$ it achieved a COD removal of 87% and a biogas production rate of 400 $ml.g^{-1}$ COD removed (63% CH_4) at 35°C. No mention of any decolourization of the waste was reported. Tables 2.5 and 2.6 summarise the waste characteristics and the results obtained.

Some work in this area has also been performed at the FRI and in the Biotechnology Department, Massey University.

At the FRI, a 8 l working volume CSTR operating at 37°C has been studied (Callander, 1983(a)). Preliminary results suggest that nitrogen and phosphorus supplements of 240 and 80 $mg.l^{-1}$ respectively should be used for the anaerobic digestion of the stillage. A high specific sludge activity of 0.60 kg $COD.kg\ VSS^{-1}.d^{-1}$ was achieved without any metal

TABLE 2.5: CHARACTERISTICS OF STILLAGE FROM FROM EUCALYPTUS WOOD ACID HYDROLYSIS PLANT AT 1:1 DILUTION (Good et al., 1982)

Parameter (all $g.l^{-1}$)	Value
Chemical oxygen demand	22.5
Total solids	17.6
Volatile solids	15.6
Ash	2.0
S tot	0.26 - 0.36
N tot	0.2
P tot	0.04
pH	5.8 - 6.3
COD:N:P	100:0.88:0.17

TABLE 2.6: PERFORMANCE OF CONTINUOUSLY LOADED FIXED FILM AND CSTR REACTORS USING EUCALYPTUS WOOD STILLAGE (Good et al., 1982)

	Reactor Number		
	(1)	(2)	(3)
Hydraulic retention time (days)	2.1	2.25	9.5
Organic loading rate (g $COD.l^{-1}.d^{-1}$)	10.7	10	2.4
COD removal (%)	86.6	84.4	85.5
Biogas production rate ($ml.g^{-1}$ COD)	400	380	400
CH_4 content (%)	63.4	62.5	58.5
H_2S Content (%)	0.01	0.01	0.01
Volatile acids, effluent, ($mg.l^{-1}$)			
Acetic acid	260	215	532
Propionic acid	680	666	1900
Temperature (°C)	35	55	35
pH	7.5	7.2	7.3

(1) Fixed film reactor, mesophilic run (35°C)
(2) Fixed film reactor, thermophilic run (55°C)
(3) CSTR reactor, mesophilic run (35°C)

supplementation. The reactor pH was controlled above 6.80 by alkali dosing using 2.5 ml of 20% W/V NaOH per litre stillage feed (at an organic loading rate of 1 kg $COD.m^{-3}.d^{-1}$). At higher loading rate (i.e. 3.5 kg $COD.m^{-3}.d^{-1}$) 4 ml 20% W/V NaOH per litre feed were required. The sCOD removal was 90% with methane and sludge yields of 78 and 12% respectively (Callander et al., 1983).

The acclimatized sludge from this CSTR was used as the seed inoculum for a 10 l UASB reactor. After 4 months operation at 37°C a non-maximal organic loading rate of 13 kg $COD.m^{-3}.d^{-1}$ was achieved with sCOD and $sBOD_5$ removals of 86 and 98% respectively (Callander et al., 1983). The reactor biomass concentration was high (at 35 $g.l^{-1}$ VSS) resulting in excellent digestion stability (acetate at 20 $mg.l^{-1}$ and propionate at 100 $mg.l^{-1}$).

In the Biotechnology Department, Massey University, two 90 l anaerobic lagoons have been studied (Archer et al., 1982). Working at 90 and 75 days HRT, approximately 80% of COD, BOD_5 and volatile solids can be removed at an operating temperature of 20°C. No apparent removal of colour was reported. The treated effluent from these lagoons will constitute the anaerobically treated feedstock used in this project on colour removal. A summary of the preliminary results of these lagoons is as presented in Table 2.7. Future work will consider shorter retention times, lower temperatures and reduced nutrient addition rates.

Thus, although anaerobic digestion is effective in removing organics from wood-ethanol stillage, no significant reduction of the intense colour has been reported to date. At most discharge locations in New Zealand, further treatment for colour removal will therefore be required and alternative colour removal methods will be considered in the following section.

TABLE 2.7: LAGOON PERFORMANCE AT 75 DAY AND 90 DAY NOMINAL HYDRAULIC RETENTION TIMES (Archer et al., 1982)

Nominal HRT (days)	90	75
Observed HRT (days)	101	84
Temperature (°C)	20	22.5
COD removal (%)	82	87
BOD_5 removal (%)	92	96
VS removal (%)	79	77
Effluent BOD_5 (mg/l)	980	480
Effluent COD	3550	2540
Effluent SS	380	365
Gas production (l/day)	8.35	11.54
Gas composition (% CH_4)	64.8	60.9
$Y^{CH_4}_{COD}$ (ml CH_4 at STP/g)	346	349
$Y^{CH_4}_{BOD_5}$ (" " " ")	470	484
$Y^{CH_4}_{VS}$ (" " " ")	824	892

No colour removal was apparent at either retention time.

2.7 COLOUR REMOVAL METHODS FOR WASTEWATER CONTAINING LIGNIN DERIVED CHROMOPHORES

The nature of the chromophoric constituents in the stillage appear to be due to lignin derived aromatic compounds and sugar condensation products. Chromophores present in a wastewater can be removed by a number of biological, physical or chemical methods. Colour removal techniques reported in the literature for lignin based aromatic compounds include: -

(i) Fungal decolourization;

(ii) Ozonolysis;

(iii) Lime treatment (precipitation);

(iv) Anaerobic digestion;

(v) Sorption techniques using resins (Chamberlain et al., 1975);

(vi) Ultrafiltration (Fremont and Kleper, 1980);

(vii) Ion Flotation (Herschmiller and Branion, 1973).

The first four options are considered more promising and cost competitive for wood-ethanol stillage and are considered in detail here. It is very doubtful if the last three physico-chemical methods are economically competitive. They are only included for completeness.

2.7.1 Fungal Decolourization

Fungi are capable of oxidizing lignin to low molecular weight degradation products, even to CO_2 and water by utilizing an active lignolytic enzyme system. Such white rot fungi have been used to decolourize the highly coloured E_1 stream originating in the Kraft bleach plant. Many white rot fungi were screened and tested and, the most promising organisms are *Phanerochaete chrysosporium* (Campbell and Joyce, 1981; Eaton et al., 1981; Sundman et al., 1981); *Coriolus versicolor* (Livernoche et al., 1981 and 1983); *Polyporus versicolor* (Marton et al., 1969); Tinctoporia p. (No. 28) (Fukuzumi et al., 1977).

Decolourization has also been shown to proceed anaerobically but its extent is much less than under aerobic condition (Marton et al., 1969). However, it is now well established that decolourization by white rot fungi is by and large an oxidative process and requires oxygen and they normally require an additional carbon source as a co-substrate (Eaton et al., 1981). Thus using glucose or cellulose as co-substrate, about 60% colour reduction (50% BOD and 60% COD reduction) has been achieved by *P. chrysosporium* within 2-4 days (Eaton et al., 1981; Smith., 1981). Also, the fungal biomass can be recycled for at least 60 days. This MyCoR (Mycelial Colour Removal) process has been considered to be more cost effective (by one order of magnitude) and reliable than current chemical and physical processes used for colour removal (Smith, 1981; Campbell and Joyce, 1981). More recent publications by Sundman et al. (1981) and Livernoche et al. (1981) have reported an 80% colour removal in one and three days respectively. It was also shown that decolourization was a result of a combination of the destruction of chromophores in the polymer and the decomposition of the polymer to low-molecular-weight, colourless, soluble/volatile products.

Fungal decolourization of wood-ethanol stillage will be investigated at FRI to determine (Callander, 1983(b)):

- the maximum loadings possible using a high productivity process e.g. a tower fermenter;
- extent of colour and BOD removal obtained, fungal biomass yield and the nutrient requirements.

Though still in its early stages of development, this method is likely to require two stages of stillage treatment for organic and colour removal (or vice versa). In a commercial operation, several reactors would be required. The reactors would then be inoculated with fungi (at various intervals), together with a nitrogen source and acidified primary sludge (Smith, 1981). Decolourization only begins after a growth period of three to four days thus resulting in a sequential nature of treatment in different reactors. Since the fungal biomass can be recycled and reused for at least 60 days, the nutrient requirement associated with this biological decolourization method can be reduced. A sterile environment is also required to prevent contamination of the fungal biomass. The requirement of co-substrate for colour removal is not likely

to be a problem since the saccharification process for ethanol production should easily meet this demand for a carbon source. A full scale plant using this treatment method has yet to be demonstrated.

2.7.2 Ozonolysis

This is a chemical oxidation method using ozone. It has been shown to be very effective in reducing colour (practical limit at 93% removals) from pulp bleachery wastes and does not produce a product which requires further processing (Melnyk et al., 1976).

Other possible advantages include the use of a small reactor (since this is a very fast reaction) and the use of recycled oxygen in a cascade system. However, like most chemical wastewater treatment processes, it has a high operating cost due to the use of chemicals. This method has also been shown to decolourize wood-ethanol stillage and is considered to be a fall-back alternative if biological decolourization (during anaerobic digestion) fails (Callander, 1983(a) and (b)).

2.7.3 Lime Treatments (precipitation)

This is a very well established method achieving over 95% colour removal for relatively concentrated kraft caustic bleach effluents. It was claimed as the most economic colour removal proposal in 1962 after a full study conducted at Louisiana State University (Winget, 1962). The method is based on the low solubility of the calcium salts of the coloured species causing their precipitation from solution. Apart from colour removal, 35-57% BOD reduction can also be obtained and, practically all the lime employed can be recovered. The clarified water after treatment also appears suitable for reuse in some process in within a kraft mill (Winget, 1962; Gellman, 1976).

This method is possibly worth considering for decolourization of wood-ethanol stillage since calcium recovery by carbonation has been shown to be feasible using a continuous carbonator-clarifier system (Winget, 1962). The fermentation process results in large volumes of CO_2 to be

released into the air (21.5 tonne.tonne^{-1} of dry wood (Table 2.3)). Though considered to present no difficulties by just venting into the atmosphere for a commercial scale operation, it may be more desirable if this effluent stream can be recovered or used. The lime treatment system provides a means of cleaning up this effluent while recovering the calcium (as calcium carbonate) used in the decolourization process. Nevertheless, there are also large quantities of calcium sludge (gypsum cake) from the neutralization process where satisfactory means of its disposal is still under review. The lime treatment method thus presents an interesting option for the decolourization of the stillage which has not been previously considered (Callander, 1983(b)).

2.7.4 Anaerobic Digestion

Studies have shown that the catabolism of lignin and related aromatic compounds by anaerobic bacterial cultures involve three different modes of decomposition of the aromatic nucleus. This occurs by photometabolism, nitrate respiration and methanogenic fermentation in which the benzene nucleus is first reduced and then cleaved by hydrolysis to yield aliphatic acids for cell growth (Evans, 1977; Zeikus, 1981). Figure 2.4 presents a common reductive pathway for anaerobic catabolism of aromatic compounds by methanogenic and denitrifying bacteria.

To date, the anaerobic degradation of aromatic compounds of lignin origin to end products of methanogenesis has been widely demonstrated (Healy et al., 1978; Healy and Young, 1979; Khan et al., 1981; Colberg and Young, 1982). Thus anaerobic decolourization of the stillage is conceptually feasible. However, negligible colour removal has been reported to date in anaerobic reactors treating wood-ethanol stillage. Conceptually, improved biological degradation of the chromophores should occur if their retention time within the reactor is increased and if close contact with the bacterial population is achieved. Packing an expanded-bed reactor with activated carbon should achieve both these purposes. To pursue this aspect further, the use of activated carbon in wastewater treatment will be considered in the following section.

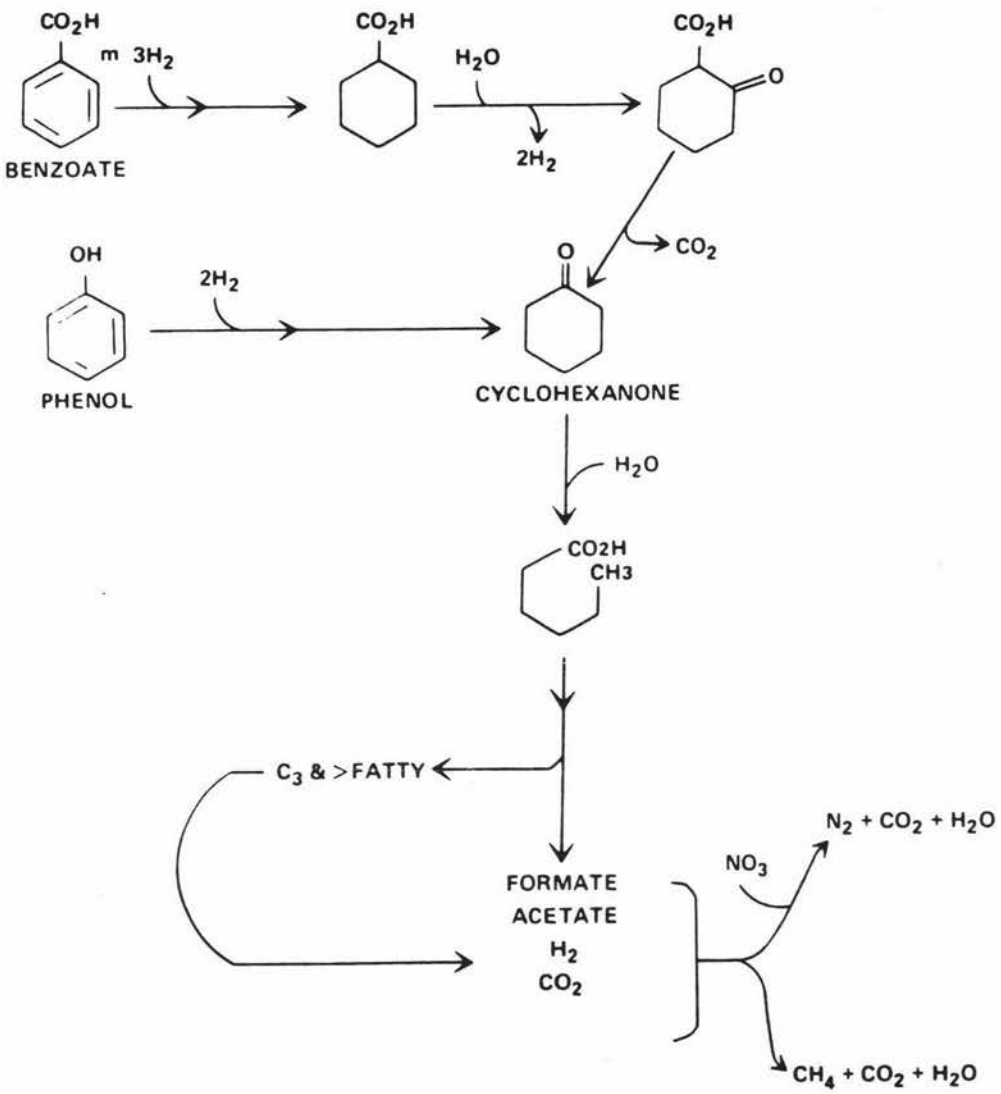


FIGURE 2.4: COMMON REDUCTIVE PATHWAY FOR ANAEROBIC CATABOLISM OF AROMATIC COMPOUNDS BY MIXED CULTURES CONTAINING METHANOGENIC OR DENITRIFYING BACTERIA (Zeikus, 1981)

2.8 THE USE OF ACTIVATED CARBON IN WATER AND WASTEWATER TREATMENT

The use of activated carbon for the removal of trace dissolved organics and colour from water and wastewater has been widely reported in the literature (EPA, 1973; Hassler, 1974; Gellman, 1976; Cheremisinoff and Ellerbusch, 1980; Cannon and Roberts, 1980; Zoltek and Melear, 1981; Culp and Clark, 1983; Robert et al., 1983). Powdered activated carbon (PAC) has been incorporated into the activated sludge process to facilitate the aerobic decomposition of organic compounds. It allows a faster adaptation of the bacteria to recalcitrant organic compounds (longer sludge age) and can compensate for the effect of low temperatures (Koppe et al., 1979; Leipzig, 1981; Kincannon and Esfandi, 1981). In fact, activated carbon has been claimed as one of the most efficient physical-chemical treatment systems available to the engineer for removal of organics (EPA, 1973). It can be applied both as a tertiary treatment process following conventional biological treatment systems, and as a unit process applied to raw or primary treated wastewaters.

Initially, it was thought that activated carbon functioned primarily as an adsorbent. But it is now widely known that the biological activity is usually quite prevalent when activated carbon is used for water and wastewater treatment (Tsezos and Benedek, 1980; Peter et al., 1983; Bancroft et al., 1983). Biological growth on the activated carbon is a consequence of the favourable environment provided by this material. Under proper conditions of design and operation, the combination of biodegradation and adsorption give stability and reliability to activated carbon column performance, can improve removal of certain types of water and wastewater components, and is known to prolong periods between required regenerations of the carbon (Weber et al., 1978; Bouwer and McCarty, 1982).

Bouwer and McCarty (1982) compared the removal of chlorinated benzenes and aliphatics in a GAC column with microbial activity versus a control column with only bacterial growth. After a suitable acclimatization period, several of the chlorinated benzenes were biodegraded in both columns but with a higher removal efficiency and a more stable operation in the GAC column. It was observed that in a favourable steady-state operation, the biodegradable organics were principally removed through

biofilm utilization. But when unfavourable conditions occured, biodegradable organics passed through the biofilm and became adsorbed by the GAC. These adsorbed species became available for degradation (by desorption) when favourable conditions returned. Thus the integrated microbial-GAC system resulted in a more stable and reliable overall removal performance.

Precise quantification of the interplay between attached biological growth and the diffusional transport processes associated with adsorption has been difficult due to the heterogeneous nature of the surface coverage on the carbon. This has been investigated by Li and DiGiano (1983), using an infinite-bath recycle reactor. Higher biodegradation rates and specific growth rates were observed on the GAC than on sand or coal. Evidence points to the utilization of internally sorbed substrates rather than better attachment on GAC. However Peel and Benedek (1983), using a bioresidual generation and adsorption system found that organic compounds that are biorefactory in conventional biological treatment were also refractory in biologically active carbon columns. This suggests that activated carbon does not possess an inherent bio-enhancement capability. This finding is contrary to many other reports (Li and DiGiano, 1980; Suidan et al., 1980; Tsezos and Benedek, 1980; Khan et al., 1982; Bouwer and McCarty, 1982).

Evsyukova et al. (1981), have shown that treatment of hydrolysis plant wastewater with granular lignin active charcoal of 2-5 mm in size was possible with 76 - 98% removal of COD and with full decolourization. However, the pollutant breakthrough slowly increased with time as more wastewater passed through the system until a stage that regeneration or replacement of the exhausted carbon was required. This regeneration of the exhausted carbon represented the major operating cost and energy utilization associated with the process. In this study, GAC was produced from the hydrolysis lignin.

In summary, the use of an integrated biological-physico-chemical treatment has already been proposed by Ying and Weber (1979). Enhanced anaerobic biodegradation of relatively resistant compounds on GAC was possible because of the favourable acclimatization environment of the GAC than, for example, sand or athracite coal (Khan et al., 1982). GAC has been used for the treatment of relatively toxic wastewaters where its ability

to sequester some components of the wastewater that were toxic to the mixed culture of anaerobic bacteria is advantageous (Suidan et al., 1980 and 1983). It has also been shown that this system can be used to treat refractory organics of aromatic origin (e.g. phenol and catechol) with continuous bioregeneration of the GAC (Suidan et al., 1980; Khan et al., 1981).

2.9 GENERAL CONCLUSIONS FROM LITERATURE REVIEW

The anaerobic digestion process has been developed to a point of reliable process application for many potential biomass feed stocks. The advancement of the high rate anaerobic digestion process in relation to efficiency and stability for wastewater treatment has made them more economically feasible and cost effective to implement. They may possibly contribute to providing net profits while overcoming major waste disposal problems.

Ethanol production from wood waste is technically and economically feasible. However, large volumes of wastewater (stillage) are generated in the process. Economic studies based on experimental work has been used to select the most attractive effluent handling process. Anaerobic digestion is favoured because it generates a usable fuel (methane) which doubles the energy efficiency of the wood hydrolysis process.

Anaerobic digestion of the stillage has been demonstrated to give 90% removal of organics and 80% conversion to methane. Nutrition and inhibition problems associated with the stillage have also been overcome. However, no significant reduction of the intense colour has been reported. Some form of treatment resulting in decolourization of the stillage will thus be needed before it can be discharged to a receiving water.

The chromophores in wood-ethanol stillage are potentially biodegradable and GAC has been demonstrated to enhance the biodegradability of a number

of refractory compounds of aromatic origin. An anaerobic expanded-bed reactor packed with GAC therefore represents a potential decolourization system worthy of investigation.

CHAPTER 3

EXPERIMENTAL PROCEDURE

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1 EXPERIMENTAL OUTLINE

Two identical reactors were constructed to assess the feasibility of using the anaerobic expanded-bed reactor packed with GAC for the decolourization of wood-ethanol stillage (including bioregeneration of the GAC *in situ*).

The first reactor was used for the treatment of raw wood-ethanol stillage from the FRI. The second reactor was fed anaerobically pretreated wood-ethanol stillage. This feedstock was obtained from the anaerobic lagoons treating raw wood-ethanol stillage and operated in the Biotechnology Department, Massey University (Archer et al., 1982). No apparent colour removal was reported for this lagoon treatment technique.

These two feedstocks were investigated because if treatment of raw wood-ethanol stillage (including decolourization) was successful, only one reactor would be required for the removal of the readily metabolizable organic compounds and the recalcitrant chromophoric material. This would constitute a major reduction in capital cost.

The anaerobic expanded bed process was selected for a number of reasons:

(i) Being a fixed film process, it would be more stable to biomass washout than a suspended growth reactor to any toxic upset or organic and hydraulic overload.

The GAC medium is able to provide a buffer against toxic or organic shock loading by interplay between adsorption and desorption from solution depending on loading conditions.

(ii) The use of GAC as a support medium should provide an increased likelihood of biological decolourization due to:

(a) a better contact between the chromphoric material and the methanogenic consortium, both being located at the carbon surface;

(b) a long retention time of adsorbed chromophoric materials is possible compensating for their slow, or negligible, biodegradation.

(iii) An expanded bed reactor is less susceptible to plugging than a packed-bed reactor.

(iv) Expanded bed reactors have been operated stably at exceedingly short hydraulic retention times and constitute an important category of the new generation of high rate anaerobic digestion processes.

In order to quantify the bioregeneration of the GAC in terms of COD and colour removal, batch isotherm tests were performed under the operating conditions used for the reactors. The same GAC was used but with no biological activity involved. The batch breakthrough curves were used to indicate when the first COD and colour breakthrough would occur and when the GAC would be fully exhausted in the absence of biological activity.

It was anticipated that the wood-ethanol stillage would contain highly inhibitory products apart from its deficiency in nitrogen, phosphorus, and alkalinity. Thus modifications of the stillage were performed to ensure optimal digester operation. The toxic nature of the stillage was overcome by the development of a highly acclimatized methanogenic consortium in the reactor.

3.2 DESIGN OF THE GRANULAR ACTIVATED CARBON PACKED, EXPANDED-BED REACTOR

Previous experience with an activated carbon packed expanded bed reactor for the anaerobic treatment of whey permeate in the Biotechnology Department, Massey University, proved invaluable in the design of the expanded bed reactor for this study. The previous reactor constructed from QVF glassware in a tower conformation had indicated a few short-comings such as the carry over of carbon, carbon slugging in the tower, carbon blockage in the recycle system and reactor carbon sampling for biomass concentration analysis. It was therefore decided that the anaerobic expanded bed reactor for this study would be designed to give a spouted bed and be constructed from 6 mm perspex. Perspex was chosen as the construction material since it can be milled to the size required and easily glued together while ensuring the visibility of the reactor contents. Apart from aiming for a compact and yet simple design of the reactor, the following features were included to overcome the problems anticipated:

(i) Carbon sampling: - A liquid sealed sampling port was located at the top of the reactor. A sampling device was designed to take an approximately 20 ml sample (carbon granules and reactor liquid) from any height within the reactor (Fig. 3.3).

(ii) Cleaning: - Detachable top and bottom covers were constructed to facilitate reactor cleaning in case of blockage (e.g. blockage of the feed inlet distributor and the overflow weir).

(iii) Carbon carry over: - A carbon trap was incorporated into the recyle line to capture any carbon carried over from the overflow weir.

(iv) Foaming problem: - A 10 cm high head space was allowed for in an attempt to prevent foam from reaching the gasline and a horizontal gas draw off was designed to permit the ready carriage of foam out with the gas. A foam trap was also incorporated in the gas line adjacent to the reactor in case of foam carry-over.

Detailed drawings of the expanded-bed reactor are as presented in Figures 3.1 - 3.3 and other important aspects of the reactor design are: -

(a) Feed Inlet Distributor

This was a closed cylindrical tube with 8 holes as the feed outlet (Fig. 3.2). The holes were directed downwards (34° to the vertical) such that they are shielded from any downflow of carbon granules. The inlet distributor was fixed to the bottom cover plate and was detachable for cleaning or modification.

The tapered end of the reactor bottom where the feed distributor is placed ensures that a high superficial fluid velocity is maintained in this area to prevent carbon blockage. A 70° angle was chosen for the sloping reactor side to prevent any carbon hold up.

(b) Reactor Body

The main bulk of the reactor carbon resided in this section in an expanded state. The dimensions were chosen such that any end effects were minimized and all sides of the reactor had equal dimensions (i.e. a square cross section).

(c) Settling Compartment

The upper part of the reactor was expanded to reduce the superficial fluid velocity and turbulance allowing any carbon granules or biomass flocs to settle back into the reactor. Two gas deflectors were used to keep gas bubbles away from the overflow weir. This created a quiescent zone around the overflow weir to minimize carry over of solids.

(d) GAC Sampling Port

This was a cylindical tube which extended from reactor top to 1.5 cm below the liquid surface. It permitted direct access to the reactor interior while maintaining a gas seal. This sampling port was used for reactor temperature measurement, alkali addition, reactor contents addition and reactor GAC sampling.

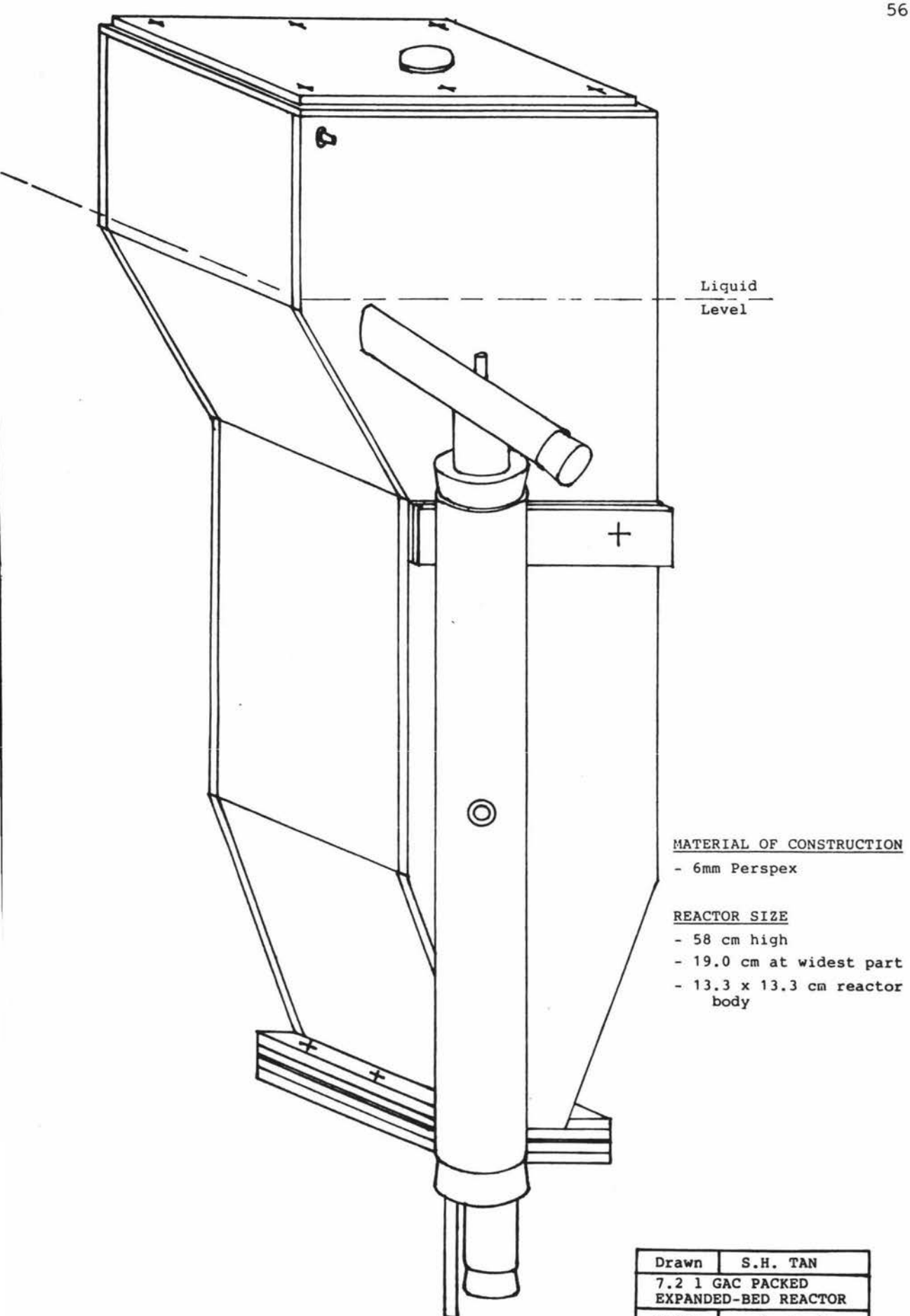
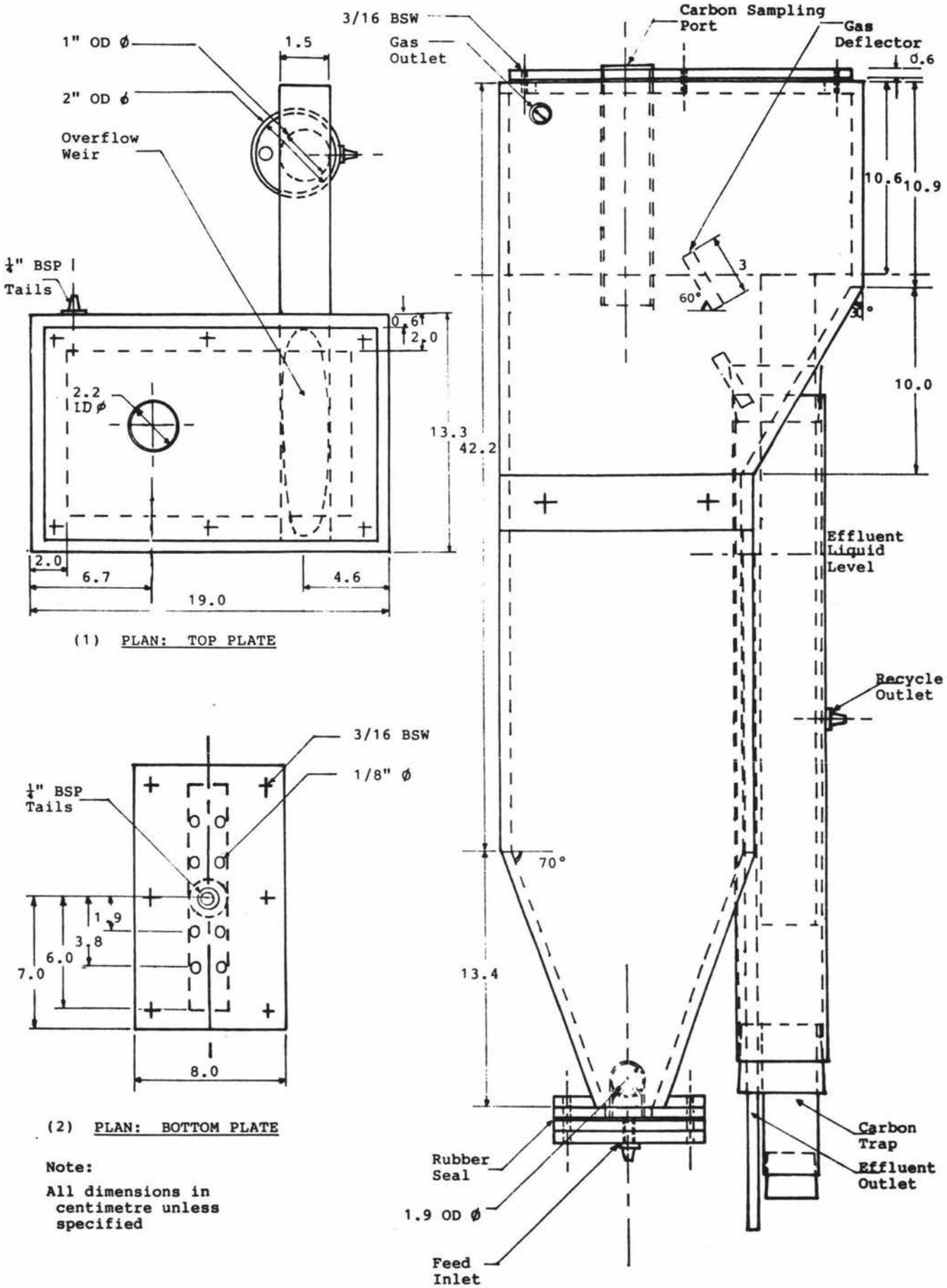


FIGURE 3.1: ISOMETRIC DRAWING OF THE 7.2 l GRANULAR ACTIVATED CARBON PACKED EXPANDED-BED REACTOR



(1) PLAN: TOP PLATE

(2) PLAN: BOTTOM PLATE

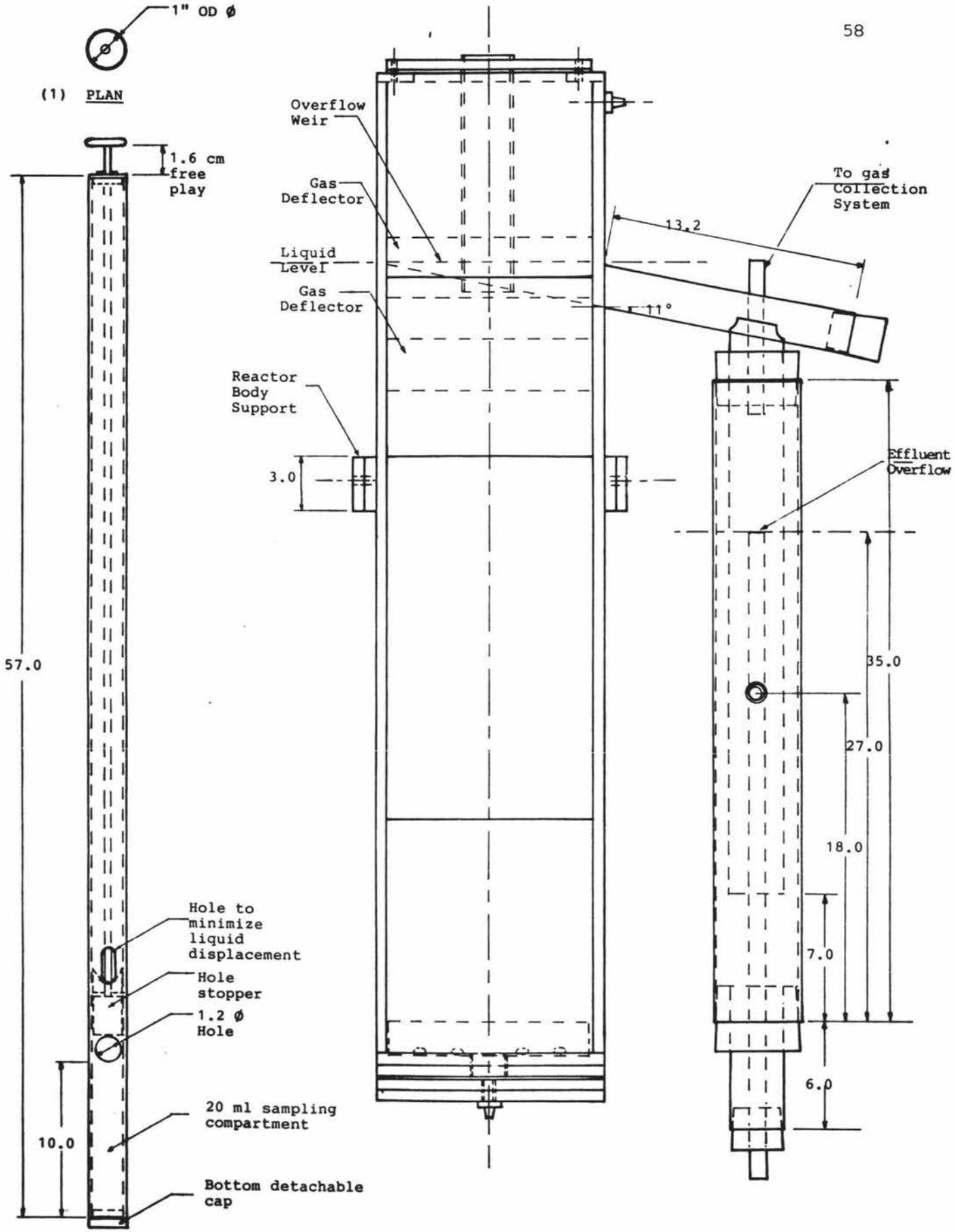
(3) SIDE ELEVATION

Note:

All dimensions in centimetre unless specified

Drawn	S.H. TAN
7.2 l GAC PACKED EXPANDED-BED REACTOR	
Scale	1 : 2

FIGURE 3.2: PLAN AND SIDE ELEVATION OF THE 7.2 l GAC PACKED EXPANDED-BED REACTOR



NOTE: All dimensions in centimetre unless specified

Drawn	S.H. TAN
7.2 l GAC PACKED EXPANDED-BED REACTOR	
Scale	1 : 2

FIGURE 3.3: FRONT ELEVATION OF THE 7.2 l GAC PACKED EXPANDED BED REACTOR AND THE GAS SAMPLER

(e) Exit Weir Assembly

This provided a simple means of collecting the effluent from the reactor while maintaining the gas seal. The exit weir assembly also consisted of an effluent reservoir for recycling and sampling of effluents. Any carbon carried over settled to the bottom into the carbon trap (Figs. 3.2 and 3.3). This was then retrieved and returned to the reactor via the GAC sampling port. However, there was very little, if any, carry over of carbon granules. An outlet for the treated effluent was provided by a tube which extended up into the effluent reservoir (Figs. 3.2 and 3.3). The recycle draw off was achieved by connecting the recycle line to the effluent reservoir at a distance below the liquid surface. This arrangement provided enough effluent buffering capacity for a single 100 ml sample drawoff without any air breaking into the effluent/feed recycle line.

The reactor had a total liquid volume of 7.20 litres. When 1.50 kg of GAC was added into the reactor, the total reactor liquid volume (including recyle system) was reduced to 5 litres. This 5 litre reactor liquid volume was used as the basis for hydraulic retention time calculations.

3.3 EQUIPMENT SET-UP

The apparatus was assembled with the minimum amount of fittings and tubes, and the arrangement was kept as simple as possible. Both reactors and their ancillary equipment were installed in a 37°C room where the temperature was controlled by an on/off hot air blower. Throughout its period of operation, the reactor liquid temperature was maintained at 37 ± 1.5°C. Occasionally, inflow of cold air through door openings had some effect on the room air temperature but little effect on the reactor liquid temperature.

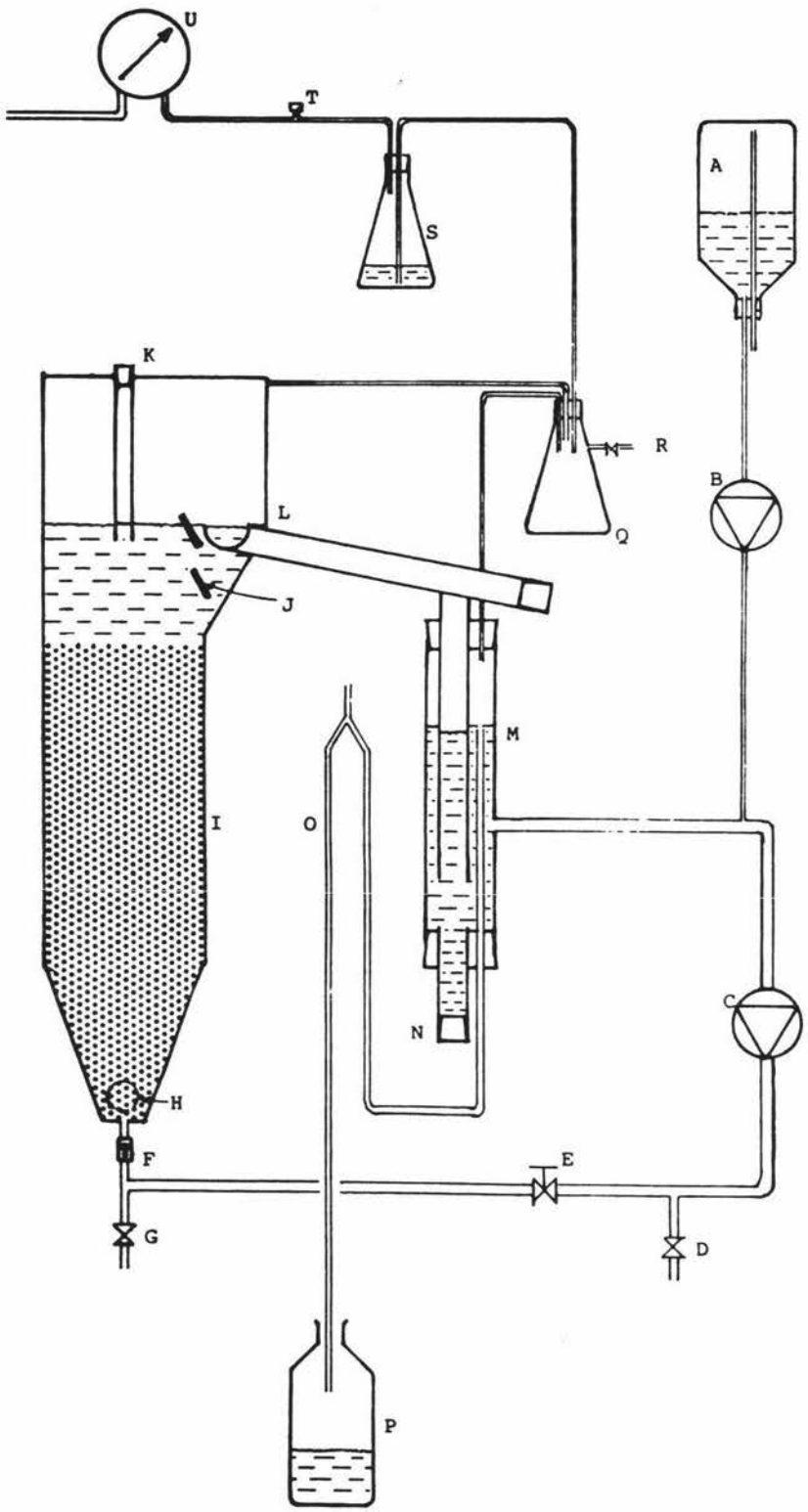
A schematic diagram is presented in Figure 3.4 and, a photograph of R1 and R2 in Fig. 3.5. The operational aspects are discussed in the following sections.

(a) The Feed and Recycle System

The stillage feed was introduced into the suction side of the recycle line (12.5 mm ID, clear PVC tube) by a peristaltic pump (Masterflex 7014 series, Cole-Parmer Instruments, Chicago, USA). This recycle flow was maintained by a recycle pump (Shacklock centrifugal pump, Auckland). This pump had a maximum flow capacity of 45 $l.min^{-1}$.

A one way check valve (3/8" BSP) was incorporated to prevent any backflow of effluent or carbon granules. The recycle flow rate was controlled by means of a ball valve (Klingers Ball-O-top, 3/8" BSP) on the recycle line. The valve setting was chosen to give a bed expansion of 6-8%, normally at a recycle flow rate of 0.73 - 0.80 $l.min^{-1}$. The recycle flow rate was measured using a Gilmont Compact Flowmeter with a flow range of 0.03 to 1.900 $l.min^{-1}$ (Gilmont Instruments, Inc.). The recyle/feed liquor then passed out of the feed distributor in an upflow mode through the bed of GAC particles which were partly fluidized. The liquid effluent then flowed out of the reactor through the overflow weir into the exit weir assembly (Fig. 3.4). The treated effluent eventually overflowed into the liquid level adjuster (also giving a gas seal to the exit weir assembly) and finally into the effluent collection vessel.

The reactor liquid contents were sampled from a tap on the delivery side of the recycle line. In order to obtain a representative sample, the first 30 ml sample was discarded to purge the sampling tube of unrepresentative material.



(1) Feed System
(A) Feed reservoir
(B) Feed pump
(2) Recycle System
(C) Recyle pump
(D) Liquid Sampling Port
(E) Recycle flow controller
(F) One way valve
(G) Water jet connector
(H) Feed distributor
(I) Granular activated carbon and biomass
(J) Gas deflector
(K) Carbon sampling and material addition port
(L) Overflow weir
(M) Exit Weir assembly
(N) Carbon trap
(O) Liquid level adjuster
(P) Effluent collection vessel
(3) Gas Collection System
(Q) Foam trap
(R) Gas sampling port
(S) Gas bubbler (scrubber)
(T) Gas sampling port
(U) Gas meter

FIGURE 3.4: EQUIPMENT SET-UP

FIGURE 3.6: A close-up view of the GAC Packed Expanded-bed Reactor.

The reactor was filled with distilled water and has not yet been inoculated. A clear view of the reactor interior was possible. Notice that no carbon particles leave the carbon bed and hence there is no problem of carbon carry over.

FIGURE 3.5: Equipment Set-Up - Photographic View.

R1 is to the right for the treatment of wood-ethanol stillage and R2 is to the left for the treatment of anaerobic lagoon pretreated stillage.

(b) The Gas Collection System

The biogas produced was collected from the top of the reactor as well as from the exit weir assembly. The biogas was first passed into a foam trap and then through a liquid bubbler. The liquid bubbler permitted a visual inspection of the biogas being produced (a quick indicator of any gas leaks) and could be used as a biogas scrubber if required (e.g. using lead acetate solution to absorb any hydrogen sulfide present in the biogas). The amount of biogas produced each day was measured by a 1 litre wet gas meter (Wilhelm Ritter, 4630 Bochum-Langendreer, West Germany) before being vented out of the hot room. Volumetric gas measurements were therefore made at 37 ± 1.5°C. ¼" ID butylrubber tubes were used for the gas line since it is less permeable to methane gas than clear PVC tube.

There were two sampling ports in the gas line. The first was an outlet tube in the foam trap allowing at least 500 ml of gas to be drawn off each time. This was required for the measurement of hydrogen sulfide concentration in the biogas using the Drager tube method (Drager, West Germany). The second gas sampling port was located after the gas bubbler and gas samples could be obtained through a rubber septum (Fig. 3.4). This was normally used for the methane composition determination where a small gas sample is required. Sampling was normally done with a 0.1 ml syringe (SGE, Australia).

3.4 METHODOLOGY

3.4.1 GAC Selection

The aim was to select a grade of commercially available GAC of suitable size but not necessarily of high purity. Some work on the decolourization of wood ethanol stillage under different adsorption conditions (e.g. pH, temperature, time of adsorption and carbon dosage) had been performed at FRI using different carbons (Callander, 1983(a), personal communication).

After considering a few options, Filtrasorb 200 from Portals Water Treatment (NZ) Limited, Auckland was considered the most suitable chiefly due to its availability for the size range required.

Filtrasorb 200 is manufactured by Calgon (Calgon Corporation) for the removal of dissolved organic material from municipal and industrial water supply. It is manufactured from selected grades of bituminous coal and claimed to yield a high density, durable granular product capable of withstanding the abrasion and dynamics associated with repeated reactivation, hydraulic transport, back washing and mechanical handling. Also, activation is carefully controlled to produce a high internal surface area with an optimum pore size for the adsorption of a broad range of high and low molecular weight impurities (Calgon Bulletins, 1974). Thus, Filtrasorb 200 appears to be particularly suitable as support medium in this study.

Table 3.1 presents a summary of the carbon physical properties and its specifications.

The product size ranges from 0.420 to 1.680 mm but the preferred size range for this study was 0.420 - 0.850 mm (18 to 36 B.S. mesh No.). Thus before using, the original carbon was sieved to obtain particles in the required size range. The carbon mixture used to pack the reactors consisted of:

0.600 - 0.850 mm	(69.13%)
0.420 - 0.600 mm	(30.87%)

At this size range, the GAC porosity was found to be 0.59 with a particle density, wetted in water of 1430 $kg.m^{-3}$. The bulk density was 515 $kg.m^{-3}$.

TABLE 3.1: PHYSICAL PROPERTIES AND SPECIFICATIONS OF FILTRASORB 200 (Calgon Bulletins, 1974)

Physical Properties	Filtrasorb 200
Total surface area, N_2 BET method ($m^2.g^{-1}$)	850 - 900
Particle density wetted in water ($g.cc^{-1}$)	1.4 - 1.5
Uniformity coefficient	approximately 1.7

Specifications	Specification	Typical Analysis
Sieve size U.S. standard series		
Larger than No.8 (max %)	-	-
Larger than No.12 (max %)	5	3
Smaller than No.30 (max %)	-	-
Smaller than No.40 (max %)	5	2
Effective size (mm)	0.55-0.65	0.6
Iodine Number (mm)	850	875
Abrasion Number (min)	70	83
Moisture as packed (max %)	2	0.5
Average pore diameter (A°)*		10-1000
Voids in dense packed column (%)*		40%
Porosity (%)*		40%
Particle internal porosity ($cc.g^{-1}$)*		2.5

* Data from Calgon Corporation, Personal Communication, 1983.

3.4.2 Seed Sludge Origin and Composition

The reactor seed inoculum was collected from a variety of sources to increase the probability of getting anaerobic bacteria capable of degrading the chromophoric compounds in the stillage. Thus the inoculum comprised samples from a dairy waste anaerobic lagoon, silage leachate, sawdust leachate and from an anaerobic lagoon treating FRI stillage (Table 3.2).

All the inocula were strained through a muslin cloth to remove much of the particulate material and fibres. Care was taken to minimize air exposure of the seed sludge.

The percentage contribution of each seed type in the inoculum is presented in Table 3.2. A detailed analysis of the seed inoculum is presented in Appendix 1.

TABLE 3.2: SEED SLUDGE ORIGIN AND COMPOSITION

Seed source	Volume (l)	% Contribution
Dairy waste anaerobic lagoon	1.50	29.5
Anaerobic lagoon for stillage	1.50	29.5
Silage leachate	0.64	12.6
Sawdust leachate	0.64	12.6
Distilled water (to 5 litres)	0.80	15.8

3.4.3 Reactor Start-UP

Trial runs were first performed with water and then with GAC to check for any leaks and to observe the liquid flow characteristics within the reactor. When a satisfactory operating procedure had been developed, the water was drained off and the reactor was seeded with the freshly prepared inoculum. Fig. 3.6 gives a view of the reactor after it was filled with the GAC (not yet inoculated). The bed had been expanded 6-8% over the original reactor carbon volume. There was no problem with

carry-over of carbon as any carbon leaving the bed and moving towards the settling compartment experienced a lower liquid superficial velocity and settled back into the reactor causing the transparency above the carbon bed as shown in Fig. 3.6.

R1 was seeded on the 3rd of June, 1983 and the reactor was allowed to run for five days before any feeding was attempted. Feeding first commenced on the 8th of July, using half-strength stillage from FRI (1:1 dilution with distilled water) supplemented with 240 $mg.l^{-1}$ nitrogen and 80 $mg.l^{-1}$ phosphorus (as ammonium chloride and potassium hydrogen phosphate respectively). The reactor was initially run at a 30 day HRT. A cam-timer operating on a 1 hour cycle was used to control the feeding rate to achieve the necessary HRT at the lowest stable pump setting.

When it was demonstrated that the reactor design was satisfactory, work commenced to construct the second similar reactor for decolourization of the anaerobic lagoon treated stillage. Using the same start-up procedure as for R1, feeding first commenced on the 10th of June for this second reactor (R2)

3.4.4 Stillage Feed Preparation and Storage

The wood-ethanol stillage to be treated by R1 was from a pilot plant for the production of ethanol from exotic forest trees at FRI. The whole stillage was trucked unrefrigerated in 200 l drums from Rotorua to Palmerston North. They were then broken down into 20 l lots and stored at 4°C. Throughout, the operation of R1, the stillage feed was not normally stored for a period of more than three months before use. For a storage period of six months, it has been reported that the total volatile acid levels rose from 1000 $mg.l^{-1}$ to 4000 mg/l and pH dropped from 4.0 to as low as 3.2 with no change in the COD (Archer et al., 1982).

At the commencement of this work, the stillage feed was first supplemented with 240 $mg.l^{-1}$ nitrogen and 80 $mg.l^{-1}$ phosphorus as recommended by Callander, 1983 (personal communication). During the start-up period the following further modifications to the raw stillage were made:

(i) Alkalinity addition - 2.5 $ml.l^{-1}$ of 20% w/v NaOH was added per litre of stillage used. This addition rate was adjusted, as required, to maintain digester operation around neutral pH and alkalinity of more than 2000 $mg.l^{-1}$ as $CaCO_3$.

(ii) Sulphate removal - the stillage soluble sulphate level (normally at 1500 $mg.l^{-1}$) was desulphated to 500 $mg.l^{-1}$ to avoid sulfide toxicity. This was done by adding a stoichiometric amounts of 10% w/v $BaCl_2.2\ H_2O$ solution (24.9 ml of the solution removes 1000 mg sulphate per litre of stillage). The mixture was magnetically stirred for 15 mins at room temperature and the $BaSO_4$ precipitate removed by filtration (Whatman No. 1 filter paper). The procedure is the same as that used at the FRI (Callander (1983) personal communication).

This sulphate level reduction step was carried out before nutrient or alkalinity addition.

3.4.5 Monitoring Parameters and Analytical Methods

Regular analyses of the stillage feed, reactor effluent and the biogas produced were performed to ensure stable digester operation and to quantify the conversion of stillage organics to biogas and biomass for reactor performance assessment.

3.4.5.1 Hydraulic and organic loading rate - chemical oxygen demand (COD) determination

The reactor feed rate was measured daily using a graduated measuring cylinder during the changing of feed in the morning. Since the expanded-bed reactor is a fixed-film system, the hydraulic loading rate is not as important in terms of cell washout as for a suspended growth system. However, the hydraulic loading rate controlled the organic loading rate to the reactor. The relationship can be described as:

$$\text{Reactor Organic loading rate } (\text{kg t COD.m}^{-3}\text{.d}^{-1}) = \frac{\text{Organic matter in feed } (\text{kg t COD.m}^{-3})}{\text{Hydraulic retention time } (\text{d})}$$

The organic loading rate is considered one of the most important parameters in digester design and operation, and it had to be closely controlled to maintain stable digester operation.

Organic matter in the feed or effluent was primarily measured by the COD concentration (normally twice a week for the effluent). The Hach COD digestion apparatus (Model No. 16500-10) was used to oxidize the samples in H_2SO_4 and potassium dichromate solution for 2 hours and the absorbance was then measured at 600 nm (Hitachi, model 101 spectrophotometer). A standard curve, linear over the range of 0-750 $mg.l^{-1}$ COD, was constructed. Duplicate samples were analysed. Samples from the untreated effluent gave the total COD (tCOD) while centrifuged (6000 rpm for 20 mins) and filtered samples (Whatman GF/C) gave the soluble COD (s COD). The method is adopted from Jirka and Carter (1975). These COD measurements allowed an assessment of reactor performance and stability.

3.4.5.2 pH measurement

A target pH value of at least 6.8 and preferably in the vicinity of 7.0 was aimed for.

The pH was measured using a pH meter (Kent, EIL 7055). The meter was calibrated daily and checked occasionally using buffer solutions at pH 7.0 and 9.2 respectively. Measurements were done as soon as possible (within 20 seconds of sampling) to minimize pH change due to loss of gas (mainly CO_2) from the solution.

pH measurement was done daily as a sharp drop in pH (especially at a low alkalinity value) could indicate an accumulation of VFAs and prewarning to digester overloading.

3.4.5.3 Alkalinity determination

Alkalinity values (as $mg.l^{-1}$ $CaCO_3$) were obtained daily by titrating with 0.0912 N HCl to pH 4.3 according to Standard Methods (APHA, 1980). This end point pH corresponds to an inflection point of the titration curve.

For adequate buffering capacity, an alkalinity value in excess of 2000 $mg.l^{-1}$ is desirable. This was achieved by the addition of an alkalinity reagent. The determination of minimum alkalinity requirement was not an objective of this research. The alkalinity doses used in this study will be expensive if used with a commercial process.

5.4.5.4 Volatile fatty acids (VFA) determination

A 5 ml sample was centrifuged (6000 rpm, 20 mins) and filtered (Whatman GF/C) prior to acidification with 0.5 ml of 100% formate. This sample was subsequently analysed in a gas chromatograph (Shimadzu, GC-5A) using a 1 µl injection sample. A hydrogen flame ionisation detector was used, with a 2 m x 3 mm I.D. glass column packed with 5% FFAP on 80/100 mesh chromosorb W AW obtained from Supelco, Inc. Flow rates of the nitrogen carrier, hydrogen and air were 80, 55 and 900 $ml.min^{-1}$ respectively. The column temperature was 145°C and the injection port temperature was 180°C. Sensitivity was set at 10^3 and range at 1. The Chromatograph output signal was recorded by a CR 650S Recorder (JJ Instruments) and the peak area was computed by an integrator (Varian CDS 111). The VFA standard was designed to measure acetate (C_2), propionate (C_3), iso-butyrate (i-C_4), n-butyrate (n-C_4), iso-valeric (i-C_5) and n-valeric acid (n-C_5) concentrations at 0-1000 $mg.l^{-1}$ for C_2 and C_3, and less than 400 for C_4 and C_5.

The VFA levels are sensitive indicator of the symbiotic balance between the methanogenic and non-methanogenic bacteria in the digester. A continued rise in acetic and propionic acids would indicate an imbalance which is normally due to organic overloading or a toxicity problem.

The individual VFA's were normally measured twice a week but when there was evidence of digester imbalance or when detailed results were required, they were determined daily.

3.4.5.5 Feed and effluent solids determination

The solids parameters measured were the total solids (TS), Volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS). Samples were dried at 105°C for at least three hours in a hot air oven (to constant weight) and ashed at 550°C overnight for TS and VS determination. Similar procedures using centrifuged (6000 rpm, 20 mins) and filtered (Whatman GF/C) samples yielded the TSS and VSS values.

The effluent solids were normally measured twice a week and these values gave a measure of the cell yield in the reactor as well as the acceptability of the effluent for disposal in terms of its solids content.

3.4.5.6 Gas production and methane content measurement

The gas production rate (recorded daily) was measured by a 1 litre wet gasmeter (Wilhelm Ritter, 4630 Bochum-Langendreer, West Germany) and the percentage methane in the biogas was determined by gas chromatography (Varian aerograph, Model 920). The column and gas thermal conductivity detector temperatures were 70 and 105°C respectively with nitrogen carrier gas at 55 ml/min. The column was a 5' x ¼" SS with 80/100 mesh Poropak S packing. A 100 µl syringe was used to inject the biogas sample and the chromatograph output signal was recorded by a Sargent Recorder, model SRG. The peak height recorded was compared to the standard curve using pure methane gas. Measurements were normally done twice a week. Both the gas production rate and its CH_4 content are very sensitive indicators of digester instability as characterized by a sharp drop in gas production rate and the CH_4 content (accummulation of CO_2 and H_2 gas). The methane gas yield was also an important consideration since the anaerobic digestion process combines wastewater treatment with energy production.

3.4.5.7 Colour measurement

Colour measurements were normally taken twice weekly to assess reactor performance and bioregeneration of the GAC in the reactor in terms of colour removal capability.

Two methods were used i.e. the Visual Comparison Method according to Standard Methods (APHA, 1980) and the Spectrophotometric Method (Carpenter and Berger, 1973). Measurements were normally done at three different pH values i.e. pH of 5, 7.6 and 9. The single colour measurement at pH 7.6 allows uniformity in comparison with reported colour concentrations.

Samples were centrifuged at 6000 rpm for 20 mins and filtered through a Whatman GF/C glass fibre filter. The GF/C filter was shown to have an effective pore size of 0.7 micron (Sheldon, 1972), which is close to the 0.8 micron membrane filter recommended by Carpenter and Berger (1973). All colour measurements quoted are for true colour determination (for clear solution) unless specified (i.e. apparent colour if sample is turbid after pretreatment).

3.4.5.8 Nitrogen determination

(a) Total nitrogen (TN)

The total nitrogen concentration was determined by the Semi-Micro Kjeldahl Method according to Standard Methods (APHA, 1980). The samples were digested in sulphuric acid followed by steam distillation of the resulting ammonia into boric acid and back-titration with 0.02 N HCl.

(b) Ammonia nitrogen (NH_3 - N)

The ammonia nitrogen concentration was determined by steam distilling the sample (after pH adjustment to 9.5 with 6 N NaOH) into boric acid followed by back titration with 0.02 N HCl according to Standard Methods (APHA, 1980).

(c) Organic nitrogen

The ammonia nitrogen was first removed from the sample (boiling with borate buffer at pH 9.5) before being acid digested as for total nitrogen determination according to Standard Methods (APHA, 1980).

NH_3 - N is the most important among these three nitrogen parameters since it is the form of nitrogen which is most readily available for bacterial growth. The NH_3 - N level in the reactor was measured once every week or after each steady state to ensure that nitrogen was in adequate supply for bacterial growth. This was taken as a residual NH_3 - N level of 50 $mg.l^{-1}$ in the reactor. TN and organic nitrogen were measured incidentally for comparison with similar work in this area and as indicator of how much the nitrogen is cell bound (as organic nitrogen).

3.4.5.9 Phosphorus determination

(a) Total phosphorus (TP)

Total phosphorus includes orthophosphate, inorganic condensed phosphates and organic phosphorus compounds. The sample was heated under acid conditions to 120°C with potassium persulphate. After digestion, the orthophosphate (i.e. total phosphorus in sample) was determined by reaction with molybdate under acid conditions to form phosphomolybdate. This is then reduced to the intense "molybdenum blue" by ascorbic acid with antimony. The resulting colour was measured at 710 nm (Hitachi Model 101 Spectrophotometer) according to NWASCO Methods (1981).

(b) Total dissolved phosphorus

The procedure used was the same as the one for total phosphorus determination but a membrane filtered sample (0.45 µm, Millipore Type HA) was used. Care was taken not to disrupt the bacterial cells during membrane filtration of the sample.

(c) Reactive dissolved phosphorus (RDP)

RDP (i.e. orthophosphate and all forms of phosphorus that are hydrolysed to orthophosphate under the conditions of the test) was determined as for the TDP method but minus the acid digestion step according to NWASCO Methods (1981).

As for NH_3 - N, RDP was considered the form that is most readily available for microbial growth. However, phosphorus forms an insoluble complex with a number of metal ions and hence may become unavailable to the bacteria. Few data are available to determine an optimal residual RDP. In this study this is taken as 1 $mg.l^{-1}$ with no sign of reactor performance deterioration or unstable operation.

3.4.5.10 Soluble sulfide measurement

Both qualitative and quantitative methods were employed since low concentrations of sulfide were involved. The tests were as recommended by Standard Methods (APHA, 1980).

For the qualitative test, both the lead acetate paper and the antimony test comparing the yellow colour of antimony sulfide to known sulfide concentrations were used.

Soluble sulfide was also determined using the titrimetric iodine method (APHA, 1980). Suspended solids were removed by centrifuging the sample at 6000 rpm for 20 minutes and interfering dissolved species were removed by precipitation of the soluble sulfide as zinc sulfide before assaying for sulfide. This method was found to be inadequate when colour was present in the effluent due to the difficulty in determining the end point of the titration.

Finally the Drager pump method was adopted (Drager, West Germany, Mod. 21/31, Drager tubes of type CH 29101). The tubes can measure hydrogen sulfide concentrations ranging from 0-2000 ppm in the gas. From a known H_2S concentration in the gas, the soluble sulfide concentration which consists of H_2S, HS^- and traces of S^{2-} species in solution (Callander and Barford, 1983(a)), can be evaluated for a known reactor temperature and pH (Lawrence et al (1964), Callander and Barford (1983(b)).

3.4.5.11 Soluble sulphate determination

The Gravimetric Method with Drying of Residue was used to measure the soluble sulphate (APHA, 1980). The sample was centrifuged at 6000 rpm for 20 mins to remove any suspended solids. The soluble sulphate was then precipitated at very low pH as barium sulphate by barium chloride addition and digested at 80-90°C for at least two hours. The granular precipitate was then filtered (Whatman 542), warm washed to remove any chloride and dried in a 105°C hot air oven before weighing.

3.4.5.12 Total soluble iron determination

The total soluble iron in the solution can be in the ferrous or ferric state. They were determined using the Thiocyanate method as described in Vogel (1961).

A centrifuged (6000 rpm, 20 mins) and filtered (Whatman GF/C) sample was allowed to react with dilute potassium permanganate solution to convert the ferrous iron to the ferric state. The ferric iron was then allowed to react with excess thiocyanate in the presence of strong acid (to suppress $Fe(OH)_3$ formation) to form an intensely red-coloured compound, which remained in true solution. The concentration was determined spectrophotometrically at 480 nm (Hitachi Model 101). A standard curve, linear over the range of 0-10 $mg.l^{-1}$ ferric iron was used.

3.4.5.13 Five days biological oxygen demand (BOD_5) determination

The Hach Manometric BOD Apparatus (Model 2173) was used for BOD_5 determination.

The sample in an air tight bottle with 10 ml seed inoculum was incubated at 20°C for 5 days. Any dissolved oxygen consumed during the oxidation of organic matter will be replenished by the air oxygen and results in a drop in air pressure. This pressure drop was registered on the mercury manometer and the BOD level computed. Any carbon dioxide produced during the oxidation process was removed by kOH solution in a seal cup in the sample bottle. Both $sBOD_5$ and $tBOD_5$ were measured. The sample pre-

treatment procedure is the same as described for COD determination. The BOD_5 test is not used as a monitoring parameter but as an indicator to reactor performance. It was only measured when the reactor had reached a new 'steady state' situation.

3.4.5.14 Biomass measurement

Usual biomass measurement techniques, most importantly volatile suspended solids, are not applicable to the reactors used in this study due to the influence that the GAC has on these methods. The biomass indicators used were: -

(a) Organic nitrogen method - The measurement method is as described under Nitrogen determination.

(b) Total phosphorus method - The measurement method is described under phosphorus determination.

(c) DNA method - DNA was extracted by 0.5N perchloric acid. Colour is developed with diphenylamine reagent to which acetaldehyde was added to increase the sensitivity of the method. Working standards ranging from 0-120 $\mu g.ml^{-1}$ DNA were used and absorbance measured at 600 nm on an Hitachi model 101 Spectrophotometer. The method is developed by Burton (1956) as described in Methods in Microbiology (Herbert et al., 1971).

DNA was chosen as a suitable biomass indicator since the DNA content was a constant proportion of the bacterial cell mass which does not vary much over a wide range of growth rates and is only found inside the living entity (Hattingh and Siebert, 1967). Both the nitrogen and phosphorus methods assumed a certain proportion of cell N and P content.

Carbon samples were taken from about 10 cm above the bottom feed plate using a carbon sampler (Fig. 3.3). The samples were then washed with distilled water. Analyses were normally done on oven dried carbon samples (105°C) but fresh samples (at about 52% water)

were sometimes used. The results obtained were compatible after appropriate corrections for moisture content.

3.4.5.15 UV-visible specrophotometric scan

UV spectra were taken with distilled water as reference using a Varian Series 634 UV-Visible Spectrophotometer and in 1 cm fused silica cuvettes. The absorbance was recorded on a CR 650S Recorder (JJ Instruments) at wave lengths (λ) between 160-400 nm. The samples were filtered (Whatman GF/C) to remove any suspended solids. The spectra were also recorded after ionization (ionization difference spectrum) by adding 3 drops of 1 M NaOH to a 10 ml sample (Sundman et al., 1981).

Visible spectra were taken using the same procedure but using 1 cm glass cuvettes for wavelength ranges from 300-800 nm. Appropriately diluted samples with and without pH adjustment to 7.6 were used. Note that, the spectrum with sample pH at 7.6 and λ of 465 nm corresponds to the results from spectrophotometric colour measurement method.

3.4.5.16 Electron microscopic scans

The carbon samples (from R1 and R2) and bacterial flocs (from R1) were taken and kept submerged in the reactor liquor on the 16th of December, 1984 and sent to the FRI for electron microscopic scans. The carbon samples or bacterial flocs were placed on aluminium studs and coated with carbon and gold palladium prior to observation in a Philip's 500 Scanning Electron Microscope.

3.4.6 Analytical Precision

The systemic and analytical errors associated with the analyses carried out in this study are considered to be within the range of values reported in Standard Methods (APHA, 1980). Other systemic and analytical errors were calculated from at least 5 replicate analyses of the same sample. Table 3.2 presents a summary of the general analytical scheme and the analytical errors that could be expected for this study.

TABLE 3.2: SUMMARY OF ANALYTICAL PRECISION

Analysis	Measurement Method	General Analytical Scheme	Percentage Recovery	Precision Reference Value (x)	Standard Deviation (σ)	Percentage Deviation (σ/x x 100%)	Units
Feed loading rate	Measuring Cylinder	Daily	-	2.00	0.09	4.5	$l.d^{-1}$
Temperature	Mercury thermometer	Incidentally	-	37.0	0.4	1.1	°C
pH	Glass electrode	Daily	-	7.00	0.05	0.7	pH units
Alkalinity	Potentiometric titration	Daily	-	2500	60	2.4	$mg.l^{-1}$ as $CaCO_3$
TS	Drying and ashing	Twice weekly	-	10.00	0.44	4.4	$g.l^{-1}$
VS	Drying and ashing	Twice weekly	-	5.00	0.43	8.5	$g.l^{-1}$
Acetate	Gas Chromatography	Twice weekly	Relative to standard	150	13	8.7	$mg.l^{-1}$
Propionate	Gas Chromatography	Twice weekly	Relative to standard	100	14	14	$mg.l^{-1}$
Percentage CH_4	Gas Chromatography	Twice weekly	Relative to standard	60.0	1.2	2.0	%
Gas flow	Wet gas meter	Daily	Subject to Calibration	30.0	-	-	$l.d^{-1}$
TN	Semi-Micro Kjeldahl Method	Incidentally	-	-	-	-	$mg.l^{-1}$
Organic N	Semi-Micro Kjeldahl Method	Incidentally	-	0.60	0.03	5	$mg.g^{-1}$ carbon
NH_3-N	Titrimetric Method	Weekly	93-99	300	10	3.3	$mg.l^{-1}$
TP	colourimetric	Incidentally	Relative to standard	300	20	6.6	$mg.l^{-1}$
TDP	colourimetric	Incidentally	Relative to standard	50	2	4.0	$mg.l^{-1}$
RDP	colourimetric	Weekly	Relative to standard	40	2	5.0	$mg.l^{-1}$
tCOD	Hach COD digestion Apparatus	Twice weekly	Relative to standard	25000	1200	4.8	$mg.l^{-1}$
sCOD	Hach COD digestion Appartatus	Twice weekly	Relative to standard	23000	600	2.6	$mg.l^{-1}$
$tBOD_5$	Hach Manometric BOD Apparatus	Steady state operation	-	10000	730	5.2	$mg.l^{-1}$
Soluble Sulfide	Drager pump Method	Incidentally	-	0.03	-	-	$mg.l^{-1}$
Soluble Sulphate	Gravimetric Method	New batch of feed	97-99	1500	45	3.0	$mg.l^{-1}$
Colour	Visual Comparison Method	Twice Weekly	Relative to standard	5000	280	5.6	CPU
Colour	Spectrophotmetric Method	Twice Weekly	Relative to standard	9000	580	6.4	CPU
DNA	Colourimetric Method	Incidentally	Relative to standard	-	-	-	$\mu g.l^{-1}$
Soluble Iron	Thiocyanate Method	New batch of feed	Relative to standard	170	5	2.9	$mg.l^{-1}$

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

4.1 OUTLINE OF SECTION

This section considers the adsorption of sCOD and colour from raw and anaerobically treated wood-ethanol stillage with and without biological activity. The objectives of the research were to determine whether bio-regeneration of the GAC occurred, in terms of sCOD and colour removal.

Batch experiments were carried out in the absence of a microbial inoculum to determine the adsorptive capacity of the GAC by purely physical and chemical means. The breakthrough curves from these studies indicated when colour and sCOD breakthrough would occur and, when the carbon would be exhausted in the absence of bioregeneration. These results are presented in Section 4.2.

To assess GAC treatment with anaerobic biological activity, two expanded bed reactors were operated on raw and anaerobically treated stillage, for 7½ and 6½ months respectively. These reactors were operated as high rate anaerobic digesters and sufficient data were obtained to describe the reactors performance in terms of organic removal, colour removal and stability.

The operation of the reactors will be described in four phases:

(i) The Acclimatization phase;
(ii) The Increased Organic Loading Phase;
(iii) The Operational Phase;
(iv) The GAC Bioregeneration Confirmation Phase

The results for each of these stages are presented in Sections 4.3 and 4.4 and, constitute the major emphasis of this study.

4.2 ADSORPTIVITY OF GAC WITH NO BIOLOGICAL ACTIVITY

The adsorptive capacity of the GAC with no biological activity was assessed in terms of both colour and sCOD removal. Two sets of experiments were carried out using raw and anaerobically treated stillage with the aim of determining: -

(i) The time for the carbon adsorption process to reach steady state;

(ii) The Freundlich Isotherm Plots (see Section 4.2.2);

(iii) Adsorption capacity of the GAC for colour and sCOD (i.e. the breakthrough curves).

These experiments were carried out using the same GAC used to pack Reactors 1 and 2 and were performed under the reactors' operating conditions (i.e. at 37°C and at a pH of 7.0).

4.2.1 Carbon Adsorption-Time Relationship

30 g of the GAC were added to 100 ml of the stillage to give the same carbon to liquid ratio as in the reactor and the pH was adjusted to 7.0. The mixture was magnetically stirred at 250 rpm and 4 ml samples were taken at 15 minute intervals to assess for the residual colour and sCOD concentrations. The results are as presented in Figures 4.1 and 4.2.

For the raw stillage, colour and sCOD adsorption reached an equilibrium after approximately 60 minutes. Using the spectrophotometric method, the residual colour concentration was 22.5 CPU while the visual comparison method gave 0 CPU (i.e. visibly colourless). Not all the sCOD was adsorbed by the GAC. The residual sCOD was approximately 2400 $mg.l^{-1}$ (Fig. 4.1). Even after 5 days, this value was not reduced. For purely physical and chemical adsorption, the concentration of the unadsorbed species is the most important variable influencing the equilibrium adsorption capacity (Neely and Isacoff, 1982).

For the anaerobic lagoon treated stillage, the adsorption of both colour and sCOD reached an equilibrium after about 30 minutes (Fig. 4.2). This was half the time observed using raw stillage.

FIGURE 4.1: CARBON ADSORPTION-TIME RELATIONSHIP FOR RAW STILLAGE

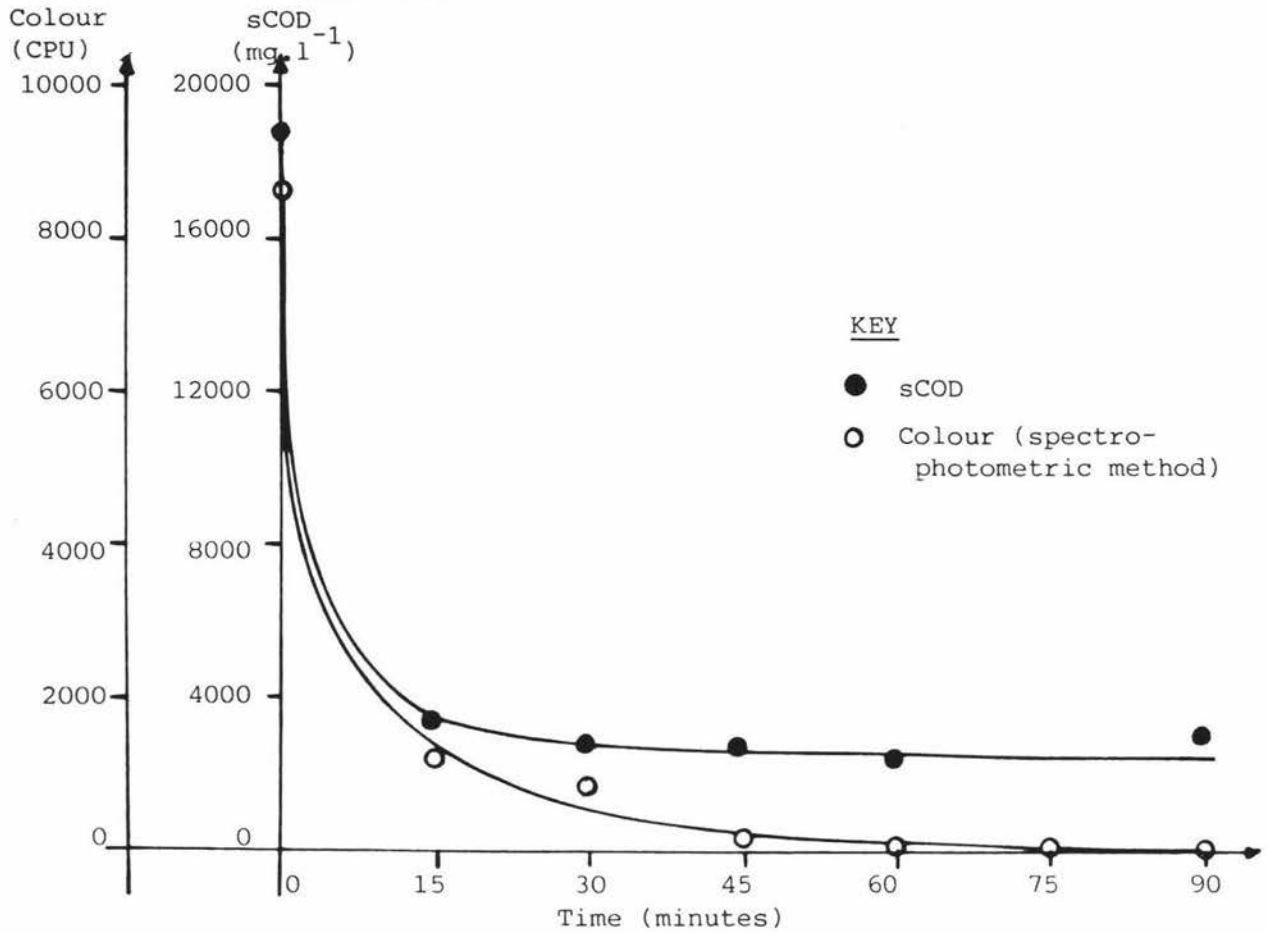
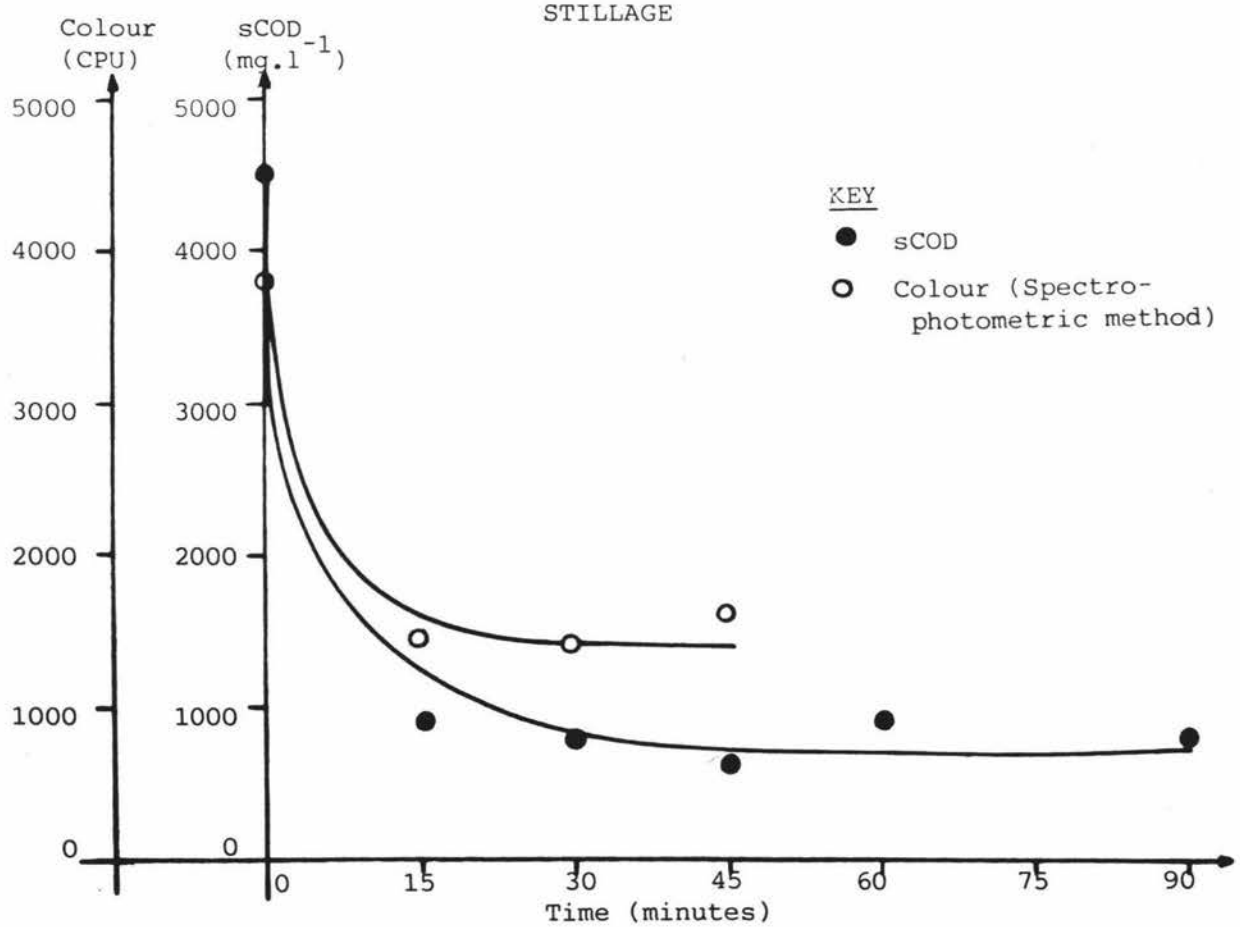


FIGURE 4.2: CARBON ADSORPTION-TIME RELATIONSHIP FOR ANAEROBICALLY TREATED STILLAGE



4.2.2 The Freundlich Isotherm Plots

Batch runs were carried out using weight to volume ratios of carbon and stillage ranging from 0-30% w/v. The environmental conditions described in Section 4.2.1 were used. The mixture was allowed to equilibrate for 90 minutes before colour and sCOD measurements were taken. These values were then plotted as shown in Figures 4.3 and 4.4.

However straight-line plots of the adsorption capability versus concentration are deemed more useful since results can be more readily interpolated or extrapolated (Neely and Isacoff, 1982). Two plotting schemes are possible based on Langmuir or Freundlich Isotherms. In this case, the Freundlich Isotherm has been used. The Freundlich equation can be defined as (Weber, 1972; Neely and Isacoff, 1982): -

$$q_e = K_F C^{\frac{1}{n}}$$

where,

q_e = the amount of solute adsorbed per unit weight of solid adsorbate ($g.g^{-1}$)

C = concentration of solute remaining in the solution at equilibrium ($g.l^{-1}$)

K_F and n = constants

The details of this method are presented in Appendix 2.

These plots, derived from Figures 4.3 and 4.4 to smooth out the data are presented in Figures 4.5 and 4.6. The linearity of the plots in Figures 4.5 and 4.6 indicate that the adsorptive capacity of the GAC can be adequately assessed using the Freundlich Isotherm. Table 4.1 gives a summary of the K_F and n^{-1} values derived from the Freundlich equation.

FIGURE 4.3: CARBON ADSORPTION CAPACITY VERSUS CONCENTRATION PLOTS FOR RAW STILLAGE

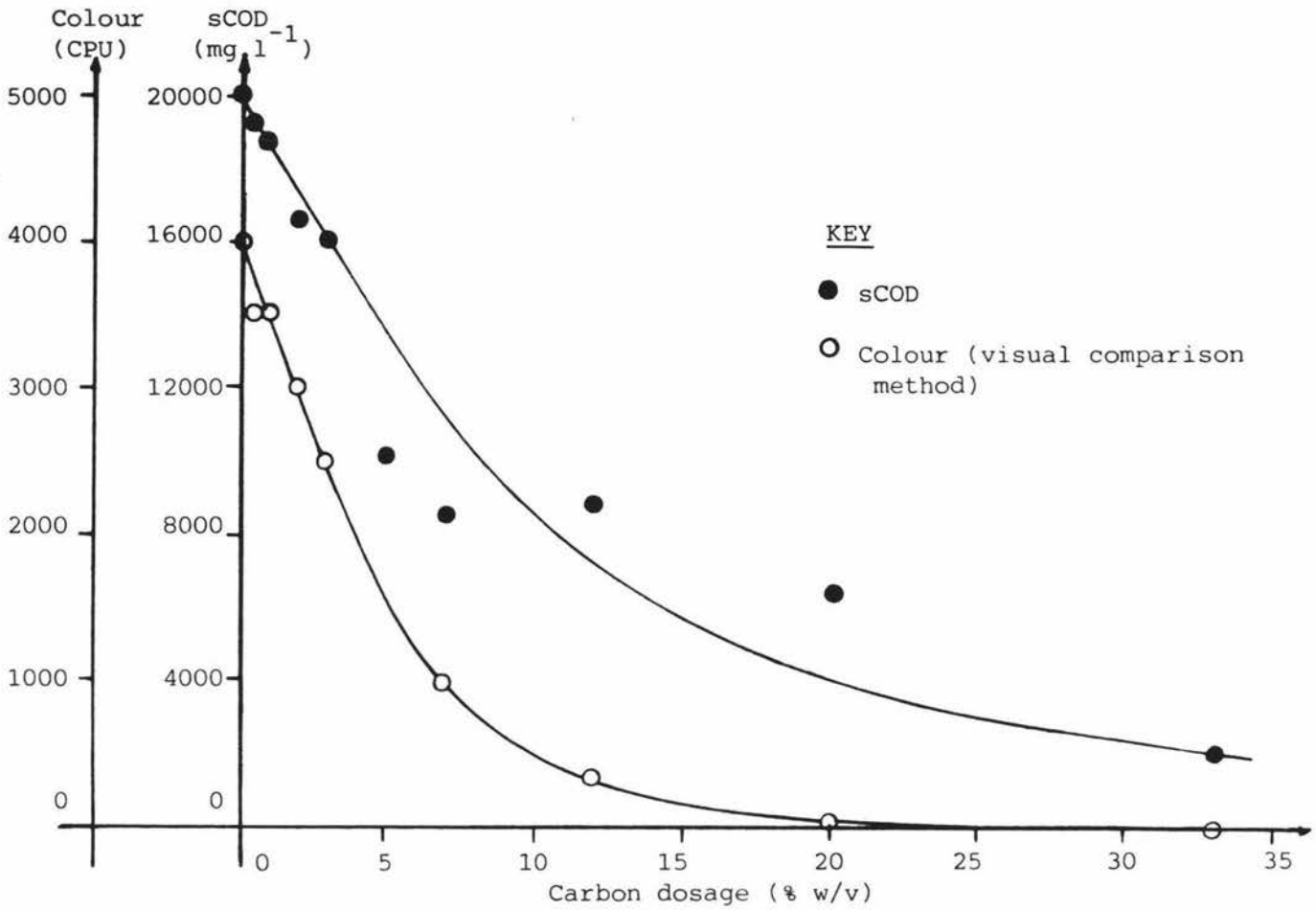


FIGURE 4.4: CARBON ADSORPTION CAPACITY VERSUS CONCENTRATION PLOTS FOR ANAEROBICALLY TREATED STILLAGE

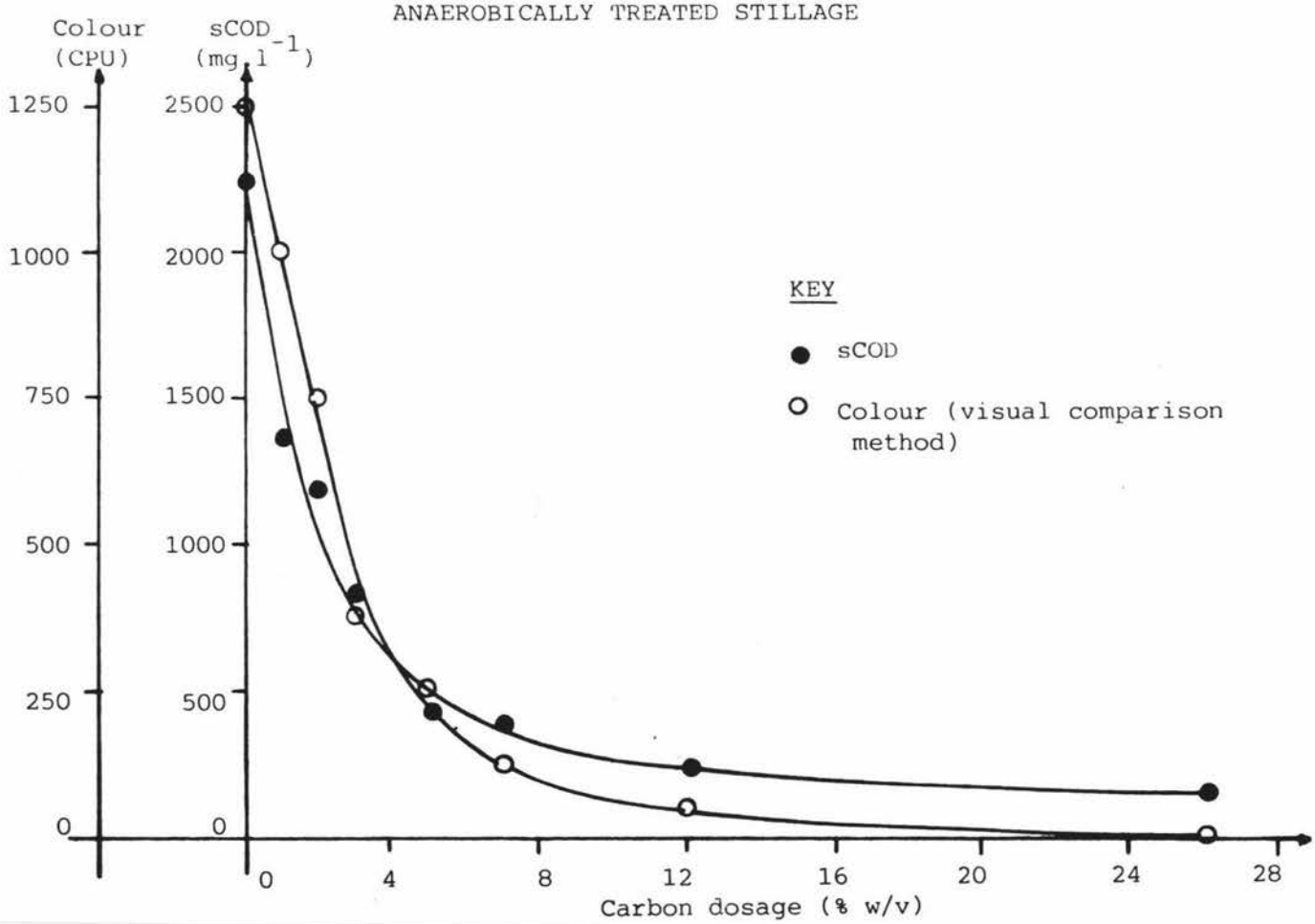


FIGURE 4.5: FREUNDLICH ISOTHERM PLOTS FOR COLOUR REMOVAL

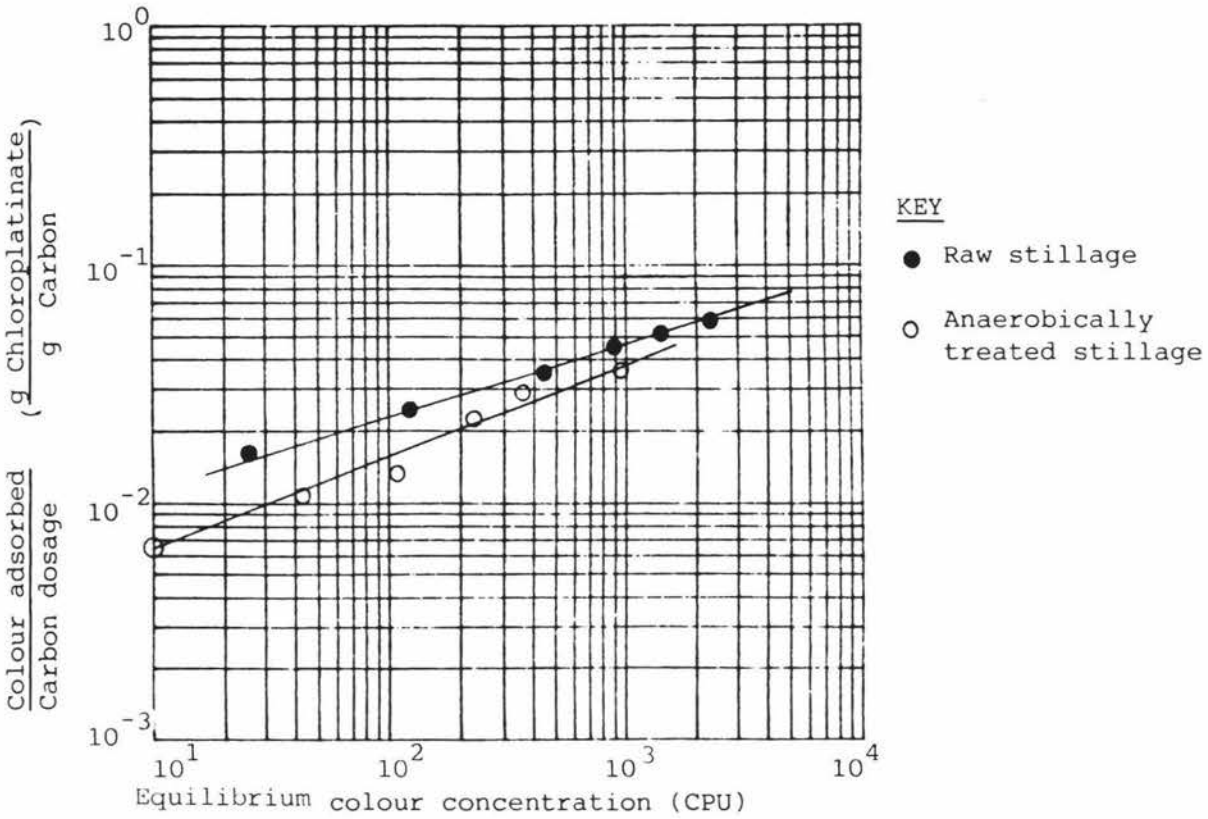


FIGURE 4.6: FREUNDLICH ISOTHERM PLOTS FOR sCOD REMOVAL

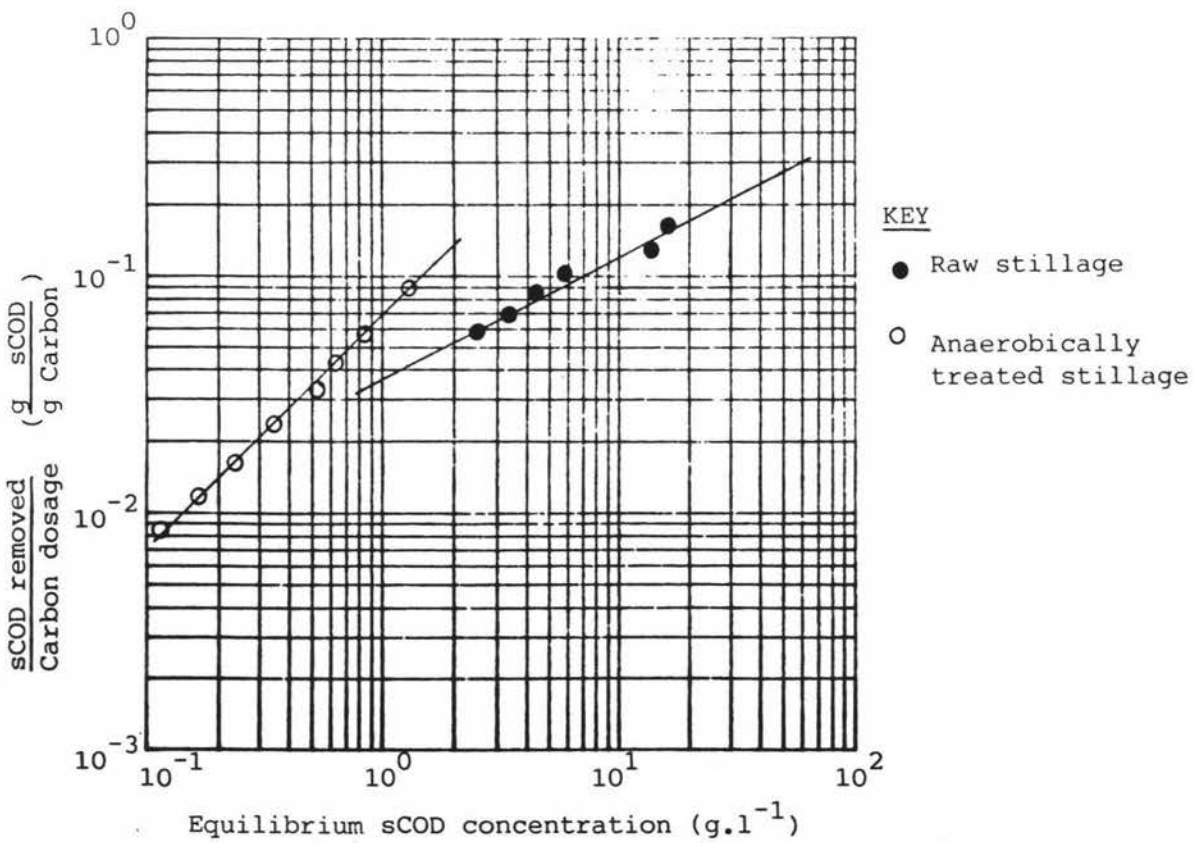


TABLE 4.1: FREUNDLICH ADSORPTION PARAMETERS FOR RAW AND ANAEROBIC LAGOON TREATED STILLAGE

Stillage feed	Adsorbate	K_F	n^{-1}
raw stillage	Colour	6.40	0.281
	sCOD	9.50	0.538
anaerobic lagoon treated stillage	Colour	2.23	0.408
	sCOD	1.02	0.945

K_F is a constant that is related to adsorption capacity while n^{-1} is related to adsorption intensity (Lee et al., 1983). Adsorption capacity of the GAC increases as the K_F value increases and that of n^{-1} decreases (El-Dib and Badawy, 1983). Thus the GAC has a higher adsorption capacity for raw stillage than anaerobic lagoon treated stillage in terms of colour removal (Table 4.1). This agrees with previous observations that adsorption is increased as the compound's solubility decreases and its molecular weight increases (El-Dib and Badawy, 1983). The isotherm for the raw stillage is at a higher level and has a lower slope than for the anaerobically treated stillage (Fig. 4.5). Thus colour is more readily adsorbed from the raw stillage over the entire range of conditions studied (EPA, 1973). This is because for any equilibrium colour concentration in the solution (x axis), the colour adsorbed per unit carbon dosage is always higher for the raw stillage than the lagoon treated stillage (y axis). The plots also show that adsorption is improved at higher concentrations.

The adsorption capacity for both cases in terms of sCOD removal depended on the range of concentrations considered. Thus GAC adsorption capacity was higher for the raw stillage case at high influent sCOD (e.g. at 20 $g.l^{-1}$) but lower at low influent sCOD (e.g. at 2 $g.l^{-1}$) as compared to the case with anaerobic lagoon treated stillage.

The adsorption intensity value (n^{-1}) correlates well with carbon adsorption-time relationships in Section 4.2.1 i.e. the n^{-1} values for anaerobic lagoon treated stillage are about twice those for raw stillage. This means the adsorption of colour and sCOD reached an equilibrium in half the time for anaerobic lagoon treated stillage when compared to raw stillage (i.e. 30 minutes instead of 60 minutes).

4.2.3 Breakthrough Curves for Colour and sCOD Removal from Raw and Anaerobically Treated Stillage

The breakthrough curves for colour and sCOD removal were assessed by adding batches of stillage to the GAC representing different bed volumes of liquid through the reactor. They were then allowed to equilibrate under the conditions prevailing in the expanded-bed reactor (see Section 4.2.1) for 24 hours before measurements of colour and sCOD were taken. The breakthrough curves give good indication of when the adsorption capacity of the GAC becomes exhausted. Figures 4.7 and 4.8 give a summary of the results.

Using raw stillage, the first colour breakthrough (i.e. detectable colour appearing in the effluent) occured within 1 to 3 equivalent stillage bed volumes. Without bioregeneration, the GAC will be fully exhausted in 11 and 15 bed volumes for colour and sCOD respectively (Fig. 4.7).

For the anerobic lagoon treated stillage, colour and sCOD breakthrough occurs within one equivalent bed volume of feed and, GAC exhaustion occurs after 21 and 49 equivalent bed volumes for colour and sCOD respectively (Fig. 4.8). Thus colour breakthrough occured at a lower number of equivalent bed volumes compared to raw stillage. This was despite the fact that raw stillage is about four times more strongly coloured than the anaerobically treated stillage suggesting that the chromophoric species are different for the raw and anaerobic lagoon treated stillage. It is probable that the change of form for the chromophores is due to microbial activity as approximately 75% of the raw stillage colour is removed on passage through the anaerobic lagoon (Tan and McFarlane, 1983).

FIGURE 4.7: BREAKTHROUGH CURVES FOR COLOUR AND sCOD REMOVAL FROM RAW STILLAGE USING GAC WITH NO BIOLOGICAL ACTIVITY

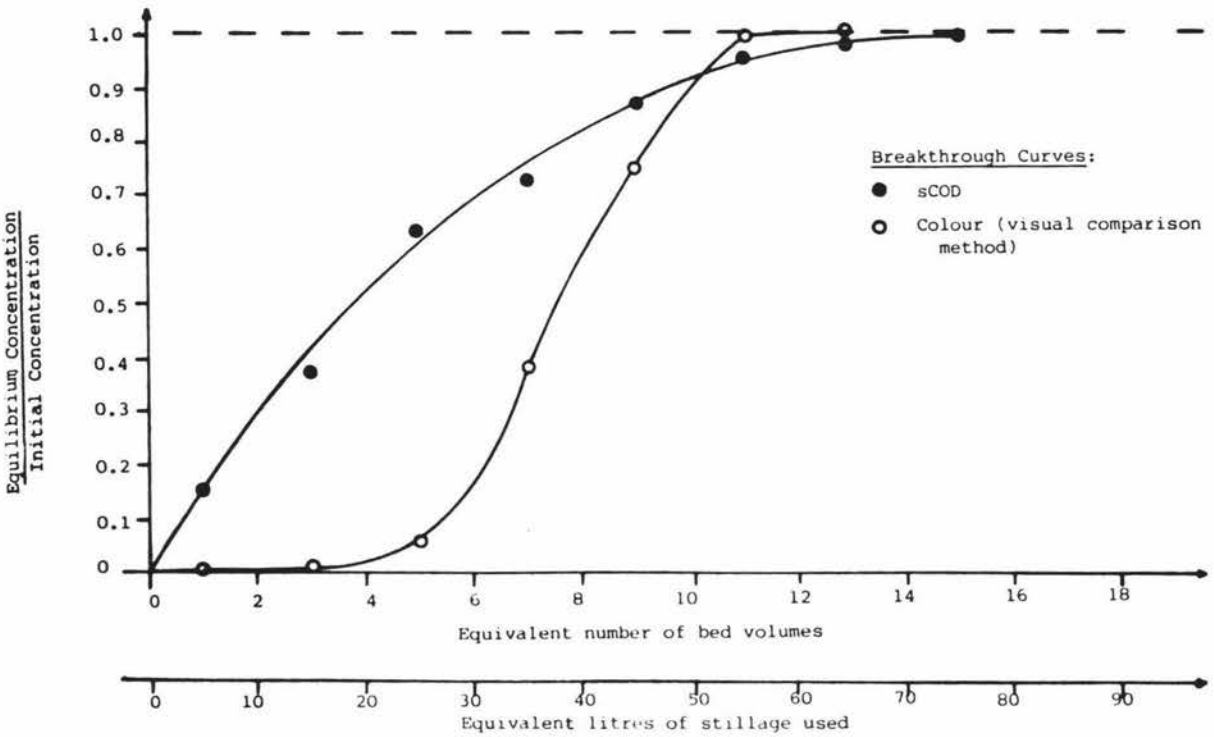
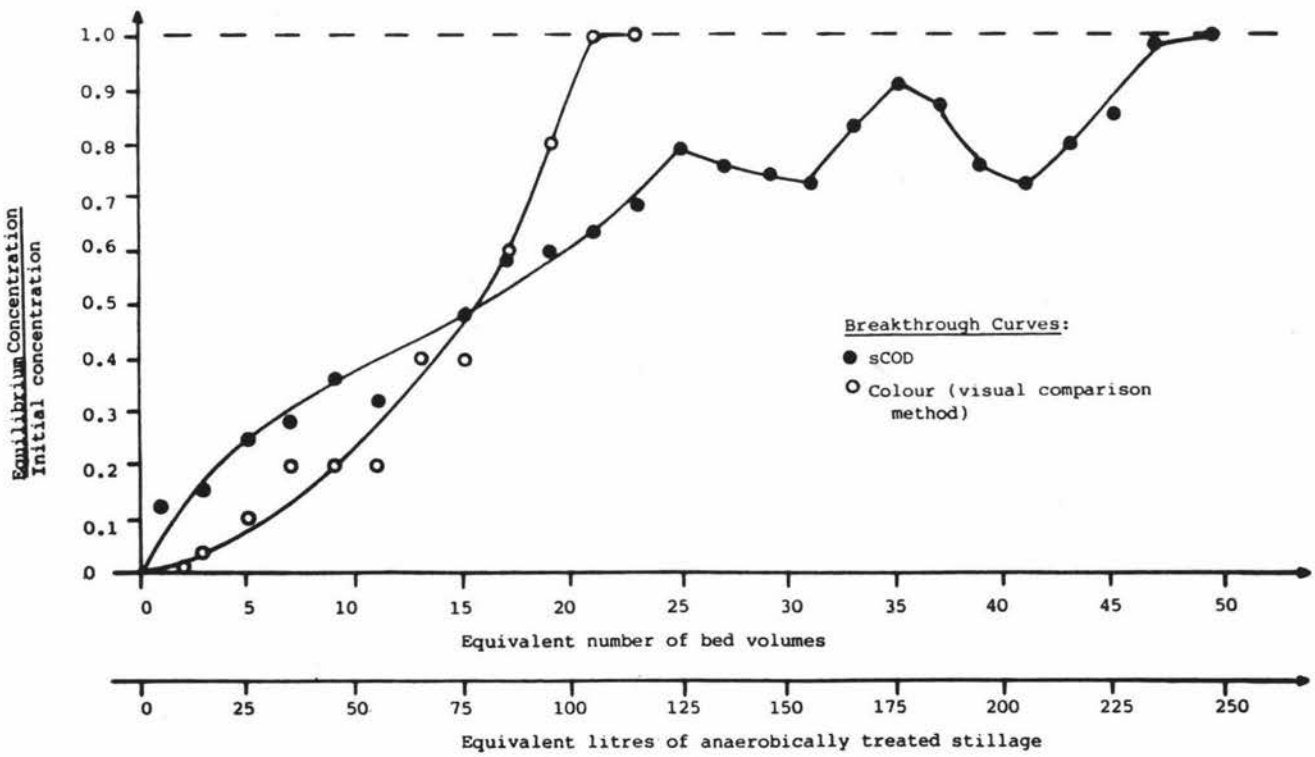


FIGURE 4.8: BREAKTHROUGH CURVES FOR COLOUR AND sCOD REMOVAL FROM ANAEROBICALLY TREATED STILLAGE USING GAC WITH NO BIOLOGICAL ACTIVITY



4.2.4 Conclusion

The adsorptive capacity of the GAC for sCOD and colour from raw and anaerobically treated stillage has been determined in the absence of biological activity.

Using the Freundlich Isotherm plots, it was demonstrated that the GAC had a higher colour adsorption capacity for raw stillage than anaerobic lagoon treated stillage over the entire range of colour concentrations studied. Using raw stillage, colour breakthrough was found to occur within 1-3 equivalent bed volumes and the GAC became fully exhausted in 11 equivalent bed volumes. With anaerobically treated stillage, colour breakthrough occured within one equivalent bed volume of the feed and GAC exhaustion occured after 21 equivalent bed volumes.

The adsorptive capacity of the GAC for sCOD depended on the range of concentrations considered. GAC adsorption capacity was found to be higher for raw stillage at high influent sCOD concentration but lower at low influent sCOD concentration as compared to the case with anaerobically treated stillage. From the sCOD breakthrough curves, it was found that sCOD breakthrough occured within one equivalent bed volume for both cases and, the GAC became fully exhausted after 15 and 49 equivalent bed volumes respectively.

From these observations, it was concluded that the chromophoric species involved are different for raw and anaerobically treated stillage. This difference is probably due to microbial degradation of chromophoric materials for the anaerobically treated stillage.

4.3 ANAEROBIC DIGESTION OF RAW STILLAGE - R1 OPERATION

4.3.1 Introduction

R1 was seeded on the 3rd of June, 1983 and feeding was first attempted 5 days later. Apart from one reactor 'upset' during the start-up period due to barium inhibition, digester operation was generally stable indicating a balanced methanogenic consortium was present in the digester The raw data for the 227 days of R1 operation are presented in Appendices 3.1-3.3 and will be discussed in detail in four phases.

The data were stored in the Prime Computer System at the Massey University Computer Centre. The Minitab II package (Pennsylvania State University, 1982) and the SPSS Batch System (SPSS Inc) were used for statistical analyses and plottings of the data.

4.3.2 R1 Acclimatization Phase

This was the reactor start-up phase where the bacteria were acclimated to the stillage and will be termed Phase 1. The operational period was from day 1 - 95 and when R1 was generally operating at or greater than 30 days nominal HRT (Fig. 4.9).

Feeding first commenced on the 8th of July, 5 days after the reactor was seeded. The reactor was initially operated at a 30 day nominal HRT (approximately 0.4 kg tCOD.m^{-3}.d^{-1}) using half strength stillage at 1:1 dilution with distilled water supplemented with 240 mg.l^{-1} nitrogen and 80 mg.l^{-1} phosphorus (Appendix 3.2). The bed expansion was set at 6-8% and a cam-timer was used to control the feeding rate to achieve the necessary HRTs at the lowest stable pump setting.

Diluted feed was used in the beginning to minimize any toxic shock to the methanogenic consortium. The feed strength was slowly increased as the bacteria became more acclimated to the feed (Fig. 4.10). This was gauged by a stable reactor operation with good COD removals.

FIGURE 4.9:

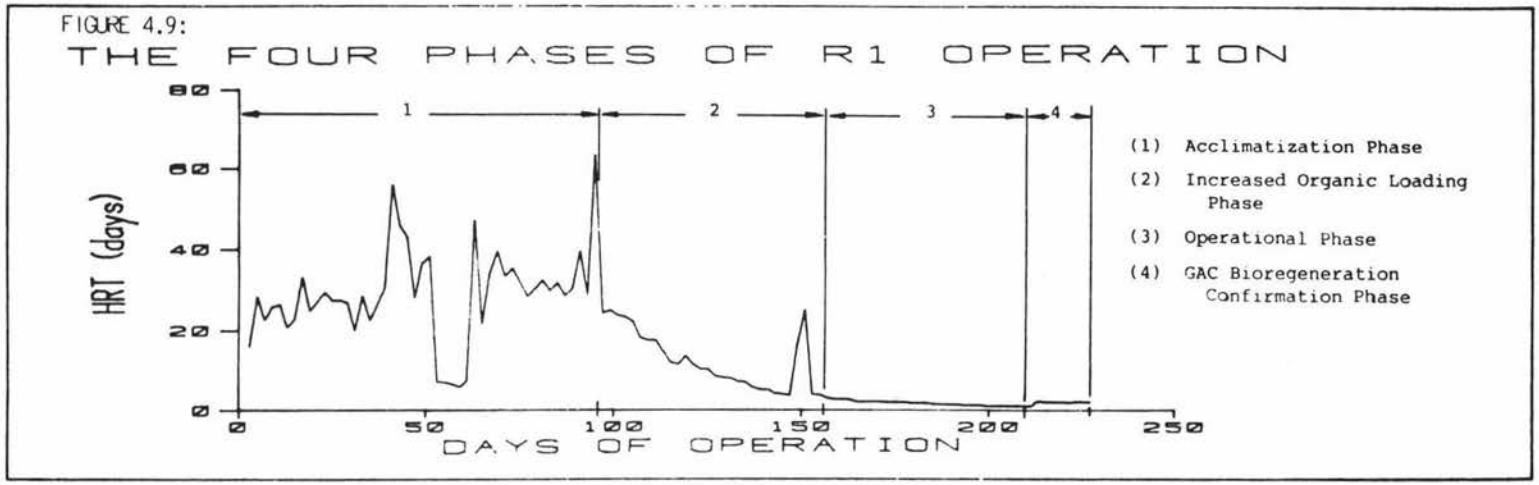
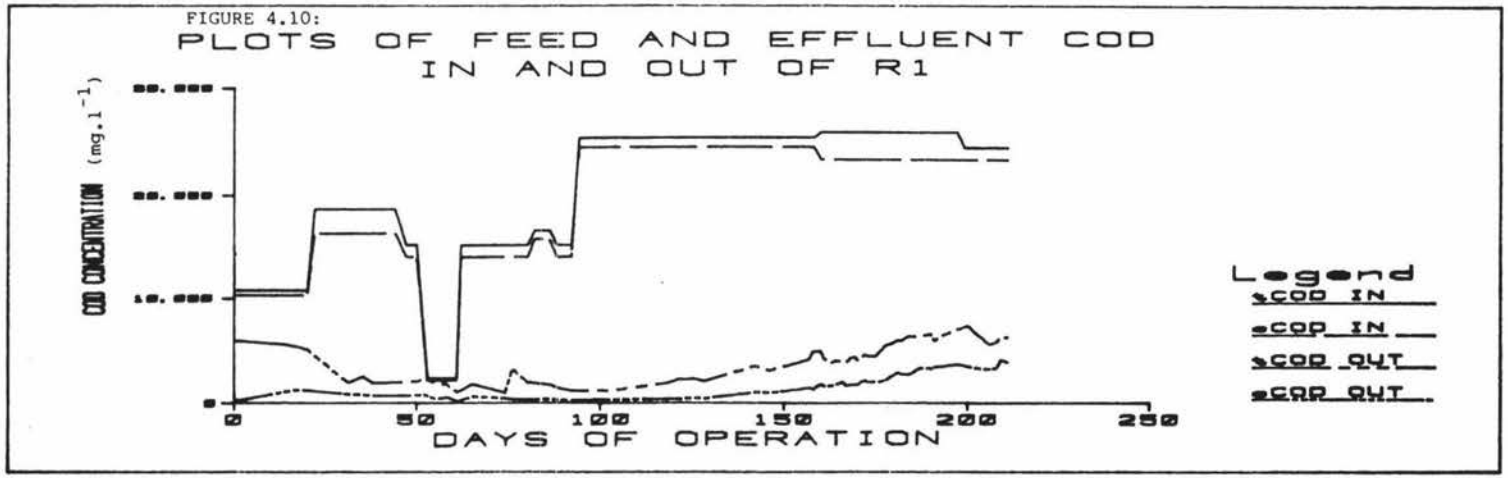


FIGURE 4.10:



4.3.2.1 Reactor performance and stability

Good removals of tCOD and sCOD were achieved in this phase of operation (Fig. 4.10). This was expected as most of the organics were adsorbed onto the GAC. Some methanogenic activity was evident from the gas produced (Fig. 4.11), the lower residual sCOD than by GAC adsorption alone (Section 4.2.1) and reduction in feed VFA levels after going through the reactor. Thus there was a competition between the bacteria and the GAC for the substrate. However, the GAC gradually became exhausted as more bed volumes of stillage were treated and this resulted in an increase in gas production as more substrate became available to the bacteria. At the same time, the bacteria were becoming more adapted to the substrate and the micro-environment of the system.

The high initial effluent tCOD concentration was a result of seed biomass washout from the reactor (Fig. 4.10). A steady state value of around 2000 $mg.l^{-1}$ was reached in about 30 days of operation. There was also an initial increased effluent sCOD concentration as the bacteria became more acclimatized to the feed (Fig. 4.10).

An attempt to measure the biomass concentration using the DNA method prove unsuccessful at this stage because the DNA content of the virgin carbon (as a control) was approximately twice that of the carbon sample from R1. Other potential biomass indicators will be considered for biomass analysis in the later stages of operation (see Section 4.3.5.2).

(a) pH and Alkalinity

A target pH value of at least 6.8 and preferably in the vicinity of 7.0 was aimed for. When the pH dropped too far an alkalinity reagent M4, was added. M4 had been used previously for anaerobic lagoon stillage treatment (Archer et al., 1982) and its composition is presented in Table 4.2.

FIGURE 4.11:

GAS PRODUCTION RATE, GAS METHANE COMPOSITION AND OLR FOR R1 OPERATION

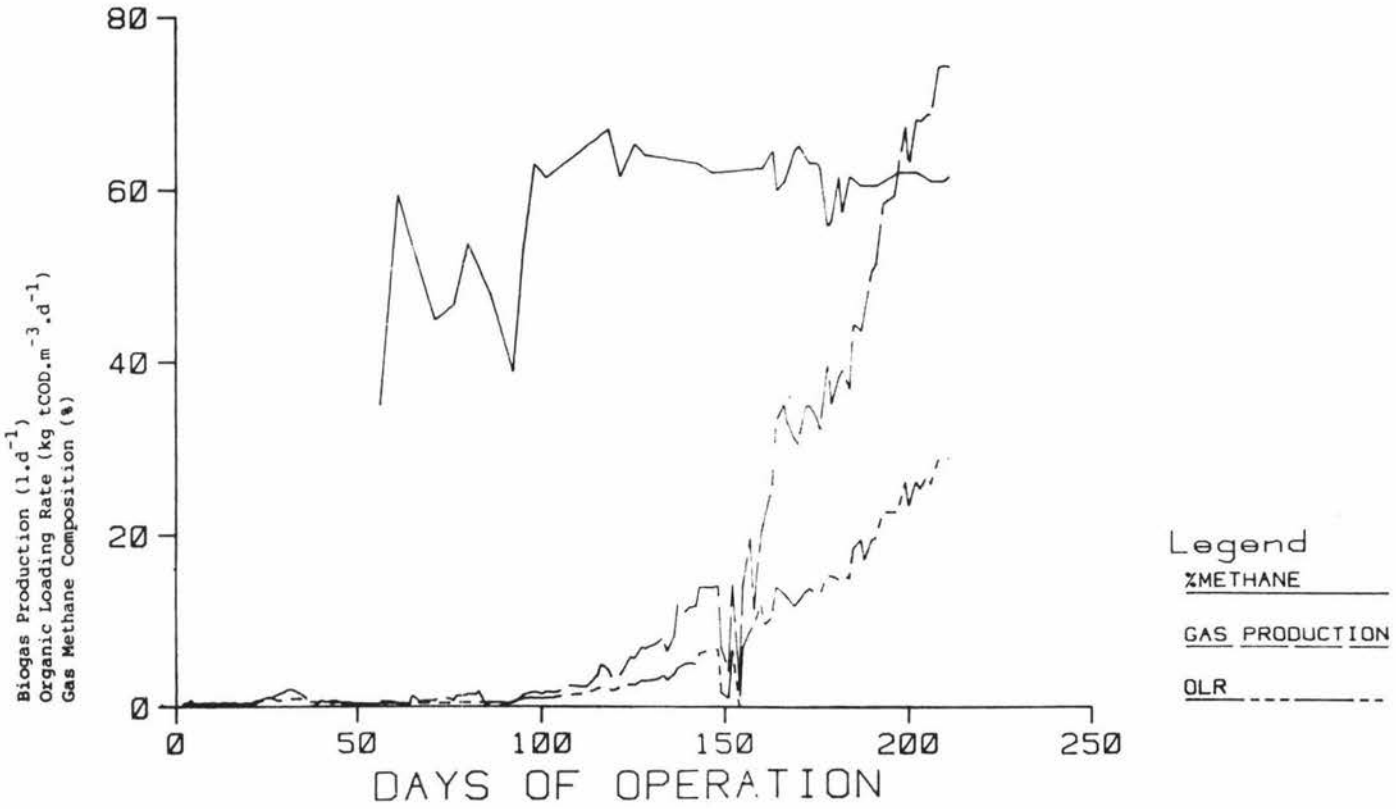


TABLE 4.2: THE COMPOSITION OF M4 ALKALINITY REAGENT

Chemical formula	Weight in Mixture (g)
$K_2 CO_3$	120
$Na HCO_3$	240
$Ca (OH)_2$	120
$(NH_4) H_2 PO_4$	16
$(NH_4) H CO_3$	110

The pH value was dangerously low on the 11th of June when the HRT was inadvertantly reduced to 16 days as shown in Appendix 3.3 (this was then quickly returned to its original HRT). However, the VFA levels were low at that time. The low pH could have been due to dissolved CO_2 (rather than VFA accumulation) because the effluent pH was considerably raised (to pH 8) after sample centrifugation where dissolution of the CO_2 had occurred. There was also a low buffering capacity then with an alkalinity of 365 $mg.l^{-1}$ as $Ca CO_3$. This low level of alkalinity was raised to greater than 1500 $mg.l^{-1}$ after M4 dosing (Fig. 4.12).

It was later decided that a constant alkalinity dosing would be more appropriate. 2.5 ml of 20% w/v NaOH was added initially to 1 litre of feed (before feeding) as the alkalinity reagent. This method was preferred to M4 dosing when the reactor pH was below 6.8 because:

(i) It allowed independent control of nutrients and alkalinity;

(ii) It was easy to prepare;

(iii) The sodium level was low enough (i.e. sodium cation at 574 $mg.l^{-1}$ with 4.5 ml 20% NaOH per litre fed) to avoid sodium toxicity (Kugelman and McCarty, 1964; McCarty 1964 (b)). Thus a mixture of alkalinity reagents was not necessary;

(iv) There was less likelihood of pH shock to the bacteria in the reactor as the NaOH was steadily added with the feed.

FIGURE 4.12: pH AND ALKALINITY PLOTS FOR R1 OPERATION

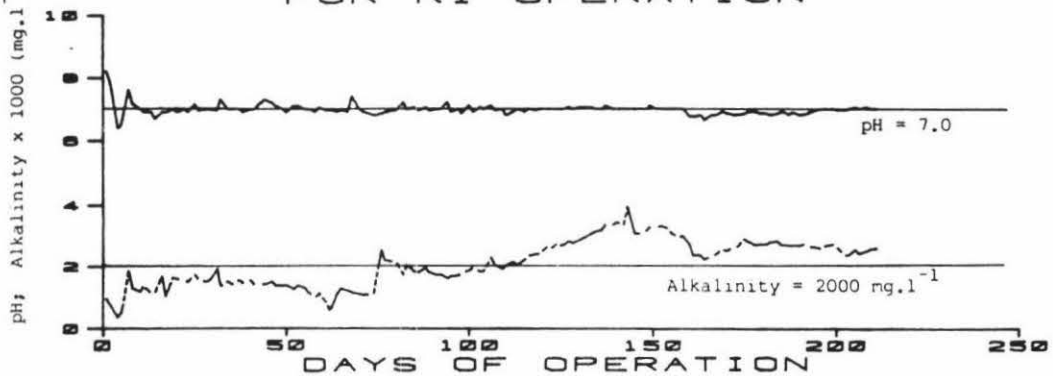
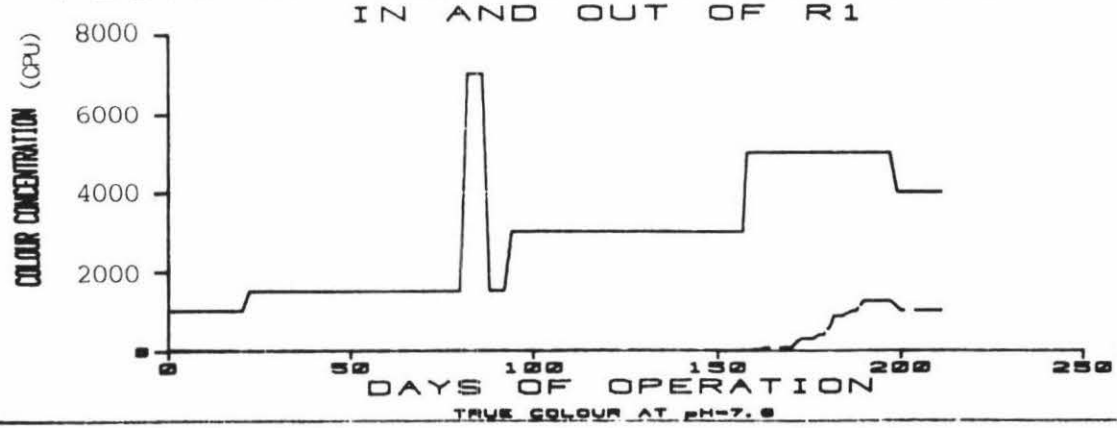


FIGURE 4.13: PLOTS OF FEED AND EFFLUENT COLOUR IN AND OUT OF R1



The NaOH dosing rate was chosen such that it resulted in a reactor pH of around neutrality and an alkalinity of approximately 2000 $mg.l^{-1}$ as $CaCO_3$. The initial NaOH dosing rate of 2.5 $ml.l^{-1}$ feed solved the pH and alkalinity problem satisfactorily (Fig. 4.12). However, higher alkalinity doses were subsequently required at higher organic loading rates.

(b) Colour Considerations

The results for colour measurement depended a lot on the pretreatment steps (e.g. pH adjustment, centrifugation and filtration) and the colour measurement methods used.

For a sample with no pretreatment, visual comparison with the standard colour solution was difficult while results can be misleading using the spectrophotometric method. Thus pretreatment, consisting of centrifugation and filtration through a Whatman GF/C glass fibre filter was considered to give a more meaningful result. This meant the colour values reported in Appendices 3.1 and 3.3 are for true colour as defined by Standard Methods (APHA, 1980), unless indicated (i.e. if solution was still turbid after pretreatment).

The two methods of colour measurement gave different results. The spectrophotometric method consistently gave a significantly higher colour concentration for both the effluent and the raw stillage (Appendices 3.1 and 3.3). The visual comparison method gave a more reasonable result, especially at low colour concentrations, since a visibly colourless solution will give a zero CPU but the spectrophotometric method will record a positive result (e.g. 20 CPU for a visibly colourless solution). This is possibly due to other non-chromophoric absorbing species at the spectral band used in the measurement. At times, it can be difficult to visually compare colour, especially when the effluent differs from the yellow of the chloroplatinate standard. The visible colour is pH dependent and the pH effect is reversible. In this study the visible comparison method was the preferred technique because the effect of receiving water discolouration

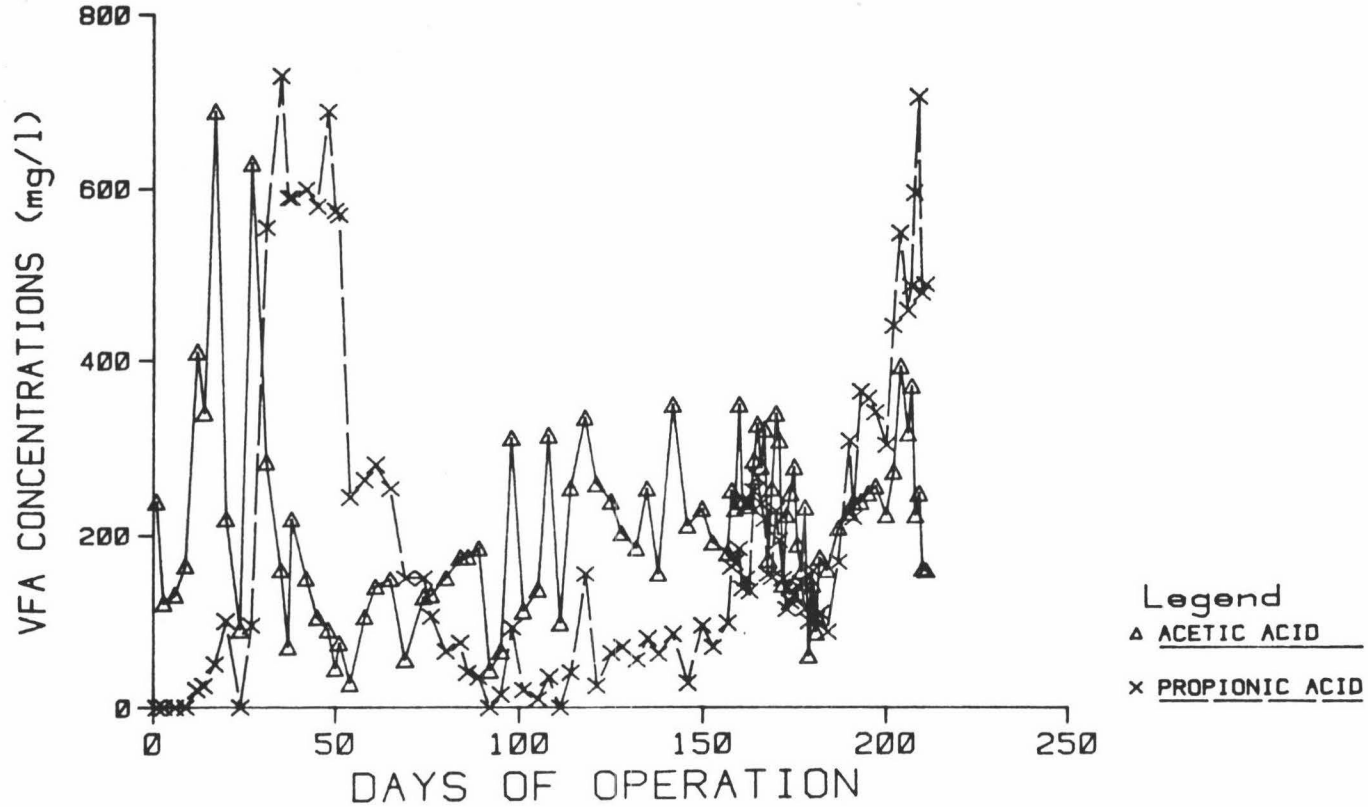
by stillage effluent discharge is visibly perceived by the human eye. It is this perception which really matters to the general public. Using the visible spectrophotometric scans, the absorbance were dependent on the wavelength considered (Section 4.3.5.4.2 (b)) resulting in different percentage colour removals for different reference wavelengths used thus questioning the validity of the spectrophotometric method. However, the absorbance at 465 nm is still the most widely accepted and used colour measurement at present. Results from the spectrophotometric method will be given and taken as being complementary to the visual comparison method.

At the end of this phase of operation, R1 had received approximately 3 equivalent bed volumes (EBV) of stillage. Except for the initial seeding stage, full colour removal had been achieved throughout this period of operation due to adsorption onto the GAC (Fig. 4.13). The experiment to assess the physical and chemical adsorption limits of the GAC for raw stillage indicated that colour breakthrough occurred within 1-3 EBVs of raw stillage (Section 4.2.3). Thus the number of equivalent bed volumes of stillage treated by R1 at this stage was near to that for physical-chemical saturation limits of the GAC and no colour breakthrough had been observed.

(c) Reactor Upset and Remedies

Following day 27, the VFA levels were high e.g. propionate at 600 $mg.l^{-1}$ on day 42 (Fig. 4.14; Appendix 3.3). This was attributed to sulfide inhibition since the feed sulphate concentration was in excess of 1500 $mg.l^{-1}$ at this time (Appendix 3.1). Digestion instability for cane juice stillage at a sulphate level of 1470 $mg.l^{-1}$ has been reported by Callander and Barford (1983(d)). The feed was then desulphated with 10% w/v $BaCl_2$ solution. A stoichiometric amount of this solution was added to remove 1800 $mg.l^{-1}$ SO_4^{2-} (data for SO_4^{2-} concentration in the feed was obtained from Dr I.J. Callander, personal communication (1983(a)). The mixture was magnetically stirred for 15 minutes at room temperature and the $BaSO_4$ precipitate removed by vacuum filtrat-

FIGURE 4.14: VFA LEVELS IN R1



ion (Whatman No. 1 filter paper). This procedure was the same as that used at the FRI (Callander, 1983(a)) and less elaborate than the one used by Stander and Hide (1950) for molasses slops.

However, when this desulphated feed was used, a dramatic drop in gas production was observed on day 48 (Fig. 4.11; Appendix 3.3). There was no sign of overloading since good sCOD removals and stable VFA levels with a high percentage methane in the gas were obtained at this time (Fig. 4.10, 4.14 and 4.11). The low gas production was suspected to be due to barium inhibition. It is clear now that the amount of $BaCl_2$ used was in excess of that required to precipitate the SO_4^{2-} because the stillage soluble SO_4^{2-} was about 15% lower than the concentration measured at the FRI. Some soluble SO_4^{2-} is believed to have precipitated out on the journeyto Massey University.

To remedy this situation, three options were considered:

(i) Replacement of the reactor liquor with previously treated effluent of the same reactor.

(ii) The use of the diluted feed at low HRT to wash out any barium left in the reactor.

(iii) Reseeding reactor, preferably with stillage acclimatized seed.

No abortive attempt was considered (i.e. empty out reactor contents and start again) because the toxic effect was believed to be bacteriostatic (rather than bacteriocidal) at that concentration range encountered. The reversibility of toxic inhibition of the methanogenic consortium has been widely demonstrated (Yang et al., 1980; Speece, 1983(a); Speece, 1983(b); Speece and Parkin, 1983).

In this case, Options (ii) and (iii) were chosen. One-tenth diluted stillage (unsulphated) was fed to the reactor at 6.2 d HRT giving an organic loading rate equivalent to that previously used. Gas production resumed after 3 days but at a lower product-

ion rate. The reactor was then reseeded with 500 ml of liquor from the anaerobic lagoon treating FRI stillage containing 0.5 $g.l^{-1}$ VSS (Archer et al., 1982) and 500 ml of UASB sludge containing 48.3 $g.l^{-1}$ VSS from the FRI (Callander et al., 1983). These remedial measures solved the problem satisfactorily as indicated by a drop in propionate level from around 600 $mg.l^{-1}$ to less than 100 $mg.l^{-1}$ (Fig. 4.14; Appendix 3.3).

(d) Nutrient Assessment

The major nutrients measured and closely monitored were nitrogen and phosphorus.

Nitrogen can be present in various forms in the reactor. The NH_3-N was considered the most relevant because it is readily available to the bacteria. The added N level at 240 $mg.l^{-1}$ of feed was considered adequate and in excess of that required for bacterial growth since the effluent residual NH_3-N levels were in excess of 80 $mg.l^{-1}$ (Appendix 3.2).

As for P, the reactive dissolved phosphorus (RDP) was considered to be the form that is most readily available to the bacteria.

A low residual RDP in the reactor and the feed was observed on the 4th and 7th of August (Appendix 3.2) at a stage when 80 $mg.l^{-1}$ P was being added to the feed. The same low concentration was obtained at the FRI from the UASB reactor treating the same stillage (Callander 1983(a); personal communication). It was soon discovered that the iron level in the stillage had risen to 1170 $mg.l^{-1}$ instead of the usual 50-150 $mg.l^{-1}$ (Callander, 1983(a), personal communication). Thus, most of the added phosphate was being precipitated as iron phosphate which explained the low RDP concentrations observed.

The high iron concentrations also explained the low soluble sulfide concentration observed in the reactor even with the use of feed with high SO_4^{2-} levels. Subsequently, a stoichiometric quantity of P (as phosphate) was added to precipitate the soluble

iron (Fe^{2+} and Fe^{3+}) level to 200 $mg.l^{-1}$, additional to the P added as nutrient (Appendix 3.2). It was also considered essential to measure the feed Fe concentration for every new batch of feed before using.

4.3.2.2 Conclusions from Phase 1 operation

The anaerobic expanded-bed reactor physicaly achieved the objectives it was designed for. Some problems were encountered with high soluble sulphate and iron concentrations in the raw stillage. These resulted in reactor upsets which were subsequently remedied.

Very good tCOD and sCOD reductions (in excess of 95.7% and 98.9% respectively) with full colour removal were obtained after treating 3 equivalent bed volumes of stillage due to adsorption onto the GAC. Some methanogenic activity was also evident from the biogas produced. At the same time, the sCOD removal efficiency gradually increased as the GAC gradually became exhausted while microbial degradation became more evident.

The stable reactor operation at end of Phase 1 (acetate at 65 $mg.l^{-1}$ and propionate at 15 $mg.l^{-1}$) implied that a well balanced and acclimatized methanogenic consortium had been developed. Thus the reactor was well prepared for its second stage of operation - the increased loading phase.

4.3.3 R1 Increased Organic Loading Phase

This was termed Phase 2 and was a period of increasing organic loading rates from 1-10 kg $tCOD.m^{-3}.d^{-1}$. It represented the transition from the acclimatization phase to the operational phase and operated from day 96 to 157 corresponding to nominal HRTs of 25 days down to 3 days.

To minimize shock loading on the reactor, the organic loading rate was normally increased in less than 15% step increments. They were generally done when the reactor was performing stably with acetate and propionate levels at less than 250 and 100 $mg.l^{-1}$ respectively.

The aim was to complete this stage of operation in the shortest time possible without causing any reactor upset.

4.3.3.1 Reactor performance and stability in relation to increased organic loading rates

This period of operation was characterised by its low VFA concentration (especially propionate, butyrate and valerate) as can be seen in Fig. 4.14. This indicated that a well balanced methanogenic consortium was present in the reactor. Even at a loading rate of 9.0 kg tCOD.m^{-3}.d^{-1}, very good sCOD, tCOD and colour removals were achieved at 94.1, 83.5 and 99.6% respectively (Fig. 4.15). It appeared that approximately 5 months were required for reactor start-up. Recently, the total time for start-up of the anaerobic fluidized bed (for complete methanogenic activity) has been reported as 6 months (Dunn, 1984). However, the COD removals were gradually dropping as the HRT was been slowly decreased. This was demonstrated by an increase in effluent sCOD from 260 to 1450 mg.l^{-1} and tCOD from 1120 to 4175 (Fig. 4.10; Appendix 3.3).

(a) pH and Alkalinity

No pH or alkalinity problems were encountered throughout this period of operation. The pH values remained above 6.8 all the time and generally stabilized at around 7.0 (Fig. 4.12). With 2.5 ml 20% NaOH l^{-1} feed addition, the alkalinity gradually increased from about 1680 to about 3000 mg.l^{-1} as $CaCO_3$ (Fig. 4.12). At times, the alkalinity was above 3400 mg.l^{-1} indicating a very good buffering capacity was present in the reactor. This rate of alkalinity addition was thus above that required for this stage of operation.

(b) Nutrients and Toxicity

The nitrogen supplement was maintained at 240 mg.l^{-1} with no signs of any deficiency as the effluent NH_3-N was consistently in excess of 28 mg.l^{-1} (Appendix 3.2). As for phosphorus, the supplement of 120 mg.l^{-1} feed was initially thought to be

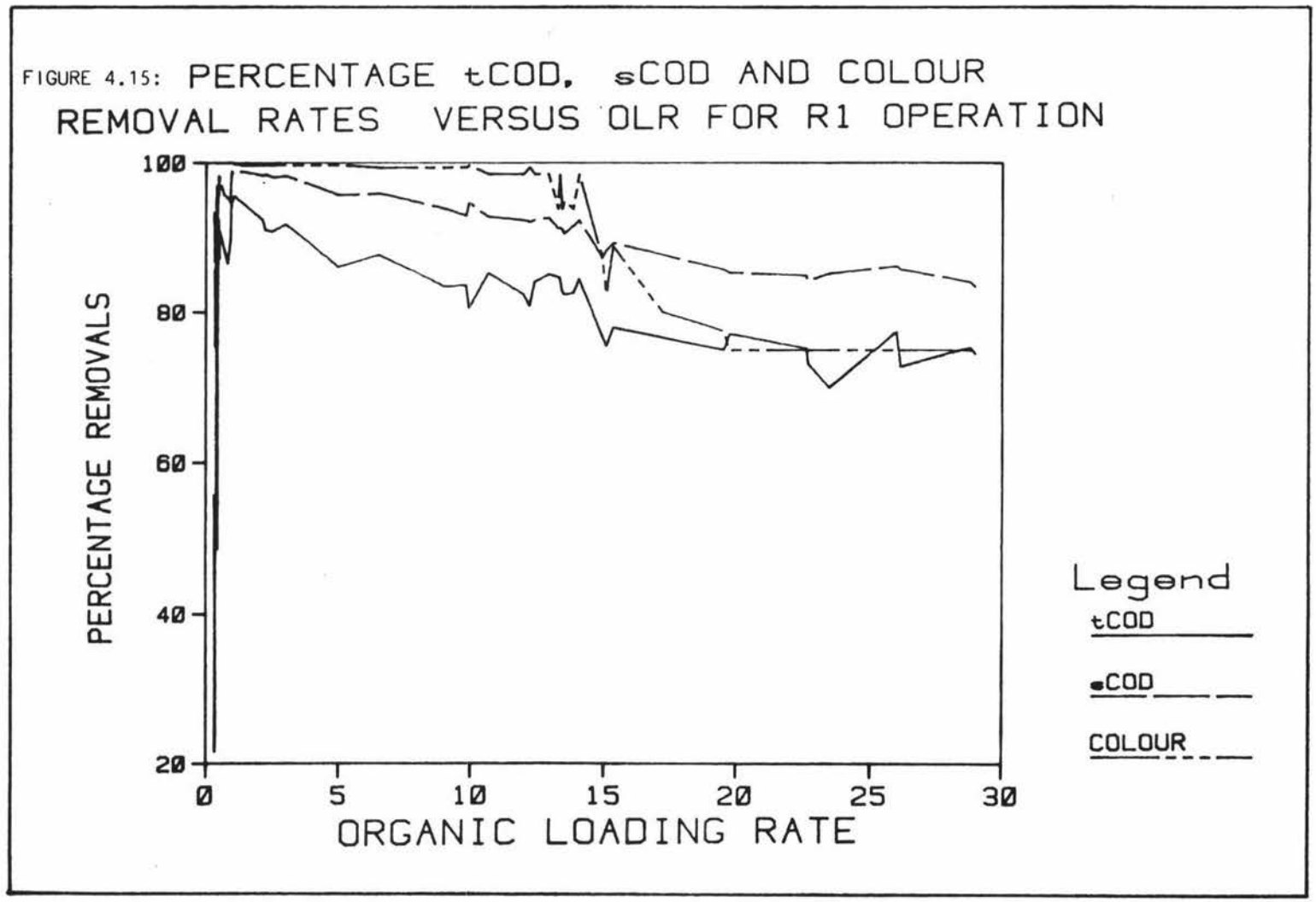


FIGURE 4.15: PERCENTAGE tCOD, sCOD AND COLOUR REMOVAL RATES VERSUS OLR FOR R1 OPERATION
PERCENTAGE REMOVALS
100
80
60
40
20
0
5
10
15
20
25
30
ORGANIC LOADING RATE
Legend
tCOD
sCOD
COLOUR

inadequate since low levels of RDP (less than 0.3 $mg.l^{-1}$) were present in the effluent at the beginning of Phase 2 operation (Appendix 3.2). However, there were no signs of nutrient deficiency in the reactor since the reactor continued to perform stably and efficiently. Larger phosphorus supplements were not used as they would precipitate essential metallic micronutrients. Since the reactor was performing consistently well, this phosphorus supplement was considered to be adequate.

The soluble sulfide concentration was below detection throughout this period of operation with a feed SO_4^{2-} concentration of 424 $mg.l^{-1}$ (Appendix 3.1 and 3.2). The soluble sulfide may have been precipitated with the soluble metals (chiefly Fe^{2+} and Fe^{3+}) present in the reactor or been adsorbed by the GAC. GAC has in fact been used in the large scale removal of sulphur-containing compounds (particularly H_2S) from industrial stack gases (Baki and Tanada, 1983).

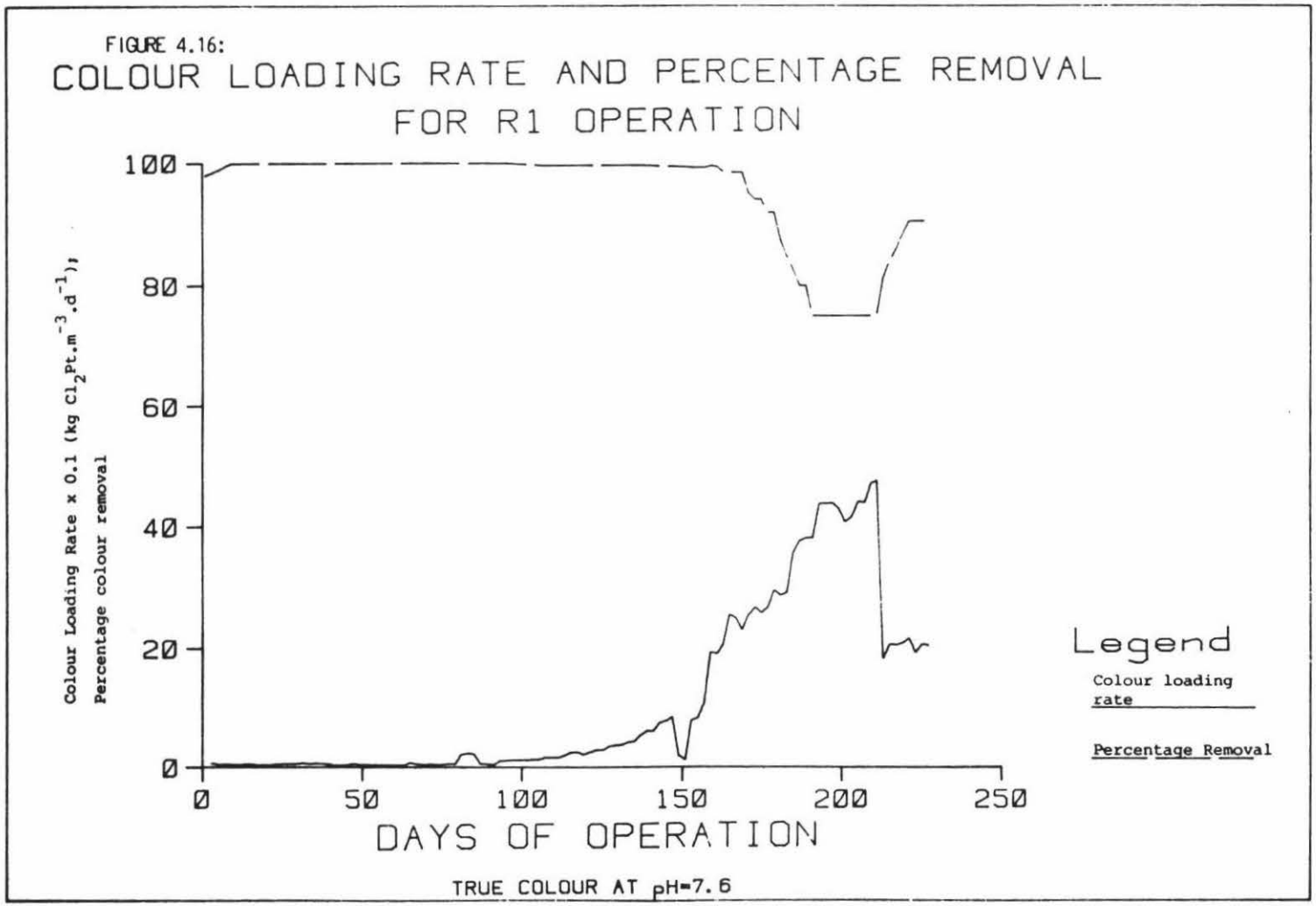
The fact that there were no toxicity problems from the stillage feed proved that Phase 1 operation had been very successful in building up an acclimatized methanogenic consortium. The biomass concentration in the reactor at the end of this stage of operation was estimated to be at 0.59 g $Vss.l^{-1}$ using the organic nitrogen method. A detailed discussion on biomass measurement is given in Section 4.3.5.2.

(c) Colour Considerations

Using the visual comparison method, the percentage colour removals ranged from 100-99.6% at colour loading rates of 0.12 to 1.0 kg chloroplatinate.$m^{-3}.d^{-1}$ (Fig. 4.16). They corresponded to HRTs of 25 d and 3.0 d respectively.

First colour breakthrough (i.e. at 5 CPU) was observed on day 101 at 3.3 estimated equivalent bed volumes of feed (Fig. 4.13; Appendix 3.3). In this calculation, the seed inoculum was estimated to contribute one equivalent bed volume of stillage. This observation correlated well with studies on GAC adsorption

FIGURE 4.16:


COLOUR LOADING RATE AND PERCENTAGE REMOVAL
FOR R1 OPERATION
Colour Loading Rate x 0.1 (kg Cl_2Pt.m^{-3}.d^{-1});
Percentage colour removal
100
80
60
40
20
0
0
50
100
150
200
250
DAYS OF OPERATION
Legend
Colour loading rate
Percentage Removal
TRUE COLOUR AT pH=7.6

with no biological activity (Section 4.2.3) which predicted first colour breakthrough within 1-3 EBVs of raw stillage (Fig. 4.7). However, the effluent concentrations remained low, i.e. at 10-20 CPU throughout this period of operation (Fig. 4.13). By day 157, 12.9 EBVs of stillage had been treated and the GAC should have been fully exhausted if no biological activity was involved (Fig. 4.7). The colour breakthrough curve in Fig. 4.7 also indicated a very sharp deterioration in percentage colour removal within 6-10 EBVs of stillage which was not observed in this phase of operation. These observations strongly suggested that bio-regeneration of the GAC in terms of colour removal had been occurring in R1. This inference was subjected to confirmation in later stages of operation (Section 4.3.5).

4.3.3.2 Conclusions from Phase 2 Operation

The organic loading rates to R1 had been increased from 1-10 kg tCOD.$m^{-3}.d^{-1}$ while maintaining stable reactor operation. This was achieved in a period of 61 days with no apparent nutrient deficiency or toxicity problem. A highly acclimatized and well balanced methanogenic consortium had been developed in the reactor. However, the biomass concentration was low and estimated at 0.59 g VSS.l^{-1}.

At the end of this stage, the reactor had treated 12.9 EBVs of stillage and achieved good sCOD, tCOD and colour removals at 94.1, 83.5 and 99.6% respectively. Bioregeneration of the GAC in terms of colour and sCOD is believed to have occurred but the extent has yet to be quantified.

4.3.4 R1 Operational Phase

This stage will be termed Phase 3 and was a period when the reactor achieved acceptably high organic loading rates (in excess of 10 kg tCOD.$m^{-3}.d^{-1}$) and was running stably i.e. low level of VFAs and not rising, good COD removals, stable pH around neutrality, stable gas production and biogas methane composition around 60%.

The organic loading rate was only increased (in less than 20% step) when the reactor had operated for at least two or three mean HRTs under the same running conditions and when the reactor was performing stably. Two to three mean HRT's is a commonly accepted time for a reactor to reach a steady-state operation after a change in reactor running conditions (Colin et al., 1983).

This period of operation was characterized by a high gas production rate, increasing from 20 to 75 $l.d^{-1}$ at about 61% CH_4 composition (Fig. 4.11). The organic loading rates increased from 10-29 kg $tCOD.m^{-3}.d^{-1}$ corresponding to HRTs of 2.5 to 0.85 d respectively. Sample analyses were carried out more frequently as the reactor performance and stability were more crucial at these high organic loading rates. For the reactor operating at 2.5, 2.0, 1.7 d and 0.85 d nominal HRTs, monitoring parameters were analysed on a day to day basis (Appendix 3.3).

4.3.4.1 Reactor performance and stability

The VFA levels remained at a satisfactory level throughout this period of operation though the values were slightly higher than during the previous phase of operation (Fig. 4.14). Increased acetate and propionate concentrations were generally observed after each increase in the loading rate but these values dropped after 1 to 2 HRTs to a concentration that was only slightly higher than that of the previous steady state. The ability to bring down the acid concentrations (especially the propionate level) meant that the reactor was able to cope with that loading rate with no danger of reactor overloading.

As can be seen in Appendix 3.3, the highest loading rate to the reactor averaged 28.9 kg $tCOD.m^{-3}.d^{-1}$ at 0.85 d HRT. This was taken as a non-maximal loading rate since the acid levels which rose initially (C_2 and C_3 from 225 and 597 $mg.l^{-1}$ to 250 and 708 $mg.l^{-1}$ respectively) dropped to steady values of 160 and 490 $mg.l^{-1}$ for C_2 and C_3 respectively after operating for 3-5 HRTs (Appendix 3.3). Table 4.3 gives a summary of the performance of R1 operating at the 2.0, 1.14 and 0.84 HRT stages (for days 174, 195 and 211 respectively).

TABLE 4.3: SUMMARY OF RESULTS FOR R1 OPERATING AT THE 2.0, 1.15 AND 0.85 DAYS NOMINAL HRTS

R1 Operational Data			
Organic loading rate (kg tCOD.m^{-3}.d^{-1})	13.4	22.6	29.0
Hydraulic retention time (d)	2.0	1.14	0.84
Temperature of operation (°C)	37	37	37
pH	6.82	6.95	7.00
Alkalinity (mg.l^{-1} as $Ca\ CO_3$)	2590	2479	2588
tCOD removal (%)	81.0	76.5	74.5
sCOD removal (%)	91.3	85.0	83.5
$tBOD_5$ removal (%)	88.2	85.4	79.2
Effluent tCOD (mg.l^{-1})	4950	6075	6220
Effluent sCOD (mg.l^{-1})	2030	3500	3843
Effluent $tBOD_5$ (mg.l^{-1})	2365	3642	3014
Total solids (g.l^{-1})	10.22	9.78	10.20
Volatile solids (g.l^{-1})	4.57	5.33	5.94
Total suspended solids (g.l^{-1})	3.77	-	2.66
Volatile suspended solids (g.l^{-1})	2.64	2.35	_
Colour loading rate (kg.Cl_2Pt.m^{-3}.d^{-1})	2.59	4.38	4.75
Colour removal (%)	94.0	75.0	75.0
Effluent colour (CPU)	300	1250	1000
Gas production ($l.d^{-1}$)	34.0	61.2	74.2
Specific biogas production (m^3.m^{-3}.d^{-1})	6.8	11.3	14.8
Methane in gas (%)	63.0	61.0	61.5
Theoretical gas production ($l.d^{-1}$)	34.1	56.3	70.0
VFA level (mg/l): Acetate	250	250	160
Propionate	122	359	490
Butyric acid	0	0	0
Valeric acid	0	0	0
Nutrient addition (mg.l^{-1}): Nitrogen	240	350	350
Phosphorus	200	250	200
Feed NH_3-N (mg.l^{-1})	148.4	316	378.7
Feed RDP (mg.l^{-1})	16.0	44.3	60.0
Effluent NH_3-N (mg.l^{-1})	9.0	54.2	185.9
Effluent RDP (mg.l^{-1})	0.45	1.00	1.04
Soluble sulphate level (mg.l^{-1})	224	224	-
Soluble sulfide level (mg.l^{-1})	0.45	0.19	-
Alkalinity addition (mls)			
20% w/v NaOH.l^{-1} feed	4.5	4.5	4.5
Biomass concentration (g VSS.l^{-1})	0.92	-	1.96
Specific Sludge activity (kg tCOD. kg VSS^{-1}.d^{-1})	3.09	-	6.30

As can be seen in Fig. 4.15 and Table 4.3, the percentage COD and colour removals dropped with increasing OLR but remained at a satisfactory level (84% and 75% for sCOD and colour removals respectively at 0.85 d HRT stage). No pH or alkalinity problems were encountered in this period of operation (Fig. 4.12) with the maximum alkalinity addition at 4.5 ml 20% NaOH.l^{-1} feed (Appendix 3.2).

Foam carry over problems were not experienced until the reactor was operating at 1.3 d HRT and below (or 19.8 kg tCOD.m^{-3}.d^{-1} and above). Generally, about 180 ml.d^{-1} of foam were produced and this foam was removed from the foam trap on a day to day basis. Problems were only encountered at the 0.85 d HRT loading stage when the amount of foam produced each day nearly filled the 1 l foam trap due to the large amounts of gas produced (74-75 l.d^{-1}). This meant that H_2S measurement was not possible since the gas sample had to be taken from the foam trap. However, the sulfide level was expected to be zero, or very low, at this stage (Appendix 3.2).

Higher levels of N addition were used (at 350 mg.l^{-1}) for this phase of operation. This was to ensure that the bacteria were not N limited. The data indicated that N was present in excess (Appendix 3.2 and Table 4.3). The same level of phosphorus addition was used as in the previous phase of operation except when the reactor was operating at 1.15 and 0.95 d nominal HRTs. The increase in P addition from 200 to 250 mg.l^{-1} at 1.15 and 0.95 d HRT appeared to result in a slight increase in effluent RDP from 0.81 to greater than 1.0 mg.l^{-1}. The aim of using this increased P addition was to assess the effect on availability of trace metallic nutrients. Samples had been collected and stored for metal analysis to be done at the Department of Scientific and Industrial Research (N.Z.). Unfortunately, the results would not be available in time for incorporation into this thesis.

These nutrient addition levels were adequate for R1 operation as indicated by the very high specific sludge activities i.e. specific organic utilization rates of 3.09 and 6.30 kg tCOD.kg VSS^{-1}.d^{-1} at HRT'S of 2.0 and 0.84 d respectively. The specific organic utilization rate was calculated from the amount of tCOD removed each day by the total biomass concentration (attached and suspended) in the reactor.

The specific sludge activity for conventional high rate processes (e.g. the anaerobic fluidized bed process) has been reported as 1.8 kg COD.kg VSS $^{-1}$.d^{-1} (Dunn, 1984). Higher sludge activities (in excess of 10g acetate.g^{-1}VSS.d^{-1}) have recently been reported by Speece et al., (1983) using an acetate feed supplemented with the necessary organic and inorganic nutrients in a nickel stimulation study. They were only achieved when optimal conditions existed for the anerobic digestion of an easily degradable substrate (e.g. acetate with a COD equivalent of 0.95 g.g^{-1}). Also the high specific biogas production rate of 14.8 m^{3}.m^{-3}.d^{-1} of reactor liquid volume at the 0.85 d HRT stage indicated a healthy reactor.

4.3.4.2 Bed expansion and recycle ratio considerations

The effluent recycle flow rate was measured at one stage (23/11/83) by incorporating a flowmeter (Gilmont Instruments, Inc.) into the recycle system on the delivery side of the recycle pump. Table 4.4 summarises the results obtained.

TABLE 4.4: RECYCLE FLOWS AND PERCENTAGE BED EXPANSIONS

Recycle Flow Rate (l.min^{-1})	Bed expansion (%)	Carbon Bed Characteristics
0.32	4	Carbon bed was partly expanded. Presence of 'dead' zones in the reactor.
0.73	7	Normal stable operating range. Carbon bed was just expanded. No 'dead' zones in the reactor.
0.80	8	
0.93	10	Carbon bed fully expanded. Turbulent carbon bed surface.
0.97	15	Carbon bed was very turbulent with 'jagged' bed surface. Occassional 'bursting' or carbon leaving bed surface.

The reactor was normally operated at 7-8% bed expansion which was at the lowest stable bed expansion. Considering an average recycle flow rate of 0.765 $l.min^{-1}$, the superficial liquid velocities were 3.2 $m.h^{-1}$ at reactor body (12.1 x 12.1 cm) and 15.8 $m.h^{-1}$ at regions adjacent to the feed outlet (12.1 x 2.4 cm). For a fluidized bed process using 0.3 to 0.55 mm Ø sand particles, superficial liquid velocities of 15-35 $m.h^{-1}$ has been reported (Mosey, 1982(b)). Thus the biomass attrition rate for R1 was expected to be significantly lower than the reactors using the sand carrier.

The aim of using the recycle rate that resulted in the lowest stable bed expansion was to minimize recycle energy consumption, carbon attrition and carry over of carbon. During Phase 3, the maximum percentage carbon bed expansion possible was approximately 15% at the maximum recycle flow rate. Initially, during Phase 1, a maximum bed expansion of 32% can be achieved.

The recycled effluent also served as a diluent for the influent to the reactor. This is important because it reduces any pH, organic and toxic shocks to the bacteria from the full strength feed. The effect of effluent recirculation has been studied by Duff and Kennedy (1983). They concluded that recirculation has little effect on start-up but the maximum loading rate which could be applied to the reactor was higher at higher rates of effluent recirculation using synthetic sugar waste. A maximum COD loading of 25 $kg.m^{-3}.d^{-1}$ at a recirculation ratio of 500:1 was achieved compared to 18 $kg.m^{-3}.d^{-1}$ for recycle ratios of 100:1 and 0. The % COD removals were 84, 81 and 78% respectively. The progressive improvements in the treatment efficiency for recycle ratios up to 7 has also been observed for the UASB reactor (Lettinga et al., 1982).

Using an average recycle flow rate of 0.765 $l.min^{-1}$, the recycle ratio for R1 when operating at 0.85 d HRT (highest non-maximal OLR period) would be at 187. The actual feed dilution ratio was less than this figure since the effluent still contained some undegraded organics. This high recycle ratio is believed to have contributed to the stable performance of R1.

The high recycle rate also resulted in more of a completely mixed system than a plug flow system. Though tracer studies have not been carried out to determine the residence time distribution of the system, R1 was considered to be operated as a completely mixed system. Measurements for tCOD, sCOD and solids concentrations for samples taken from the recycle line, the reactor settling compartment and the final effluent show little difference with regards to the sampling points (Table 4.5) supporting this assumption.

The reactor final effluent generally had a lower solids content and hence lower tCOD as compared to results from other sampling points. This was due to settlement of solids in the carbon trap. This small difference is of little importance to R1 operation and performance. Subsequently, samples taken from the recycle line were considered to be equivalent to the treated effluent from R1.

4.3.4.3 Conclusions from Phase 3 Operation

A non-maximal organic loading rate of 29.0 kg tCOD.m^{-3}.d^{-1} (at 0.85 d HRT) has been achieved in this phase of operation with tCOD, sCOD and colour removals of 74.5, 83.5 and 75.0% respectively.

At this stage, R1 had achieved a very high and practical OLR with good COD and colour removals and a stable running condition. The assessment of the organic removal potential of R1 is thus considered to have been achieved. The next phase will consider the extent, and confirmation, of GAC bioregeneration.

TABLE 4.5: INFLUENCE OF SAMPLING POINTS ON tCOD, sCOD AND SOLIDS MEASUREMENTS AT THE 0.85 d HRT STAGE

Points of Sampling	Parameters					
	$tCOD$ $(kg.m^{-3}.d^{-1})$	$sCOD$ $(kg.m^{-3}.d^{-1})$	TS $(g.l^{-1})$	VS $(g.l^{-1})$	TSS $(g.l^{-1})$	VSS $(g.l^{-1})$
Recycle line	6220	3843	9.88	5.77	-	2.05
Reactor content	6580	3818	10.23	5.94	2.66	2.99
Reactor final effluent	5775	3860	9.23	5.13	1.77	1.87

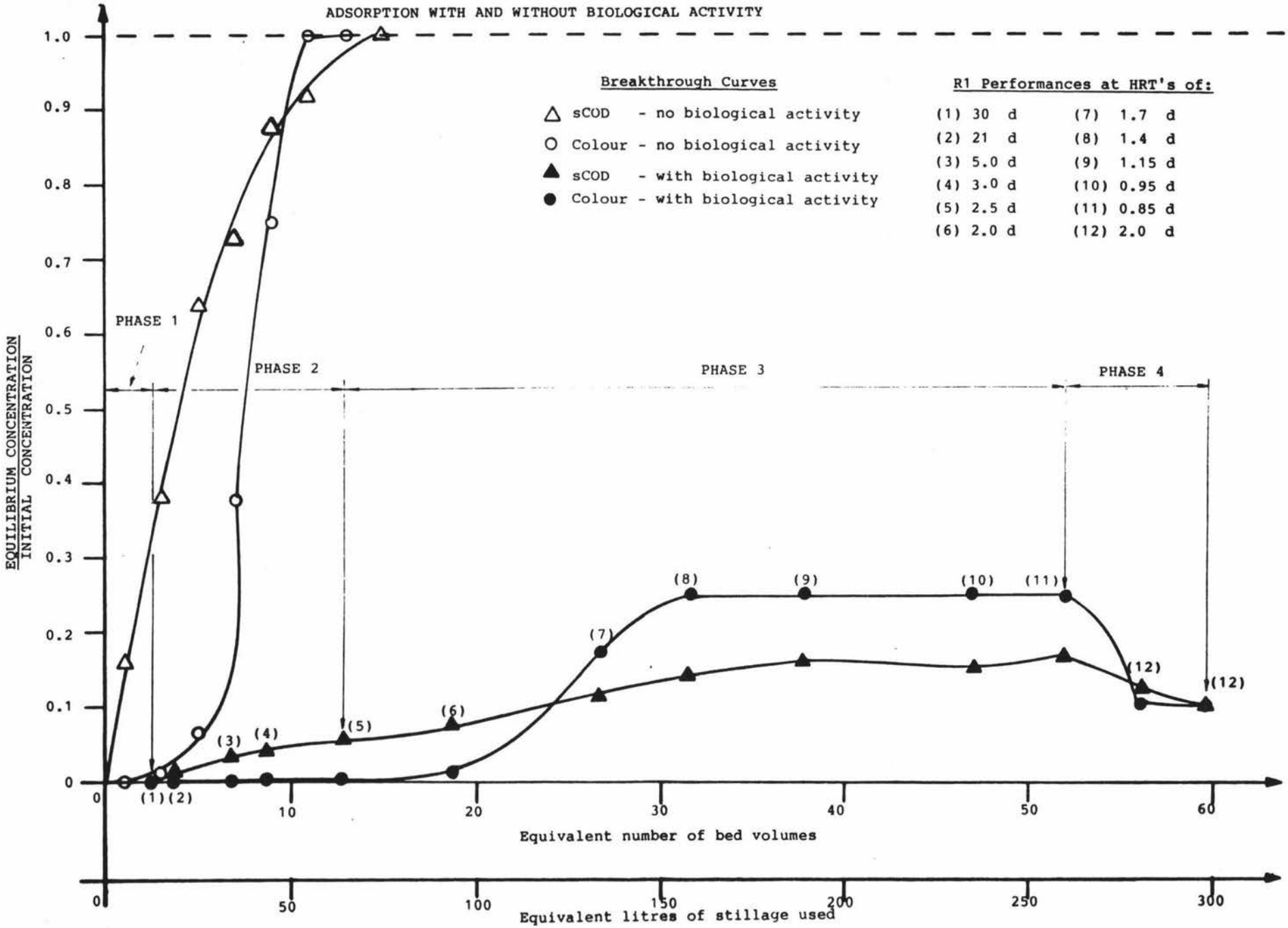
4.3.5 R1 Phase 4 Operation
- The GAC Bioregeneration Confirmation Phase

Until now, the organic loading rate had been used as the loading criterion for reactor operation. The organic loading rate had been slowly stepped up only when the reactor was running stably. The colour loading rate had not been used as the loading criterion. It is thus possible that the percentage colour removal could be apparently low when operating at low HRTs to maximize the methanogenic activity because the chromophores are expected to be more recalcitrant to microbial degradation than other substrates present in the stillage. Under these conditions, the colour loading rate may exceed the bioregeneration rate for colour and colour breakthrough will occur.

Thus R1 was operated at as high a practical methanogenic limit as possible, depending upon time constraints, to assess the organic removal potential in Phase 3 Operation. In Phase 4, the colour loading rate to the reactor was decreased to a 2 d HRT so that the percentage colour removal in relation to bioregeneration could be assessed. The 2 days HRT loading rate was chosen because it is well within the reactor operational phase and it is also the HRT that the highest organic loading rate (13 kg $COD.m^{-3}.d^{-1}$) for the anaerobic treatment of wood-ethanol stillage had been previously reported (Callander et al., 1983).

Also, the percentage colour removal deteriorated quite rapidly after the 2 d HRT period (Fig. 4.17). It is possible that the 2 d HRT loading rate stage represented the point where the colour degradation rate is in equilibrium with the colour loading rate. If this is so, probably the most promising way to reduce this 2 d equilibrium rate is to reduce GAC particle size resulted in an increased surface area to volume ratio for increased adsorption and subsequent microbial degradation. The 2 d HRT colour loading stage thus merited further investigation.

FIGURE 4.17: BREAKTHROUGH CURVES FOR COLOUR AND sCOD REMOVALS FROM RAW STILLAGE USING GAC: RESULTS FOR ADSORPTION WITH AND WITHOUT BIOLOGICAL ACTIVITY



4.3.5.1 Performance and stability of R1 in relation to a step decrease in OLR

An immediate drop in VFA levels from 160 and 490 $mg.l^{-1}$ for C_2 and C_3 to 110 and 165 $mg.l^{-1}$ respectively were observed in 24 hours when the OLR to R1 was decreased from 29.0 to about 11.7 kg $tCOD.m^{-3}.d^{-1}$ (Appendix 3.3; Fig. 4.14). Subsequently, the C_2 and C_3 levels remained low, averaging 131 and 86 $mg.l^{-1}$ respectively indicating very stable reactor operation.

As expected, a very sharp drop in the gas production rate was observed from 74 to 31.8 $l.d^{-1}$ average at 61.3% methane composition (Appendix 3.3). However, the gas yield was still close to the rate predicted by theory (30.7 $l.d^{-1}$ average). The higher observed gas yield was believed to be due to desorption of organics from R1 carbon. These organics were then subsequently degraded.

The decrease in OLR also resulted in a measurable drop in attached biomass concentration from 1.96 g $VSS.l^{-1}$ to 1.51 g $VSS.l^{-1}$ (Table 4.6). This period of operation was not considered to be sufficiently long enough for significant cell decay to occur. The decrease in biomass concentration is thus believed to be due to its dislocation from the carbon surface by attrition and subsequently being washed out of the reactor. This was reflected by a general slight increase in VSS when compared to the previous stages of operation (Appendix 3.3).

At an average tCOD loading rate of 12.4 $kg.m^{-3}.d^{-1}$, the percentage sCOD removal increased gradually from 85.4 to 90.5% over a period of 16 days (Fig. 4.17). This percentage removal was expected to approach that achieved during Phase 3 operation at the same HRT (13.2 kg $tCOD.m^{-3}.d^{-1}$), i.e. an average of 91.8% (Appendix 5). It was hypothesized that this stage of operation required a larger number of HRTs before steady state operation was achieved was due to the gradual desorption of organics from the GAC to the reactor liquor as a result of the concentration gradient between the GAC and the solution medium. As can be seen in Fig. 4.17 and Appendix 3.3, more than 7 HRTs were required before the effluent sCOD reached a steady state.

For colour removal, the steady state situation was achieved within 5 HRTs (Fig. 4.17) suggesting that the chromophoric species involved different adsorption-desorption mechanisms to the sCOD case. It was hypothesized that the chromophoric species only represented a minute proportion of the organics that were measured as sCOD.

At a colour loading rate of 2.0 ± 0.1 kg chloroplatinate $m.^{-3}.d^{-1}$, the percentage colour removal stabilized at 90.6% as compared to 94% and still deteriorating at the previous 2 d HRT loading stage (Fig. 4.17). However, the fact that the percentage colour removal improved significantly when the colour loading rate on R1 was lowered conclusively proved that colour removal from the stillage was a result of microbial degradation with continuous bioregeneration of the GAC. This conclusion will be further substantiated with results from GAC particle size analyses and the UV-Visible spectrophotometric scans (Section 4.3.5.4).

4.3.5.2 Biomass Estimation

Many attempts were made to measure the biomass concentration on the activated carbon using various techniques. With the exceptions of the organic nitrogen method, all other techniques attempted proved unsuccessful due to the influence of activated carbon on the method. The results will be described in detail here.

(a) DNA Method

As stated earlier in phase 1 operation, problems were encountered using the DNA method. The DNA extraction method resulted in the extraction of other compounds from the carbon as well, causing turbidity and precipitation problems in the final stages of the analysis. It could not have been reagent preparation or impurity problems because the DNA standard curve developed using similar procedures was the same as that reported by Herbert et al., (1971). At one stage, it was thought that the precipitation problem could be due to the centrifuge (MSE, MINOR 35)

being unable to develop the necessary acceleration of 10,000 g to settle the interfering species. The extraction stage was subsequently performed on a super speed SORVALL RC-5 refrigerated centrifuge (Du Pont Instruments) operating at 15,000 rpm (or 10,000 g). Centrifugation was carried out at 4°C for 20 mins as reported by Solomon et al., (1983). However, the problem still persisted though it was clear from the extracts (by visual observation) that progressively higher biomass concentrations were present on the carbon sample as the organic loading rate was increased.

(b) Total Phosphorus (TP) Method

This method assumed a cell composition of $C_{60}H_{87}O_{23}N_{12}P$ (McCarty, 1970) or 0.0226 g $P.g^{-1}$ cells.

The R1 carbon total phosphorus content was measured at 4.4% w/w of dried carbon as compared to 0.06% for the virgin carbon. Assuming the TP in excess that of the virgin carbon was from the bacterial cells, the VSS was estimated at 578 $g.l^{-1}$ when operated at the 0.95 d HRT stage. This value was unreasonably high and phosphorus was believed to have aggregated or precipitated on the R1 carbon giving a high TP baseload which interfered with cell phosphorus measurement. The TP extraction using this method was not able to discriminate between biomass and inorganic phosphorus content. In fact, phosphate contents of activated carbon ranging from 0-3% have been reported (Hassler, 1974).

(d) Direct Solids Measurements

Direct TS and VS analyses were done for the virgin and dried R1 carbon. Though aimed at getting the maximum VS possible as indicator of biomass concentration, some other interesting results were obtained.

The R1 carbon (sampled at 24/11/83) was found to have a lower VS content than the virgin carbon i.e. 0.8719 and 0.9356 g VS.g^{-1} dried carbon respectively. This is because the R1 carbon had a higher ash content than the virgin carbon presumably to be due to biomass present in R1 carbon. Previously, R1 carbon has been shown to have a high TP content and this was mainly due to inorganic phosphorus rather than phosphorus from the cells.

(d) Organic Nitrogen (RNH_2) as Indicator of Biomass Concentration

This method used the same rationale as the TP method with an RNH_2 equivalent of 0.122 g N.g^{-1} cells (McCarty, 1970). The nitrogen content of the cell can be dependent on the N availability and the rate of biological growth for organic waste types since the N requirements are directly proportional to net synthesis of substrate into biological solids. This difference has been shown to be minimal by Speece and McCarty (1964) who concluded that the anaerobic biological solids can be represented as $C_5H_9O_3N$ for bacteria growing on many different substrates.

The cell weight/N ratio was given as 9.4 within ± 2 standard deviations of the mean. The results obtained using RNH_2 as a VSS measurement parameter are presented in Table 4.6.

The results are considered reasonable though the attached biomass concentrations are significantly lower than those reported in the literature e.g. 15-40 g.l^{-1} using 0.3 mm Ø uniformly sized sand packed expanded-bed reactors (Rockey and Forster, 1982); 13 g.l^{-1} using sand carrier with 91% of the particles between 0.10 and 0.18 mm (Frostell, 1982) and, 25.4 g.l^{-1} using 0.3 mm Ø uniformly sized sand (Barnes et al., 1983) both in fluidized bed reactors. For these cases, enough information was only given by Frostell (1982) to enable the superficial velocity to be calculated. Since R1 had a maximum superficial velocity of approximately 15.8 m.h^{-1} (Section 4.3.4.2) as compared to 330 m.h^{-1} used by Frostell (1982), it appears that bed turbulance was not the cause of the low attached biomass concentration.

TABLE 4.6: R1 BIOMASS CONCENTRATION

Days of Operation	OLR (kg tCOD.m^{-3}.d^{-1})	Percentage Organic Removal	RNH_2 of R1 carbon in excess of Virgin Carbon (mg.g^{-1}carbon)	Attached Biomass Concentration* (g VSS.l^{-1})	Suspended Biomass Concentration ** (g VSS.l^{-1})	Total Reactor Biomass Concentration (g VSS.l^{-1})	Specific Sludge Activity (kg tCOD. kg VSS^{-1}.d^{-1})
115	2.12	93.0	0.167	0.40	0.63	1.03	1.91
148	6.68	87.7	0.238	0.59	1.13	1.72	3.41
170	12.4	84.1	0.372	0.92	2.46	3.38	3.09
206	29.0	77.5	0.795	1.96	1.61	3.57	6.30
228	12.4	83.7	0.615	1.51	1.85	3.36	3.09

* Calculations based on 1.5 kg GAC in R1 with 5 l reactor liquid content.

** Data from effluent VSS and assumed to be all from cell biomass. The contribution from carbon fines was considered to be minimal (see Section 4.3.5.4.1).

All evidence pointed to a low biomass concentration in the reactor except for its high organic reduction rate. No slimy appearance was observed on the R1 carbon throughout its period of operation. Experience with an expanded-bed reactor for whey permeate digestion at Biotechnology Department, Massey University (Archer et al., 1983) has indicated that a slimy layer of biomass was present on the GAC support after being operated for 2-3 years. An organic loading rate of 12.4 kg $COD.m^{-3}.d^{-1}$ had been achieved (Archer et al., 1983) and the biomass concentration ranged from 20-30 g $VSS.l^{-1}$ (Archer, 1984, personal communication). The absence of a slimy layer on the GAC in R1 correlated well with the low sludge yield of the system (Section 4.3.5.6).

Other evidence can be listed as;

(i) Low bacterial density on R1 carbon from the electron scanning photomicrographs (Section 4.3.5.3).

(ii) Low nutrient requirement (Section 4.3.4.1).

The use of RNH_2 as a measure of biomass concentration for a similar system using 0.840 - 1.190 mm Filtrasorb 400 GAC in a fluidized bed has already been reported (Suidan et al., 1983). A RNH_2 concentration in excess of 3.2 $mg.g^{-1}$ carbon over the virgin carbon was obtained when operated for 290 days on coal gasification wastewater. The total biomass concentration in the reactor was reported as 68.84 g. The biomass concentration can be estimated as 14.6 $g.l^{-1}$ assuming a wetted GAC density of 1450 $kg.m^{-3}$.

From Table 4.6, the suspended biomass generally represented more than 50% of the total biomass concentration. The low attached biomass concentration in R1 carbon did not prove to be a handicap for this system in terms of reactor performance (Table 4.3). Instead, the low biomass yield resulted in a system with a very high methane yield and a low nutrient requirement (Table 4.3). The high sludge activities obtained (greater than 3 kg $COD.kg\ VSS^{-1}.d^{-1}$ for the reactor operational stage, Table

4.6) suggested the enhancement of microbial degradation of organics by the activated carbon. A very long sludge age is expected for the attached biomass which could also possibly contribute to the high sludge activities. It appears that the attached biomass concentration increased significantly as the HRT was decreased (i.e. at a higher organic loading rate - Table 4.6). They contributed approximately 55% of the total biomass concentration in the reactor at the 0.85 d HRT stage (day 206) as compared to less than 40% at the previous longer HRT stages (days 170, 148 and 115). Thus this AEB system is expected to be able to withstand a high hydraulic loading rate without encountering problems of cell washout from the reactor. Previously, attempts have been made to improve reactor biomass retention in a fluidized bed system by recycling of sludge from the effluent (Frostell, 1982). A maximal biomass concentration of 22 kg $VS.m^{-3}$ (of which 13 kg $VS.m^{-3}$ was attached) has been achieved with a high COD loading rate of 22.2 $kg.m^{-3}.d^{-1}$. But, the average COD removal was low, at 43% conversion to methane (Frostell, 1982) as compared to R1 with 74.5% tCOD removal at an OLR of 29.0 kg $tCOD.m^{-3}.d^{-1}$.

4.3.5.3 Scanning electron micrographs (SEM) of GAC and baterial flocs

Scanning electron micrographs were taken for the virgin carbon (Fig. 4.18, 4.19 and 4.20), R1 carbon after 192 days of operation (Fig. 4.21 and 4.22) and the bacterial flocs from R1 (Fig. 4.23). The bacterial flocs were located on top of the R1 carbon bed surface and normally had a thickness of about 0.8 cm of reactor height. Their presence was first observed on day 32 (Appendix 3.3). The extent depended on the OLR and may fluctuate, presumably due to hydraulic effects.

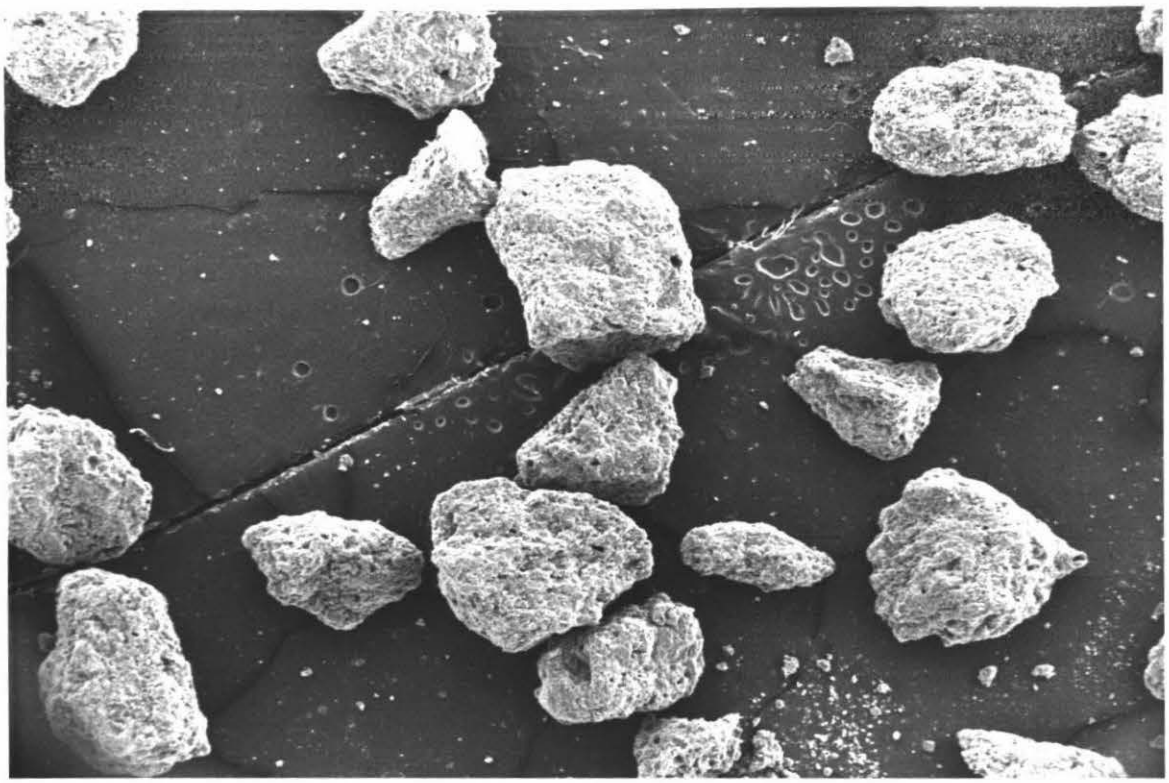
The advantages of the scanning electron microscope over the conventional light microscope, including greater magnification, increased depth of field, and higher resolution (Weber et al., 1978) were clearly demonstrated here. As can be seen in Fig. 4.18 and 4.21 the R1 carbon

FIGURE 4.18: Scanning Electron micrograph of virgin granular activated carbon surface. x 20

The large variations in the virgin carbon particle size with its angular structure is evident in this figure.

FIGURE 4.19: SEM of GAC Surface. x 80

This figure highlights the rough surface and porous structure of the activated carbon.


1 mm

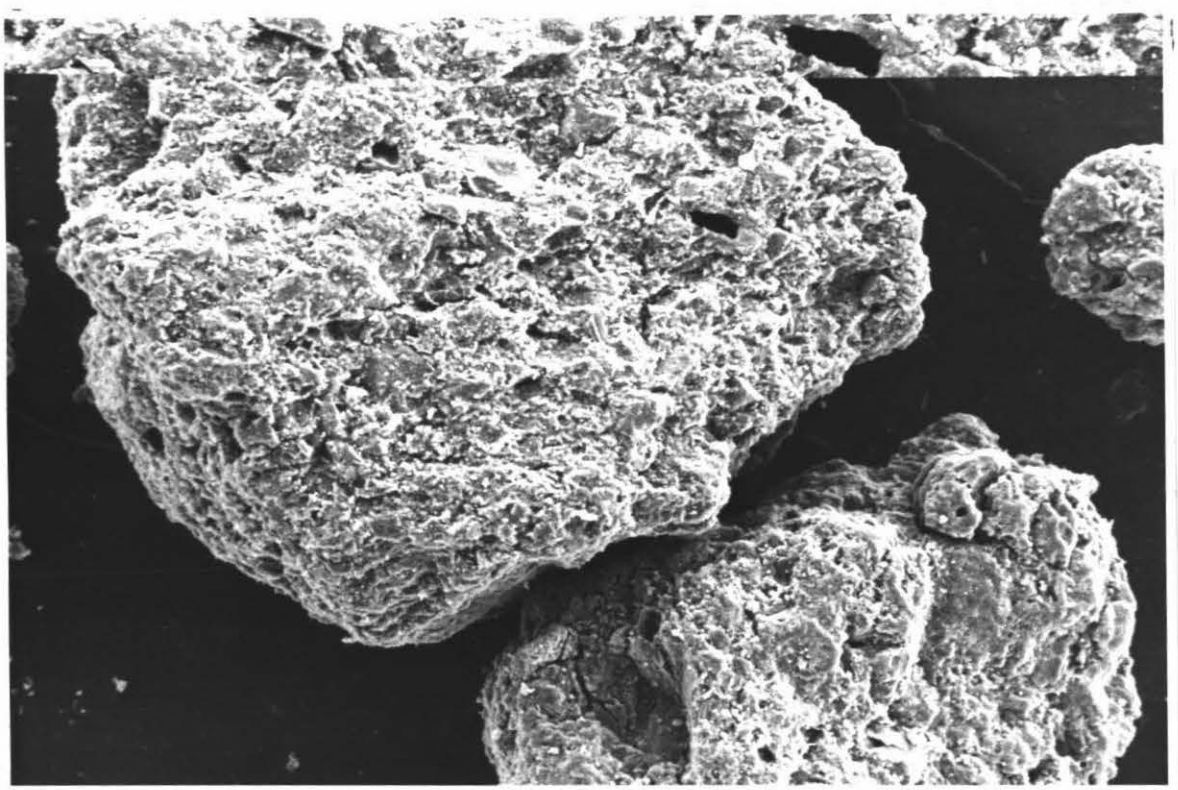

1 mm

FIGURE 4.20: SEM of Virgin GAC Surface. x 1250

The GAC surface is free of biological growth. However, the holes, crevasses, grooves and ridges are expected to provide a favourable environment for bacterial growth since the sites would be shielded from shear forces in an expanded-bed.

FIGURE 4.21: SEM of R1 Carbon after 192 days of Operation.

The R1 carbon appears to be more consistent in size and shape and were more rounded with all the sharp edges (as seen in the virgin carbon) removed by attrition in the expanded-bed.


0.01 mm

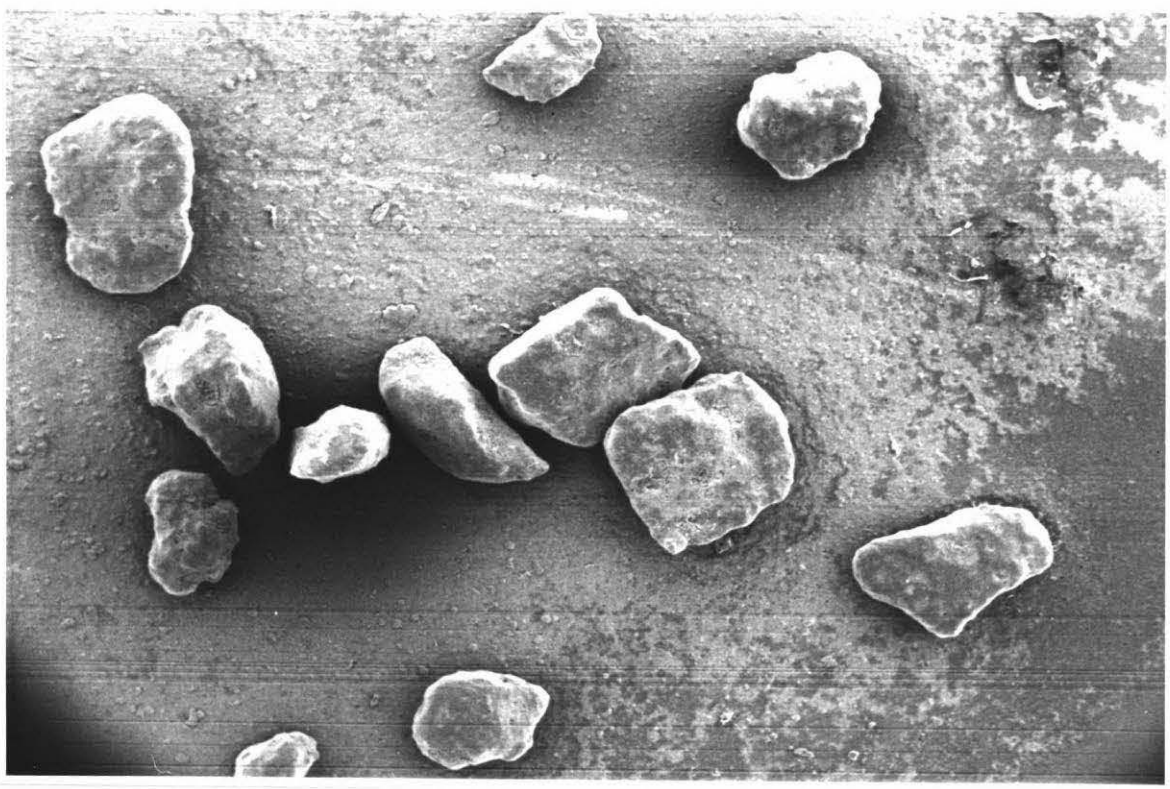

1 mm

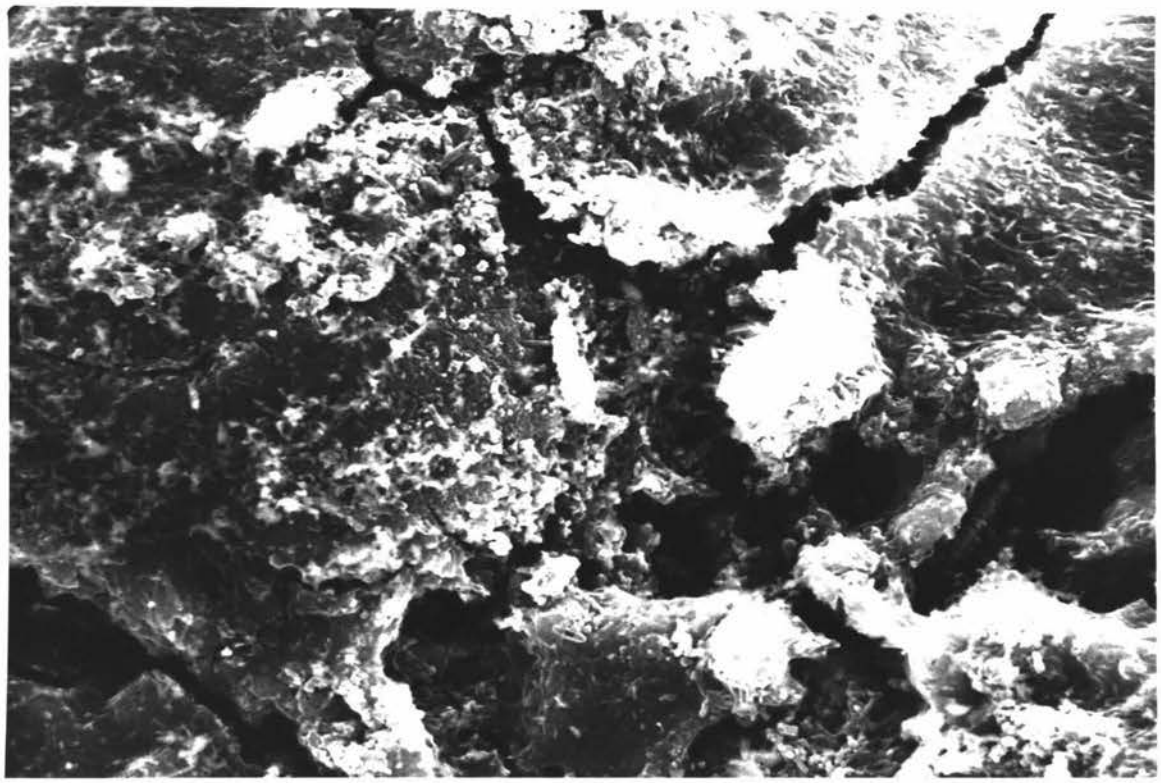
FIGURE 4.22: SEM of R1 Carbon after 192 days of Operation. x 1250

Bacterial colonies are clearly evident in the grooves in the carbon surface which were shielded from the effects of fluid shear forces and carbon attrition. The rod shape bacteria have a size of about 3.6 x 1.4 μ. It is also obvious these bacteria can grow in the micropores or crevasses in the carbon (about 4.8 μ).

There appears to be a layer of slime in the top right hand corner of the figure but this does not cover the whole carbon surface. Thus sites are still available for adsorption. Apart from the obvious grooves or holes, the carbon surface is generally flat with some sparsely scattered bacterial cells. This is expected since the outer unprotected surface does not provide such a favourable environment for bacterial attachment.

FIGURE 4.23: SEM of R1 Bacterial Floc. x 2500

A very high bacterial density is evident throughout the floc surface. These bacteria appear to be similar to the one observed in R1 carbon (rod like). Notice the 'balloon' like structure on the right hand bottom corner of the figure. SEM of R1 bacterial floc at lower magnification shows that they were scattered throughout the surface. They don't appear to be a 'living organism' and is believe to be due to secretions from the bacteria.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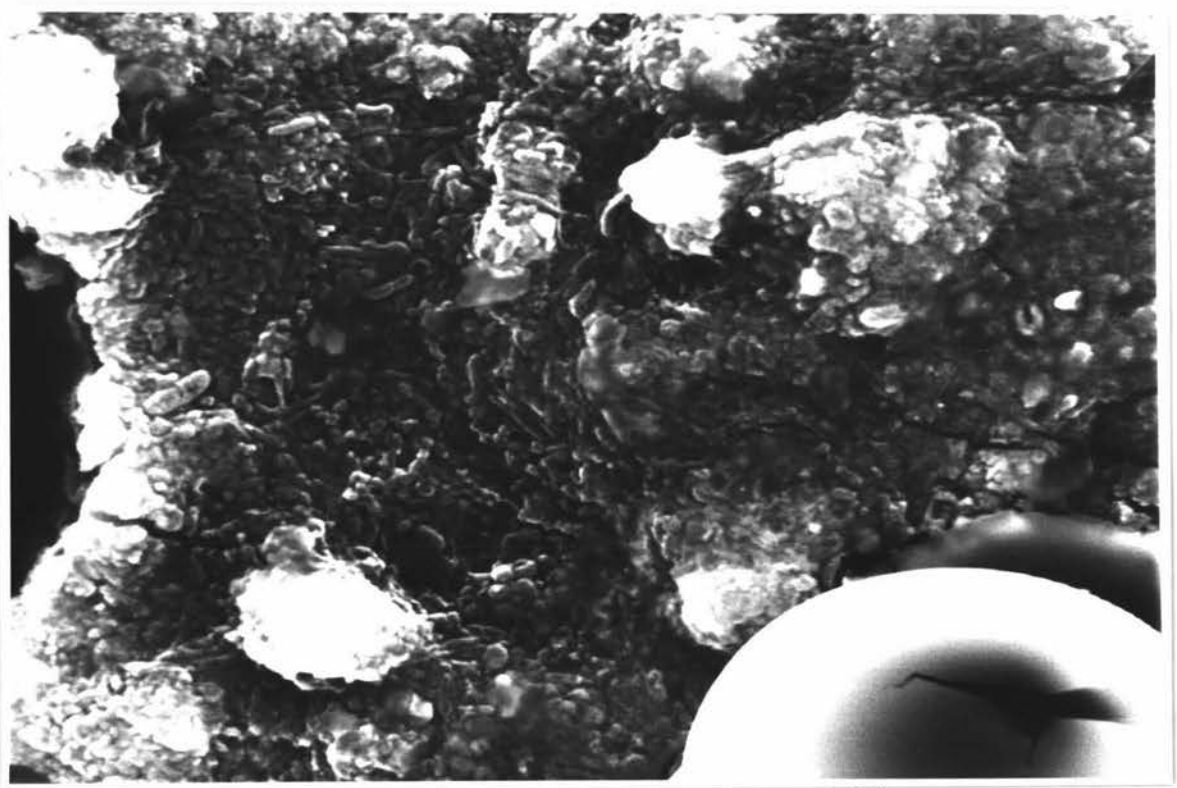
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particles are more rounded and have a smoother surface than the virgin carbon. The rough and porous structure of the virgin carbon surface is clearly seen (Fig. 4.19). The R1 carbon had all the ridges and grooves of the virgin carbon removed and replaced by a smooth surface after fluidization (Fig. 4.20 and 4.21). No bacteria were evident on the virgin carbon surface (Fig. 4.20) and as expected, they can clearly be seen in the R1 carbon. They appear as long rods and distributed sparsely throughout the carbon surface. It is also clear that these organisms can grow in the larger micropore openings in the carbon surface (Fig. 4.22). No limitation to the access of adsorbates to the internal micropore surface area was expected since only a sparse bacterial population was involved and the pores were not covered by a layer of slime. The low bacteria density correlates well with earlier observations that R1 carbon had a low bacterial population (Section 4.3.5.2). For cases with a high attached biomass concentration, the particle external surface area available for substrate adsorption is likely to be greatly reduced due to bacterial colonization (Dunn, 1984).

High concentrations of rod-like bacteria were observed, and completely covered, the bacterial floc surface. They appear to be embedded in an 'organic matrix' (Fig. 4.23). They were also some other 'balloon' like outgrowths on the floc surface. They are believed to be secretions from the bacteria colonizing the surface (e.g. slime) which were 'blown' up during sample preparation for electron microscope scanning.

4.3.5.4 Extent of GAC bioregeneration in R1

The breakthrough curves for sCOD and colour using raw stillage (with and without biological activity) are presented in Fig. 4.17.

The expanded-bed reactor had performed far better than predicted by physical and chemical adsorption (see Section 4.2.3). The GAC in R1 had treated 60 EBVs of stillage and was still achieving approximately 90% sCOD and colour removals (Fig. 4.17). This was far more than the amount required to fully exhaust the GAC when only physical and chemical

treatment were involved (14 EBVs). There are two possible explanations for the superior performance of R1 carbon:

(i) Increase in carbon surface area by attrition resulting in better chemical and physical adsorption than predicted;

(ii) Microbial degradation of adsorbed species resulting in continuous bioregeneration of the activated carbon.

Bioregeneration of the GAC in terms of sCOD removal is clear enough from the conversion of COD to methane. However, the microbial degrad- of the chromophoric species, presumably to lower molecular weight colourless products, is more difficult to quantify. Fig. 4.24 gives a photographic view of the stillage before and after R1 treatment in relation to the colour standards. The R1 effluents were taken from samples at the end of the four phases of operation. The sections to follow seek to elucidate this point by considering the particle size of the virgin and R1 carbon and, the UV-Visible absorption spectra of raw stillage and R1 effluents.

4.3.5.4.1 GAC particle size analysis

The aim was to determine if there was any generation of new carbon surface areas in R1 carbon. This was achieved by measuring the equivalent particle diameters (dp) of R1 carbon and the virgin carbon using the sedimentation technique for particle size analysis (Scarlett, 1977) and to compute any change in specific external carbon surface area (Asp). Appendix 4 gives an account of the method used and a summary of the results is presented in Table 4.7. The R1 carbon samples were taken during the end of Phase 2 and 3 operation.

FIGURE 4.24: Photographic View of the Stillage Before and After Treatment.

Shown on top of the photo is the colour measurement standards ranging from 0 - 70 CPU. The intense colour of the raw and anaerobic lagoon pretreated stillage can be clearly seen and a dilution of 100 x is required for comparison with the standards. It can be seen that the anaerobic lagoon treated stillage is less intensely coloured than the raw stillage (comparison at 100 x dilution) since approximately 75% colour removal was achieved in the lagoon treatment. Also notice that the R1 effluent at the 25 x dilution, 20 d HRT and 59 EBVs is less intensely coloured than the R2 effluent at same dilution and HRT but at 50 EBVs.

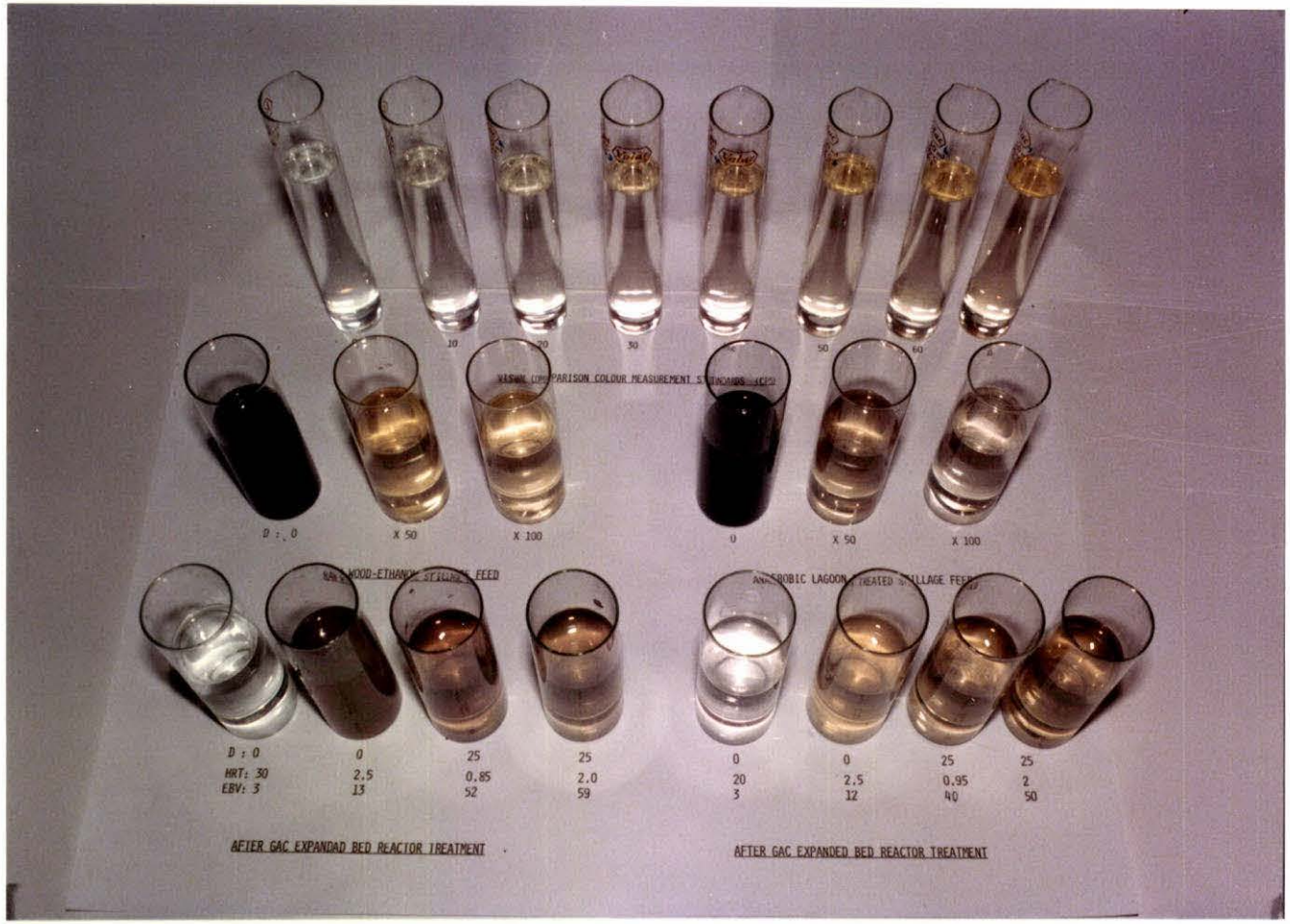

10
30
50
D : 0
X 50
X 100
0
X 50
X 100
D : 0
HRT: 30
EBV: 3
0
2.5
13
25
0.85
52
25
2.0
59
0
20
3
0
2.5
12
25
0.95
40
25
2
50
AFTER GAC EXPANDAD BED REACTOR TREATMENT
AFTER GAC EXPANDED BED REACTOR TREATMENT

TABLE 4.7: RESULTS SUMMARY FOR GAC PARTICLE SIZES AND EXTERNAL SPECIFIC SURFACE AREAS

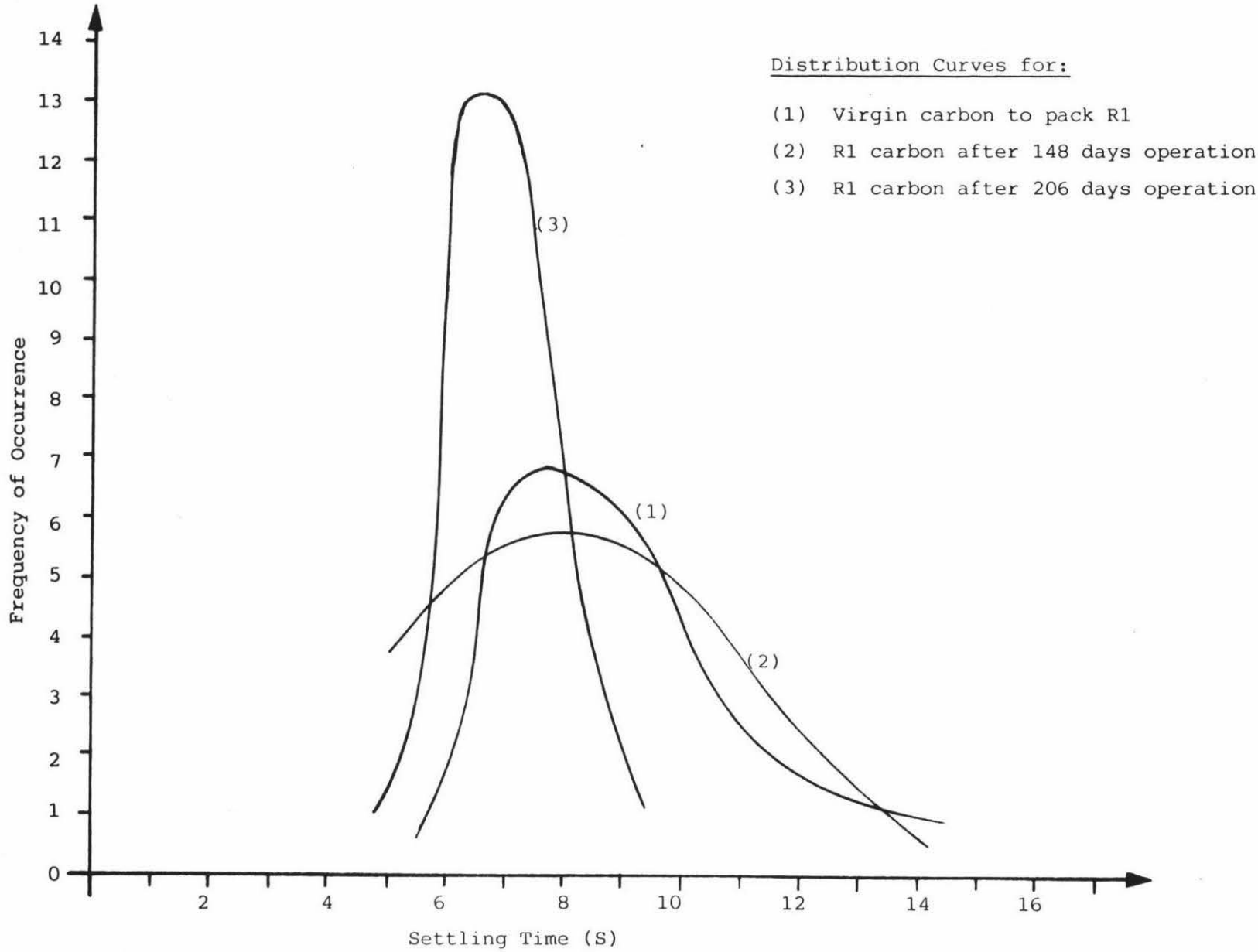
GAC Sample	Average Particle diameter (mm)	External specific surface area ($m^2.kg^{-1}$)
Virgin carbon	0.315	21.7
R1 carbon, 148 days of operation	0.323	19.9
R1 carbon, 206 days of operation	0.392	15.4

The results are comparable to those reported by Suidan et al., (1983) at 15.0 $m^2.kg^{-1}$ using 0.840-1.190 mm GAC carrier.

There was in fact a measurable increase in particle size for the R1 carbon over the duration of the study (Table 4.7). However, this slight increase in particle size is believed to be due to a change in particle size and shape after being used in R1. The R1 carbon was observed to be more consistent in size and shape and, have a more rounded and smooth surface when studied using a light microscope. In contrast, the virgin carbon appeared to be quite angular. They have been confirmed by results from the scanning electron photomicrographs (Section 4.3.5.3). Thus R1 carbon will settle faster than the virgin carbon even if both had the same dp because R1 carbon had a lower drag coefficient being more spherical in shape (Perry and Chilton, 1974). The relatively consistent R1 particle size and shape (for carbon after 206 days of operation (R_{206})) resulted in less variation in the particle settling time (Fig. 4.25).

Taking the particle sphericity into account (Appendix 4), the external specific surface areas (Asp) decreased from 21.7 to 19.9 and 15.4 $m^2.kg^{-1}$ for virgin carbon (R_0), R1 carbon at end of Phase 2 (R_{148}) and 3 (R_{206}) respectively (Table 4.7). There was not only no creation of new carbon external surface area but measurable reductions of 12.9% (for R_{148}) and 32% (for R_{206}) over that of the virgin carbon were obtained.

FIGURE 4.25: GAC SETTLING TIME DISTRIBUTION



Though there are limitations in the method due to assumptions in the analysis, this exercise shows that there is no evidence of creation of new carbon external surface area for better chemical and physical adsorption than predicted.

No significant decrease in R1 carbon bed volume was observed (2 ± 1%). Carbon loss could well be replaced by biomass growth, although this has been shown to be minimal (Section 4.3.5.2), or from adsorbed organics. This is despite the fact that some 50-60 g of dried carbon (i.e. about 4% of the GAC used to pack R1) had been sampled from R1 for biomass measurement, particle size analyses, gas chromatograph-mass spectrograph (GC-MS) analyses and electron photomicroscopic scans.

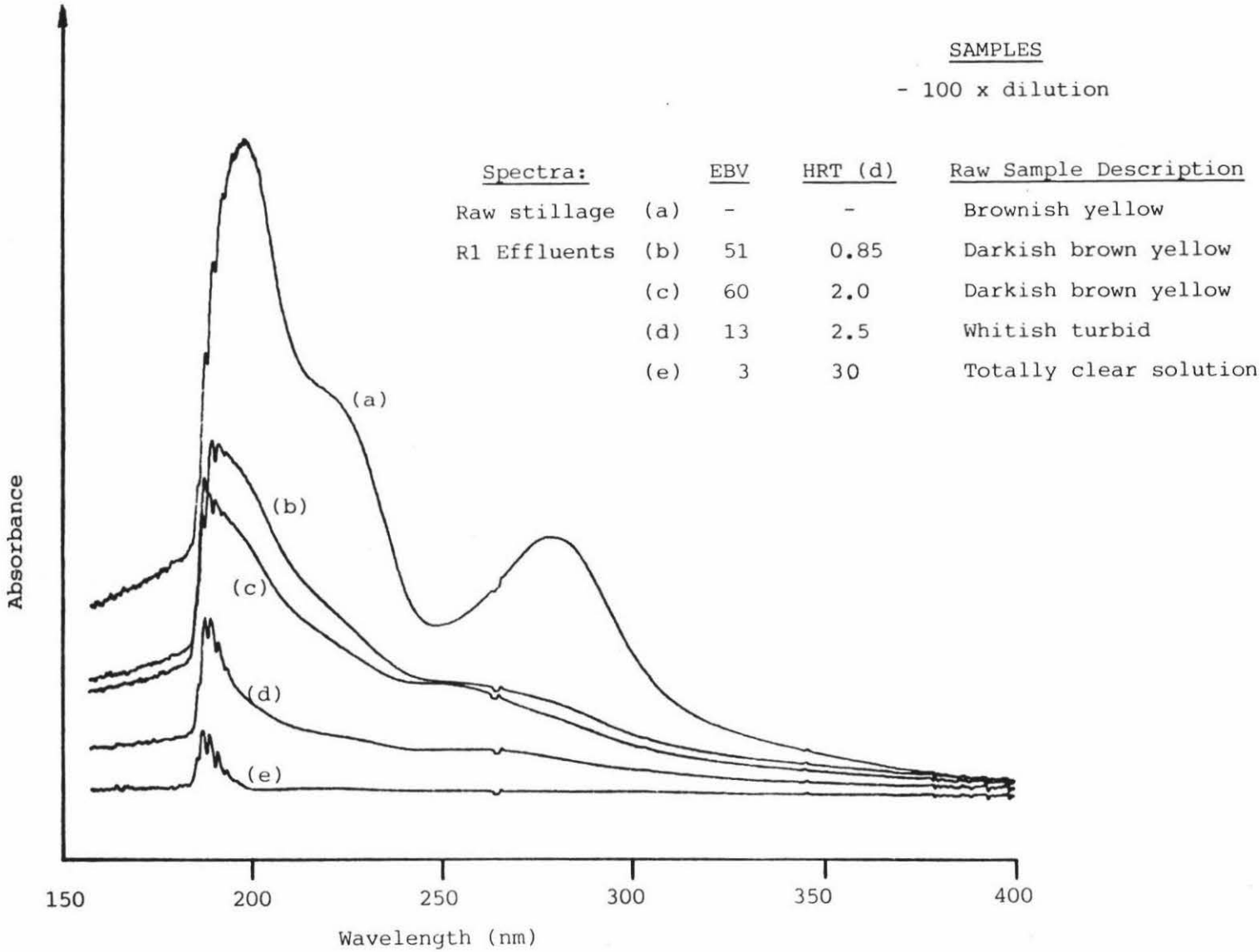
4.3.5.4.2 The UV-visible spectrophotometric scans

This technique permitted the confirmation of microbial degradation of chromophoric species (if any) by considering the presence and disappearance of UV-visible absorbing species before and after treatment in R1. The samples were chosen from all the four Phases of operation (being stored at 4°C) when the reactor was operated at the appropriate HRTs and equivalent bed volumes of stillage. They were deliberately selected such that comparisons between R1 performance in terms of HRT (microbial degradation parameter) and EBV (physical-chemical adsorption parameter) were possible. Different chromophoric species will absorb radiant energy traversing them at different light wavelengths and this has been shown to be in direct proportion to the concentration of the absorbing species present (from Beer's Law). The presence and disapearance of the absorbing species before and after anaerobic treatment (if any) can be gauged by the difference in peak heights in the UV-visible spectra.

(a) R1 UV-Spectroscopic Characteristics

A UV-spectrophotometric scan of the raw stillage indicated two peaks at 200 and 280 nm and a point of inflection at 230 nm (Fig. 4.26). The 280 nm UV absorption has been associated with aromatic content of kraft black liquor (Marton et al., 1969) or the presence of aromatic rings in the compound (Higuchi, 1980).

FIGURE 4.26: UV - SPECTRA FOR RAW STILLAGE AND R1 EFFLUENTS



For the decolourized R1 effluents, the UV-absorption spectra had a much lower absorbance and no peak was observed at 280 nm (Fig. 4.26). This confirmed the reduction in UV chromophoric species after treatment in R1.

The spectra were also found to be related to HRTs in the reactor (Fig. 4.26). Since, GAC adsorption by physical and chemical means reached an equilibrium in less than 2 hours contact time (see Section 4.2.1), the percentage removal (with only purely physical and chemical adsorption) was not expected to vary for HRT's very much greater than 2 hours. On the other hand, greater removals of the UV-chromophoric species were observed at a higher HRT resulting in much lower peak (Fig. 4.26). Also, if only purely physical and chemical adsorption were involved in effluent decolourization, the number of stillage bed volumes treated was expected to affect the percentage colour removal i.e. with a deterioration in percentage colour removals as more EBVs of stillage were treated with the GAC progressively becoming more exhausted. This has been ruled out since a greater percentage colour removal was achieved at 2.0 d HRT after treating 60 EBVs than at 0.85 d HRT and 51 EBVs (Fig. 4.26). Thus the UV-spectra provided further proof that R1 effluent decolourization was a result of microbial degradation rather than by physical or chemical adsorption by the GAC.

Similar, trends were obtained with the ionization difference spectra except that there was no point of inflection at 220 nm (Fig. 4.27). Also the first effluent peaks and the raw stillage peak occured at the same wavelength of 205 nm and were more distinct.

(b) R1 Visible-Spectroscopic Characteristics

The raw stillage visible-spectrum increased monotonously in the visible region as the wavelengths decreased from 800-300 nm with no distinct absorption maximum (Fig. 4.28). This spectrum is similar to that typical of alkaline solutions of kraft lignin (Marton et al., 1969). For R1 treated stillage, the spectrum

FIGURE 4.27: UV - IONIZATION DIFFERENCE SPECTRA

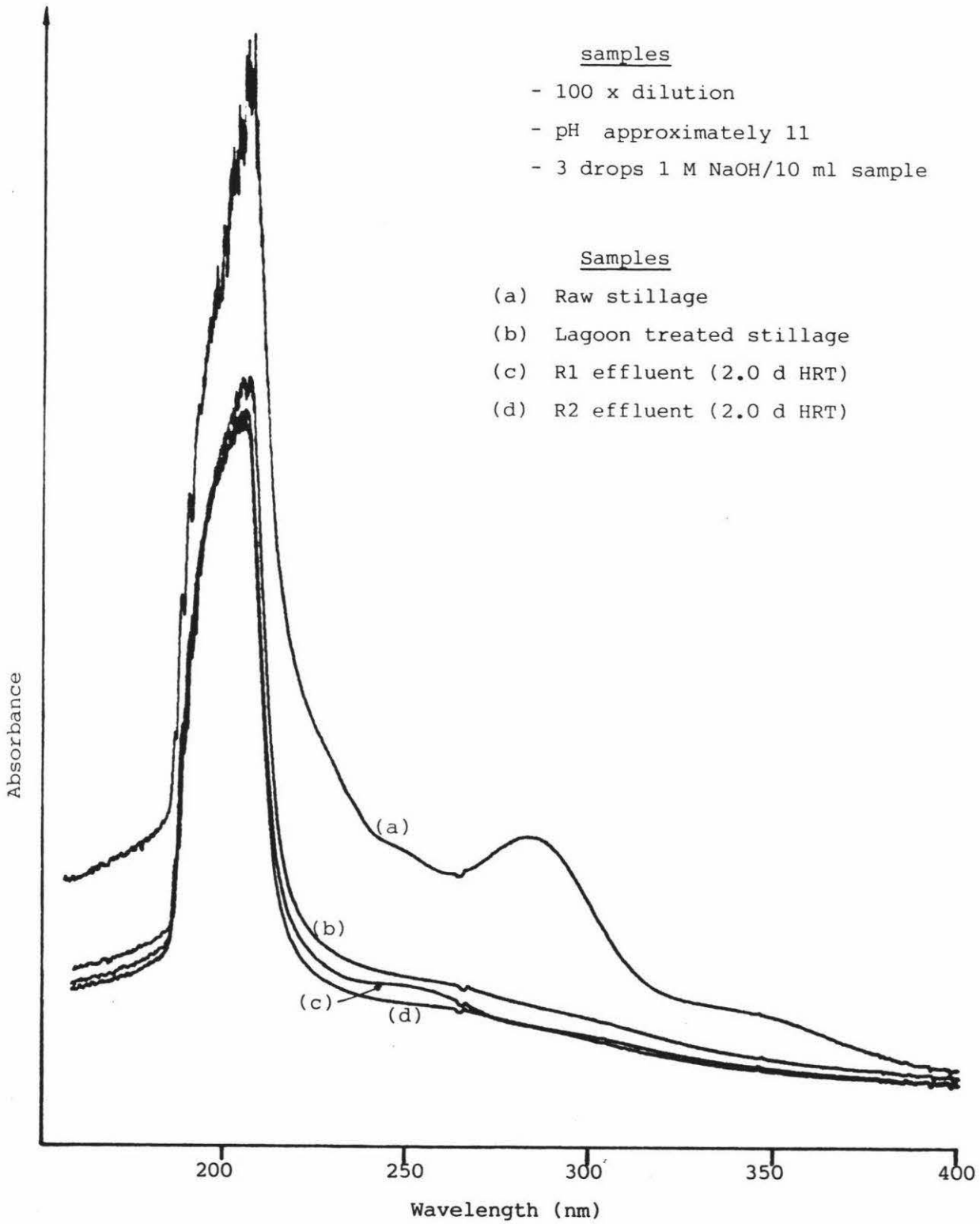
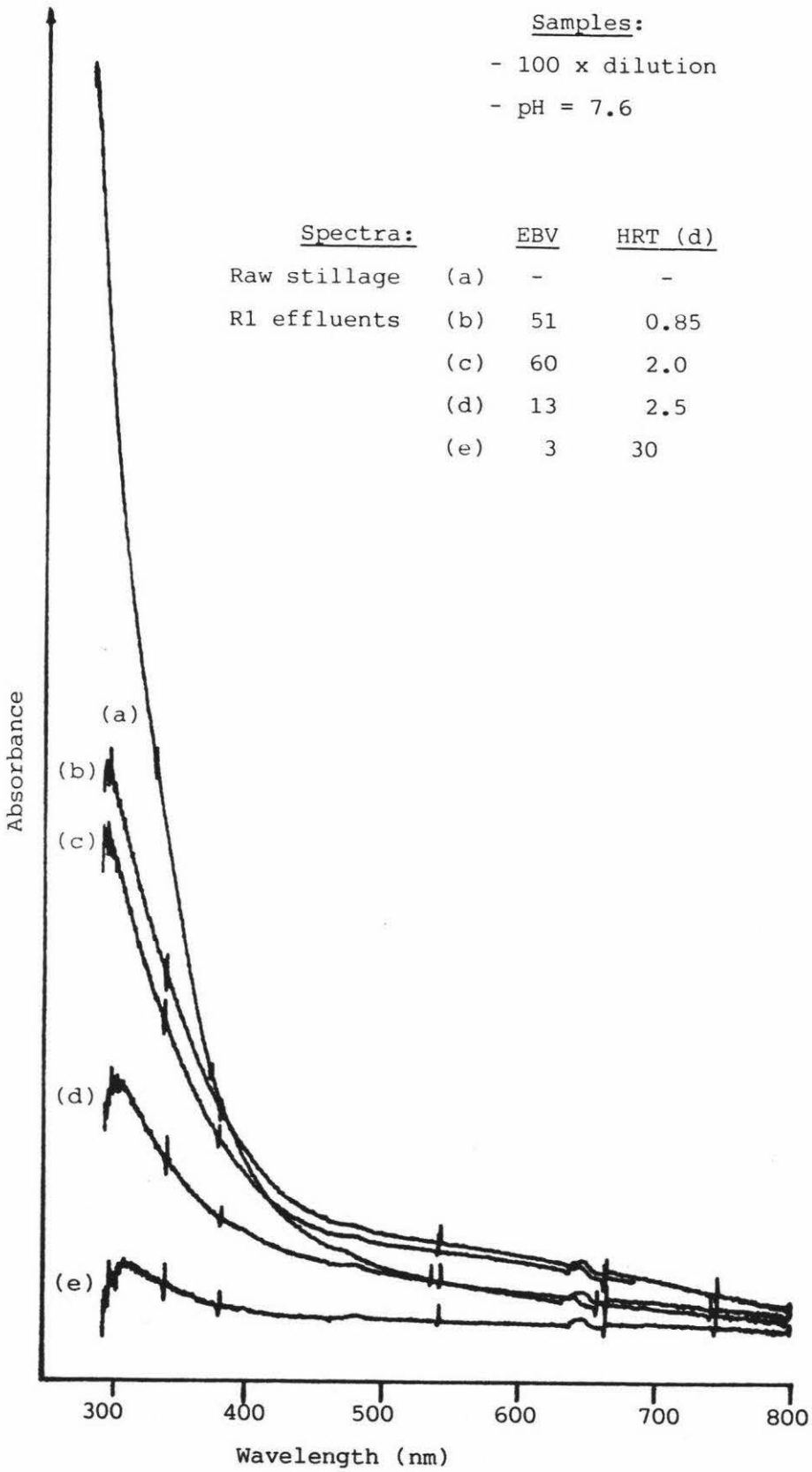


FIGURE 4.28: VISIBLE-SPECTRA FOR RAW STILLAGE AND R1 EFFLUENTS



can have higher or lower absorbances than the raw stillage depending on the wavelength considered. There appeared to be an absorption maximum for the R1 effluents at around 310 nm. The same general trend regarding colour removal and HRT can also be observed as in the UV-spectra thus confirming the microbial degradation of visible chromophoric species as well.

Samples were taken for Gas Chromatograph-mass spectrograph (GC-MS) analyses to be done at the FRI. This method allowed a qualitative and quantitative determination of aromatic compounds before and after treatment. Unfortunately, the results were not available in time for incorporation into this thesis.

Visible scans were also done for samples that were not pH adjusted to 7.6. Though the visible spectra were expected to be pH dependent, similar results were obtained as before for the raw stillage and, the R1 effluents at 0.85 and 2.0 d HRTs (Fig. 4.29).

There was no evidence to show that the superior colour removal ability of R1 carbon as compared to the virgin carbon was due to the creation of new external carbon surfaces for adsorption. The continued colour removal was in excess of that accounted for by purely physical and chemical adsorption alone. On the other hand, results from the UV-Visible absorption spectra had demonstrated that the reduction of UV-Visible chromophoric species was a result of microbial degradation. It was thus proven beyond doubt that removal of colour from the stillage was due to microbial degradation of the chromophoric material resulting in continuous bioregeneration of the GAC *in situ*.

4.3.5.5 Reactor wind down stage. Final note

Instead of stopping the reactor completely after the fourth phase of operation, the reactor was allowed to operate at a 20 d HRT. Results were only taken after 20 days of operation (i.e. 1 HRT).

FIGURE 4.29: VISIBLE-SPECTRA WITH NO pH ADJUSTMENT

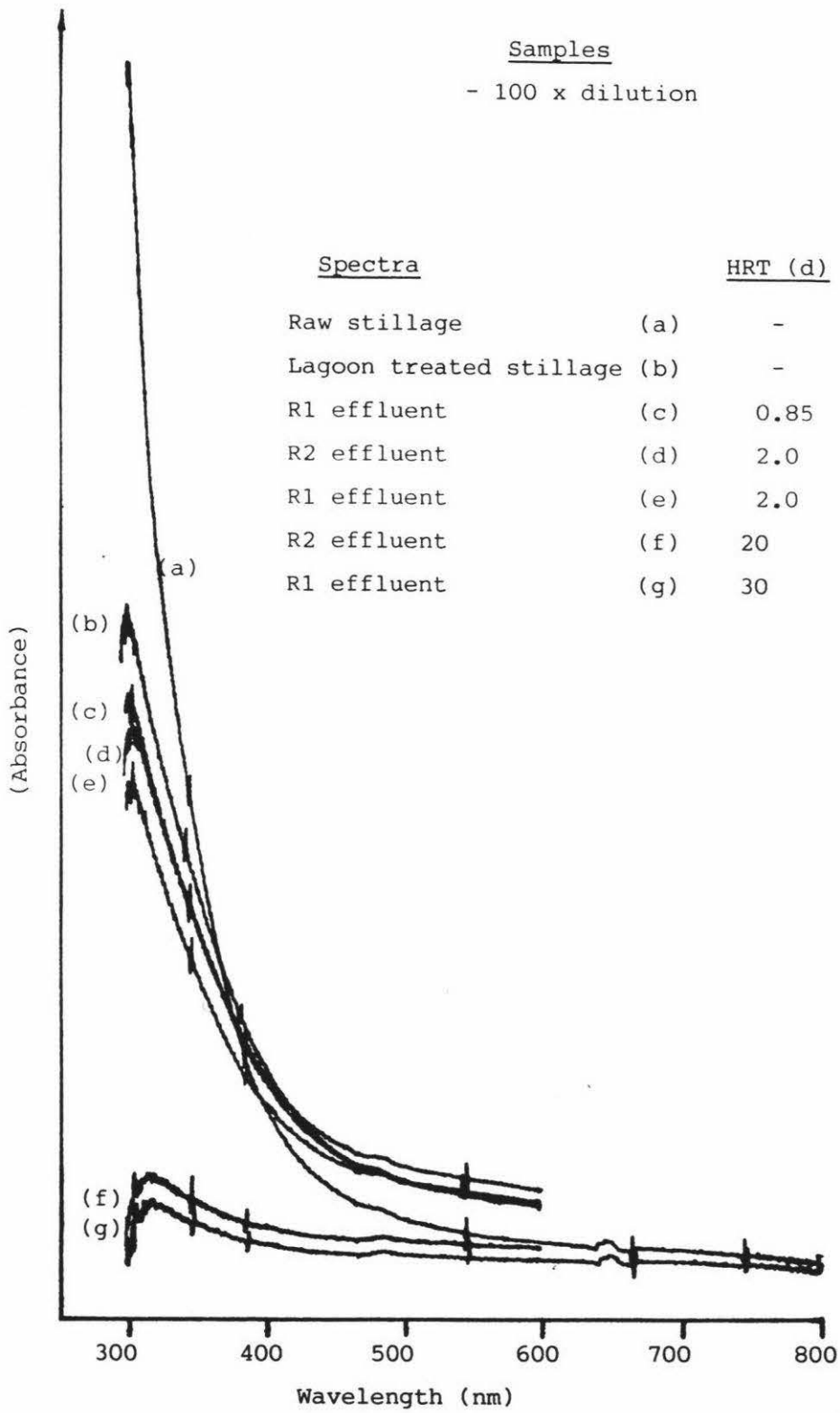


TABLE 4.8: ANAEROBIC TREATMENT OF WOOD-ETHANOL STILLAGE - A COMPARISON BETWEEN THIS STUDY AND PREVIOUS WORK REPORTED

Treatment System	Reference	Feed	Temperatures of Operation (°C)	HRT (d)	OLR achieved (kg tCOD.$m^{-3}.d^{-1}$)	pH	% tCOD removal	% sCOD removal	% $sBOD_5$ removal	Colour Removal	Acetic acid ($mg.l^{-1}$)	Propionic acid ($mg.l^{-1}$)	Gas Production ($l.d^{-1}$)	% methane	$Y_{CH_4}^{COD}$ (ml CH_4 at STP g^{-1})	% sludge yield based on sCOD removal	Biomass concentration (g $VSS.l^{-1}$)	Specific sludge activity (kg tCOD.kg $VSS^{-1}.d^{-1}$)	Specific biogas production ($m^3.m^{-3}.d^{-1}$)	Comments
Anaerobic Lagoon (90 l)	Archer et al., (1982)	Raw Stillage	22.5	84			87		96	No apparent colour removal			11.54	60.9	349	Negligible				Preliminary Results
CSTR (2 l)	Good et al., (1982)	1 : 1 diluted stillage	35	9.5	2.4	7.3	85.5			Not reported	532	1900		58.5	234					Cell washout at low HRT
Anaerobic Filter (1.8 l)	Good et al., (1982)	1 : 1 diluted stillage	35	2.1	10.7	7.5	86.6			Not reported	260	680		63.4	254					Superior to CSTR System
Anaerobic Filter (1.8 l)	Good et al., (1982)	1 : 1 diluted stillage	55	2.25	10.0	7.2	84.4			Not reported	215	666		62.5	238					Lower gas production than a similar mesophilic reactor
CSTR (8 l)	Callander et al., (1983) and Callander (1983(a))	Raw Stillage	37		3.7	7.04		90		Expected insignificant colour removal	30	38	6.3		273	12		0.60		For study of nutrients required
UASB (10 l)	**Callander et al., (1983)**	**Raw Stillage**	**37**		**13**			**86**	**98***	**Expected insignificant Colour removal**	**20**	**100**			**280**	**10-12**	**35**			**Highest COD loading reported for Wood-ethanol stillage**
GAC Packed Expanded-bed Reactor (5 l)	**This study**	**Raw Stillage**	**37**	**2.0**	**13.4**	**6.82**	**81.0**	**91.3**	**88.2**	**94.0**	**250**	**160**	**34.0**	**63.0**	**349**	**<2.8****	**3.38**	**3.09**	**6.8**	**High COD & colour removal rates**
				0.85	**29.0**	**7.00**	**74.5**	**83.5**	**79.2**	**75.0**	**122**	**490**	**74.2**	**61.5**	**371**	*******	**3.57**	**6.30**	**14.8**	**A superior system**

* $sBOD_5$ removed.
** The sludge yield is quoted as 'less than' because sCOD removed may partly be adsorbed onto the GAC.
*** Methane yield is above the predicted theoretical maximum gas yield (350 ml at STP.g^{-1} COD removed). This implied that sludge yield is minimal.

The most significant observation was an increase in reactor solids concentration (TS = 14.7; VS = 6.75; TSS = 8.46; VSS = 4.33, all in $g.l^{-1}$). This was due to dislocation of biomass from the GAC as observed in the fourth phase of operation. Biomass dislocation was more prominent at this stage is possibly due to the detachment of cells from the GAC support which was not washed out of the reactor at high HRT. They appeared as a blanket of greyish flocs in the reactor.

The high solids concentration resulted in an effluent tCOD of 7500 $mg.l^{-1}$ (about 70% removal). However, the effluent had a relatively low sCOD of 2245 $mg.l^{-1}$ (about 91% removal). The colour removal rate had not decreased much either (at 91.5%). It appeared that the last few percentages of chromophoric materials were particularly recalcitrant to microbial degradation.

4.3.5.6 Anaerobic treatment of wood-ethanol stillage - A comparison between this study and previous work reported

Similar work in this area has been performed by Archer et al., (1982); Good et al., (1982); and Callander et al., (1983). A comparison between this study and those reported in the literature for the treatment of wood-ethanol stillage is as presented in Table 4.8.

The GAC packed expanded-bed reactor used in this study is thus far superior to the other systems used in the anaerobic treatment of wood-ethanol stillage in terms of (Table 4.8): -

(a) OLR and COD Removals

The highest OLR reported to date for anaerobic digestion of wood-ethanol stillage is 13 kg $COD.m^{-3}.d^{-1}$ (non-maximal) with a sCOD removal of 86% (Callander et al., 1983). Higher OLRs were not possible using the present design due to the loss of biomass from the UASB reactor (Callander, 1983(a)). However, a higher OLR may be possible in the UASB's with improved gas/solids separator . For this study, due to time constraints, the highest non-maximal OLR achieved was 29.0 kg $tCOD.m^{-3}.d^{-1}$ with 83.5% sCOD removal. For comparison, the sCOD removal at OLR of 13.4

kg tCOD.m^{-3}.d^{-1} was 91.3% in this study (Table 4.8).

The expanded-bed reactor of this study is also superior to the anaerobic filter which achieved an OLR of 10.7 kg tCOD.m^{-3}.d^{-1} with 86.6% tCOD removal. Both these systems are fixed-film reactors.

(b) Colour Removal

A colour removal of 75% has been achieved with continuous bio-regeneration of the GAC even at a short HRT of 0.85 d and a colour loading rate of 4.7 kg chloroplatinate.m^{-3}.d^{-1} (Section 4.3.4.1) as compared to 'no apparent colour removal' using the anaerobic lagoon (84 d HRT). For this study, the anaerobic lagoon system used by Archer et al., (1982) was found to achieve about 75% colour removal from similar stillage but at long HRTs in excess of 30 days (Section 4.4.2(b)). There is no report on colour removal by Good et al., (1982) and Callander et al., (1983). However, it is clear that treatment using the UASB system (Callander et al., 1983) does not achieve significant colour removal (if any) since this study was a result of colour removal problem from FRI stillage before or after anaerobic treatment.

The success of this anaerobic expanded-bed system in the microbial degradation of the recalcitrant chromophoric species is believed to be due to enhancement of microbial activity by the GAC as discussed in Section 2.8.

A higher percentage colour removal is also possible when operated at a longer HRT (e.g. in excess of 90% at 2 d HRT).

(c) Methane and Sludge Yields

A very high CH_4 gas yield (99.7% at 2 d HRT) based on tCOD removed and sometimes slightly above the theoretical maximum had been achieved in this study (Table 4.8 and Fig. 4.30). Comparable results had only been achieved using the anaerobic lagoon system (Archer et al., 1982). The calculations for the gas yield were

FIGURE 4.30: DAILY GAS PRODUCTION THEORETICAL AND EXPERIMENTAL

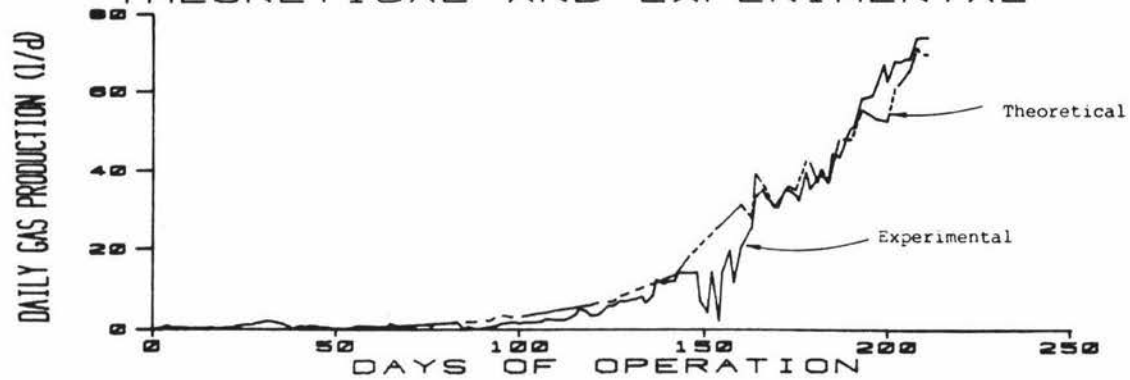
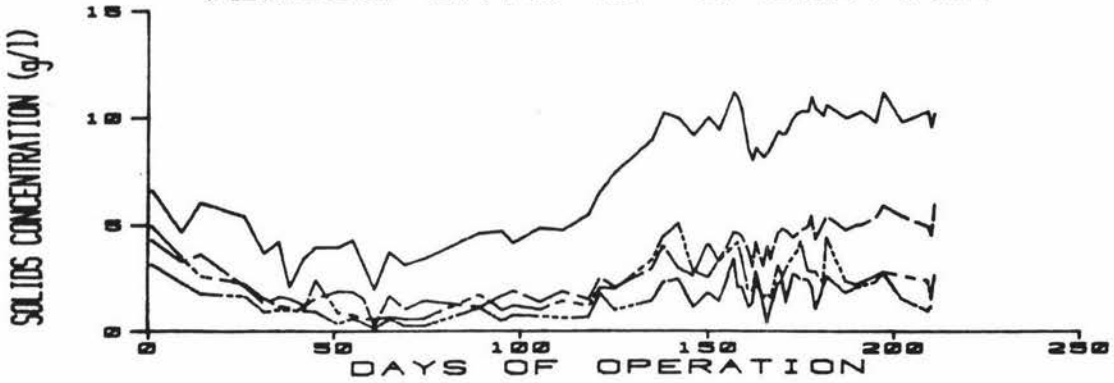


FIGURE 4.31: PLOTS OF EFFLUENT SOLIDS VERSUS DAYS OF OPERATION



based on COD balances and are presented in Appendix 5.

The high gas yield also meant a very low sludge yield and consequently, a low nutrient requirement. The low sludge yield was reflected in the effluent VSS concentration which remained low, generally less than 4 $g.l^{-1}$ and normally at 2 $g.l^{-1}$, even at the high organic loading rates experienced during Phase 3 operation (Fig. 4.31). A possible result of the slow sludge yield was a low biomass concentration in the reactor. This had not been a handicap for the expanded-bed system since very high sludge activities were obtained e.g. at 6.30 kg tCOD.kg $VSS^{-1}.d^{-1}$ for OLR of 29.0 kg $tCOD.m^{-3}.d^{-1}$ (Table 4.6).

For example, at a 2 d HRT, 33.5 ± 1.4 l of gas was produced each day, with a 62.3 ± 1.6% methane composition. The theoretical gas yield under these conditions was estimated to be 34.8 $l.d^{-1}$ (Appendix 5). Thus, 96.3% of the tCOD was removed as CH_4 gas while the rest was either used up by the cells or adsorbed onto the GAC. As for the anerobic treatment of similar stillage using the CSTR reactor (Table 4.8), the methane gas and sludge yields were 78 and 12% respectively based on sCOD removal of 90%. From Appendix 5, the methane gas and sludge yields for the expanded-bed reactor were 2.8% and 89.0% respectively based on an average sCOD removal of 91.8%. A very high specific gas production rate of 14.8 $m^3.m^{-3}.d^{-1}$ reactor liquid volume was also achieved.

(d) Reactor Stability

Being a fixed film process, the reactor can accomodate very high hydraulic loading rates without encountering problems of cell washout as in the case of the anaerobic lagoons, the CSTR's and the UASB reactor. It is also expected that the reactor has a greater toxicity sequesting potential than all the other systems considered due to the use of activated carbon as the biomass support.

4.3.5.7 Conclusions from Phase 4 operation

The same percentage COD removal was observed in this stage of operation as when the reactor was operated at the same OLR during phase 3.

The percentage colour removal rate improved significantly from 75% to 90.6% when the colour loading rate was decreased from 4.73 ± 0.01 to 2.0 ± 0.1 kg chloroplatinate.m^{-3}.d^{-1}. The fact that this can be achieved proved that stillage colour removal was a result of microbial degradation. This conclusion was further substantiated by;

(i) A comparison between colour breakthrough curves for colour removal by GAC with and without biological activity. The amount of colour removed was greatly in excess of that accounted for by physical and chemical adsorption alone.

(ii) A particle size analysis which showed no generation of new carbon surfaces for better physical and chemical adsorption than predicted. This conclusion was based on results from measurement of particle size and the electron scanning photomicrographs.

(iii) UV-visible spectrophotometric scans which confirmed the microbial degradation of UV and visible chromophores from HRT and EBV considerations (Section 4.3.5.4.2).

Continuous bioregeneration of the GAC in terms of colour removal has thus been confirmed.

The wind down stage of R1 operation at 20 d HRT resulted in little improvements in percentage colour removal. It is believed that approximately 9% w/v of the chromophoric materials are particularly recalcitrant to anaerobic microbial degradation. GC-MS analyses may be able to identify the chemical compounds which are particularly recalcitrant.

After operating for 227 days, it was demonstrated that the anaerobic GAC packed expanded-bed system is superior to the previous systems reported for the treatment of similar stillage in terms of: -

(i) OLR and COD removal

A non-maximal OLR of 29.0 kg tCOD.m^{-3}.d^{-1} has been achieved with sCOD and tCOD removals of 83.5 and 74.5% respectively.

(ii) Colour removal

A colour removal rate of 75% has been demonstrated when operated at the highest non-maximal OLR (0.85 d HRT) at a colour loading rate of 4.7 kg chloroplatinate.m^{-3}.d^{-1}. Higher colour removals may be obtained at longer HRTs (e.g. in excess of 90% removal at a 2 d HRT).

(iii) Methane and Sludge Yields

The methane gas yield is near to that predicted by theory (99.7% at 2 d HRT stage) with a very low sludge yield. Consequently, this system has a relatively low nutrient requirement for effective treatment.

(iv) Reactor Stability

The reactor can accommodate very high hydraulic loading rates without problems of cell washout. The use of activated carbon as support medium also provides a toxicity sequesting potential.

4.4 DECOLOURIZATION OF ANAEROBIC LAGOON TREATED STILLAGE - R2 OPERATION

4.4.1 Introduction

R2 was seeded on the 5th of July, 1983. The procedure for starting up this reactor was the same as for R1 (see Section 4.3.1). Although R2 has been inoculated approximately one month after R1 (seeded on 3/6/83), the loading rates to R2 were generally increased at the same rate as R1.

Initially some fluidization problems were encountered that had not been observed with R1. Also, there was little microbial activity throughout the 6½ months of operation (191 days). A summary of the raw data is as presented in Appendices 6.1 - 6.3. The operation of R2 will be discussed in the same manner as R1.

4.4.2 R2 Phase 1 Operation - The Acclimatization Phase

This period was defined as when R2 was operating at 20 d HRT and above (Fig. 4.32). Being anaerobically pretreated, the feed to R2 was of relatively low tCOD and sCOD (3250 and 2375 $mg.l^{-1}$ respectively - Appendix 6.1). The $tBOD_5$ was less than 1300 $mg.l^{-1}$ with a very low $sBOD_5$. The COD represented mainly recalcitrant materials which were not easily degraded by the bacteria during the long treatment time of up to 90 d in the anaerobic lagoon (Archer et al., 1982). Feeding was first attempted on the 10th of July at a 30 d nominal HRT. Half strength stillage was used with supplements of 240 $mg.l^{-1}$ N and 80 $mg.l^{-1}$ P. These N and P supplements were used throughout the entire R2 operation.

4.4.2.1 Reactor performance and stability

Little methanogenic activity was observed for the 73 days of Phase 1 operation. Apart from the initial seeding stage, no gas was produced as confirmed by undetected level of CH_4 in the gas collection system (Appendix 6.3; Fig. 4.33). There were also low levels of VFAs in the effluent (Fig. 4.34) reflecting very low microbial activity in

FIGURE 4.32:

THE FOUR PHASES OF R2 OPERATION

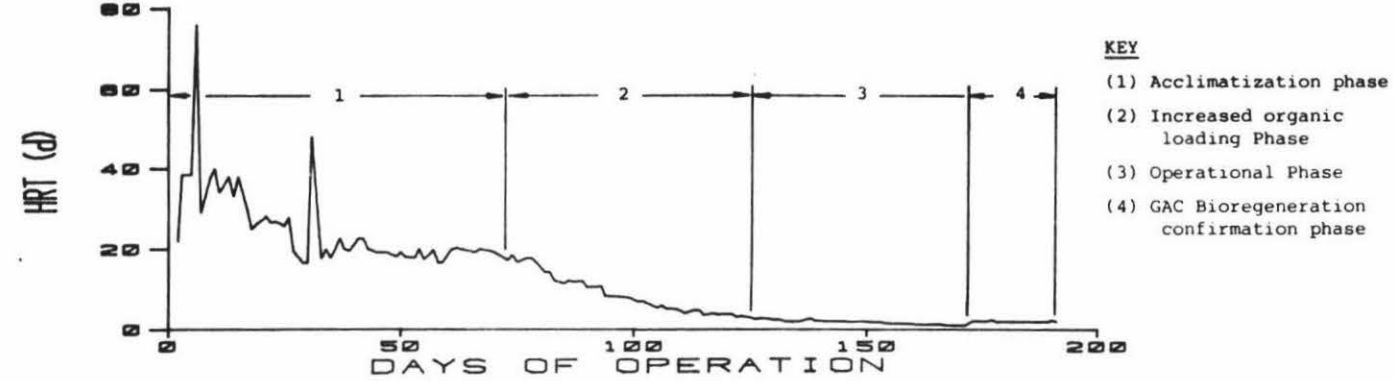


FIGURE 4.33:

GAS PRODUCTION RATE. GAS METHANE COMPOSITION AND OLR FOR R2 OPERATION

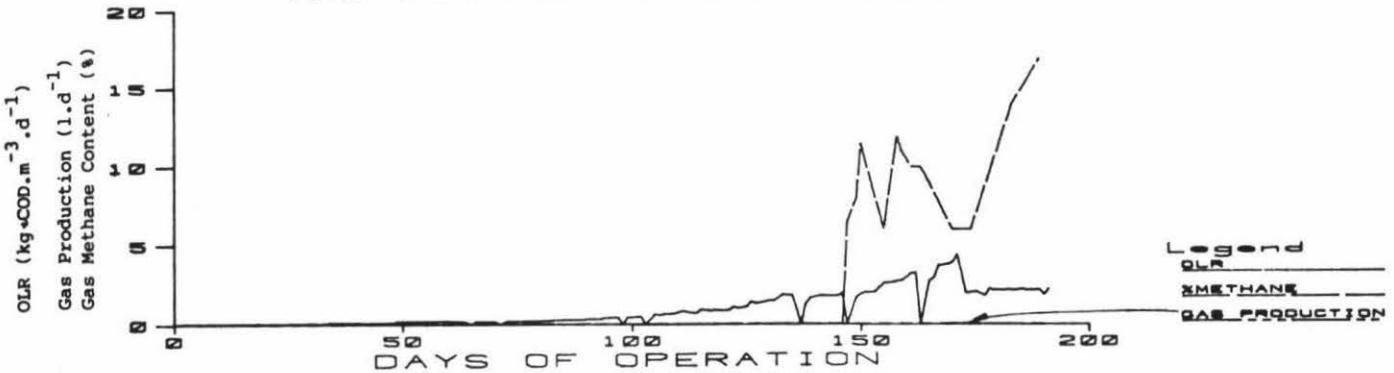


FIGURE 4.34:

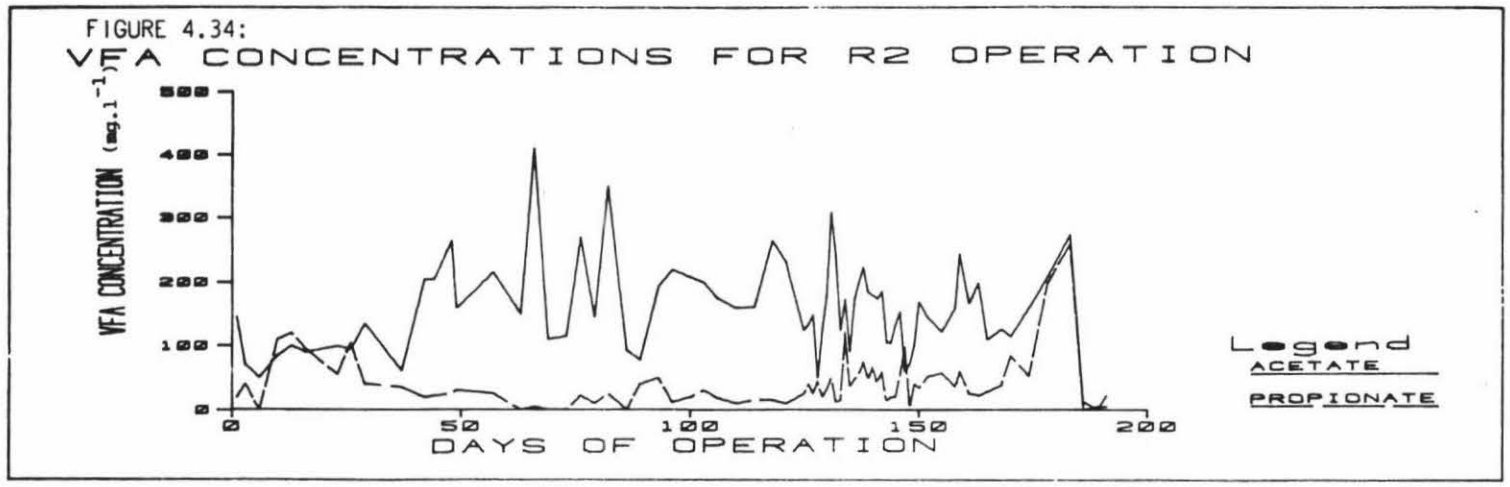
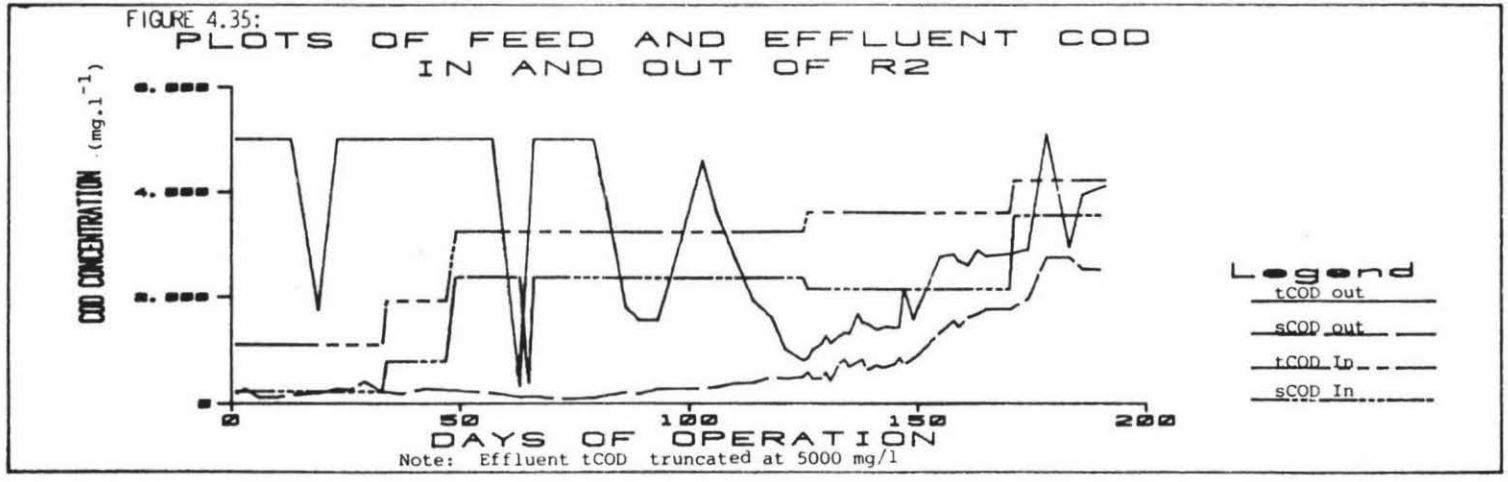


FIGURE 4.35:



Note: Effluent tCOD truncated at 5000 mg/l

the reactor. Nevertheless, very low loading rates of less than 0.2 kg tCOD.m^{-3}.d^{-1} were experienced at this stage (Appendix 6.3; Fig. 4.33).

The high and erratic tCOD and solids concentrations in the effluent (Fig. 4.35 and 4.36) were due to biomass washout from the seed inoculum and from carbon fines. The prolonged cell washout stage was due to fluidization problems in R2 which was occasionally operated as a packed bed.

The reactor pH increased gradually from slightly below 7.0 to slightly above 8.0 at the end of this phase of operation. Except on the first and second days of feeding, the pH level was at all times satisfactory (Fig. 4.37). Since the reactor pH was generally well above 7.0, no feed alkalinity addition was needed and the alkalinity level, generally in excess of 1500 mg.l^{-1}, was considered adequate (Fig. 4.37). This was found to be satisfactory for all phases of R2 operation. The high alkalinity level was due to alkalinity addition in the lagoon pretreatment stage (Archer et al., 1982).

At the end of this phase of operation, the bed was still difficult to fluidize, even at the maximum recycle flow rate due to uneven feed distribution at the bottom of the reactor. To remedy this, the feed distributor was removed from the reactor for cleaning. It was found that the whole tube was almost full of fine solids which appeared to be carbon fines from their grainy nature. Some scouring on the perspex in the region directly adjacent to a few feed outlet holes from the distributor was observed. This was a direct result of uneven feed distribution causing a high liquid flow velocity at certain regions around the feed distributor. The high attrition rate that resulted caused the loss of about one litre of GAC from the reactor. This was reflected in the high tCOD and solids concentrations in the effluent. Using virgin carbon fines, a COD equivalent of 4.5 mg.mg^{-1} carbon was obtained. Subsequently, R2 was well fluidized as indicated by a gradual drop in tCOD and solids concentrations as shown in Fig. 4.35 and 4.36.

FIGURE 4.36: PLOTS OF R2 EFFLUENT SOLIDS VERSUS DAYS OF OPERATION

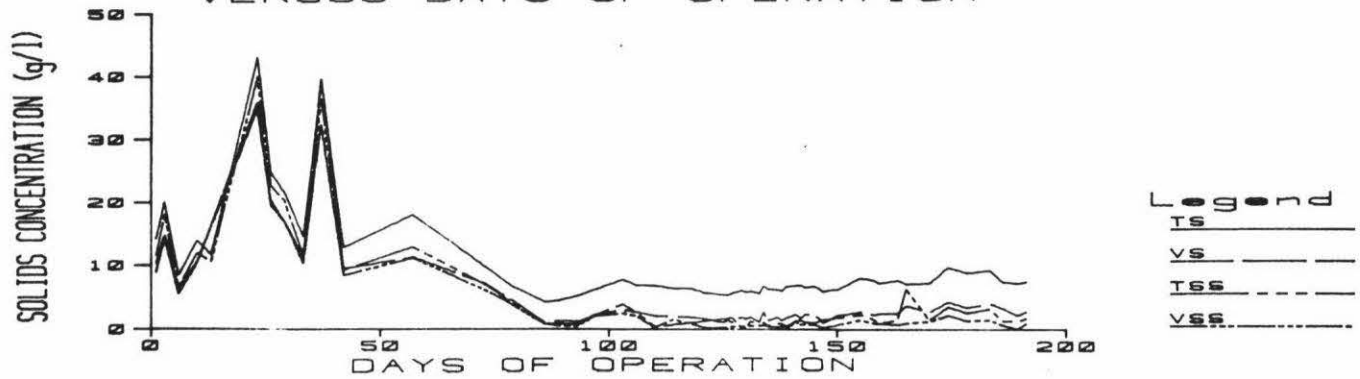
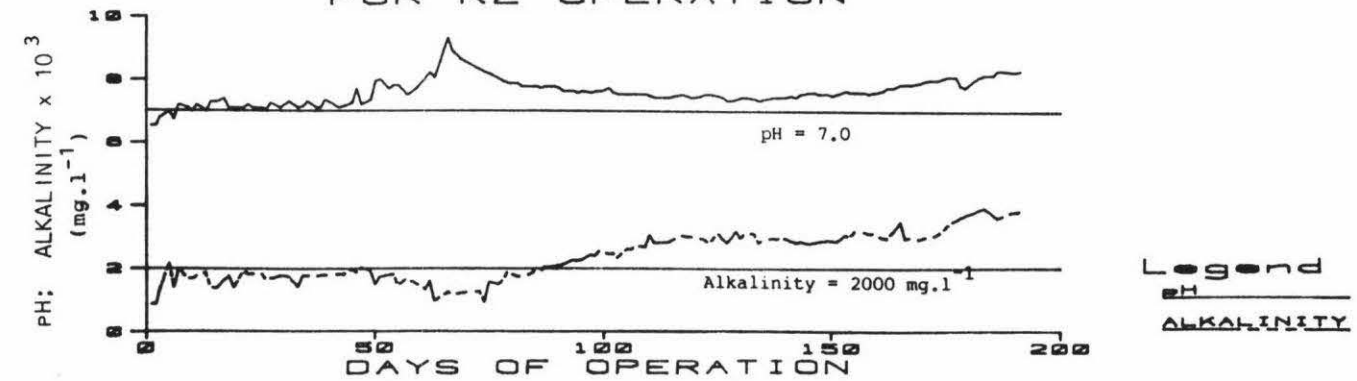


FIGURE 4.37: pH AND ALKALINITY PLOTS FOR R2 OPERATION



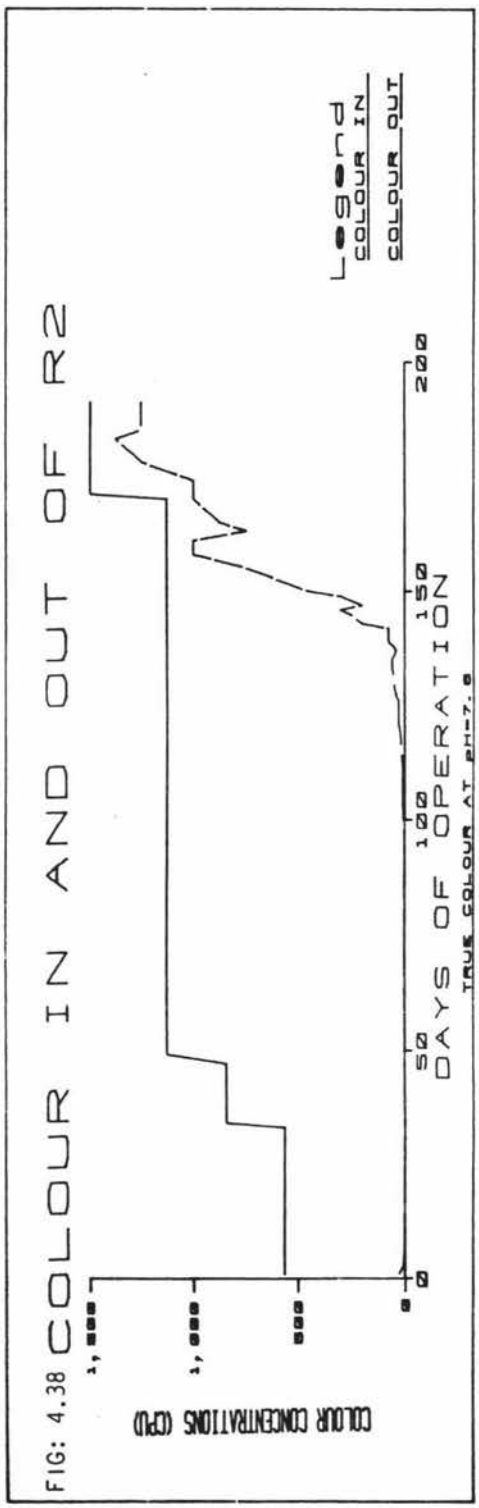


FIG: 4.38 COLOUR IN AND OUT OF R2
COLOUR CONCENTRATIONS (CPU)
1,000
1,000
800
0
50
100
150
200
DAYS OF OPERATION
TRUE COLOUR AT PH=7.0
Legend
COLOUR IN
COLOUR OUT

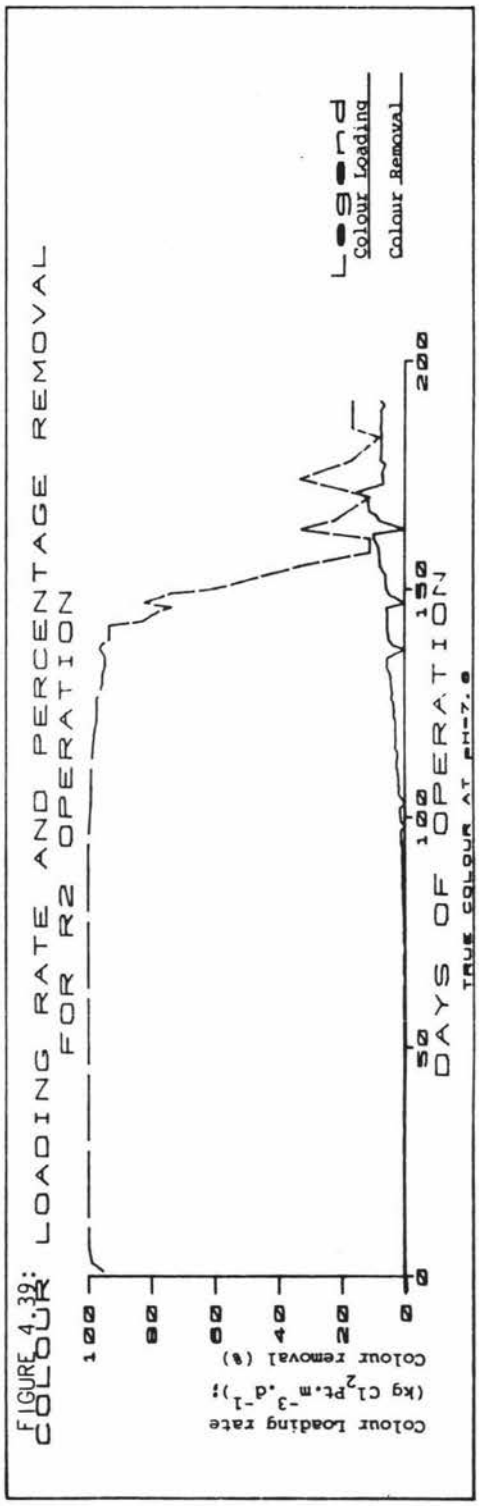


FIGURE 4.39: COLOUR LOADING RATE AND PERCENTAGE REMOVAL FOR R2 OPERATION
Colour loading rate (kg Cl2Pt.m-3.d-1); Colour removal (%)
100
80
60
40
20
0
50
100
150
200
DAYS OF OPERATION
TRUE COLOUR AT PH=7.0
Legend
Colour Loading
Colour Removal

4.4.2.2 Colour considerations

Contrary to what was originally assessed as 'no apparent colour removal' after anaerobic digestion in the lagoon treatment of raw stillage (Archer et al., 1982), some colour had actually been removed in that process. When the visual comparison colour measurement method was used, the lagoon effluent was typically 1125 CPU at pH 7.6. Assuming a lagoon stillage feed colour of 4000-5000 CPU, this represented approximately 75% colour removal. However, the effluent was still highly coloured with a dark brownish appearance.

The colour concentration in the treated stillage was quite hard to determine due to the brownish appearance after dilution (at pH 7.6) instead of the buff yellow of the raw stillage (Fig. 4.24). However, at pH 5, the diluted treated stillage was yellow in colour making a visual comparison more feasible. The fact that the lagoon treated stillage was different in colour to the raw stillage suggested that some of the chromophoric material had been degraded or converted to a different form.

Full colour removal was achieved by GAC adsorption in R2 during this acclimatization phase at colour loading rates of less than 0.07 kg chloroplatinate.$m^{-3}.d^{-1}$ (Fig. 4.38 and 4.39). At the end of this stage, about 3.3 EBVs of lagoon treated stillage were treated but the effluent was still colourless and was crystal clear when filtered. Thus, R2 carbon performed better than physical and chemical adsorption predicted (i.e. colour breakthrough within one EBV from Fig. 4.8). However, this was believed to be due to increased carbon adsorption capacity from the generation of new carbon surfaces by attrition that resulted in the loss of 1 l of carbon.

4.4.2.3 Conclusions from R2 Phase 1 operation

Very little methanogenic activity was observed throughout this stage of operation. This was expected since the feed had been pretreated anaerobically and was very low in tCOD and sCOD which consisted mainly of biologically recalcitrant materials.

Full colour removal and very good sCOD removal (96%) were achieved. This was believed to be due to adsorption onto the GAC rather than by microbial degradation.

4.4.3 R2 Phase 2 Operation - The Increased Organic Loading Phase

This stage was defined as when the reactor was operating at HRTs between 20 and 2.5 d or organic loading rates from 0.19 to 1.5 kg tCOD.m^{-3}.d^{-1} (Fig. 4.32).

Like the first stage of operation, negligible methanogenic activity was observed as signified by an undetectable gas production rate throughout this stage of operation. No faults were detected in the gas collection system (e.g. leaks and gas meter not working). The zero gas production was also confirmed by measuring the methane composition in the gas.

Once the fluidization problem was overcome, the effluent tCOD dropped gradually to a more reasonable value but was still high as carbon fines were still being washed out of the reactor (Appendix 6.3; Fig. 4.35). The effluent solids concentrations also returned to normal with TS at less than 10 g.l^{-1} throughout this Phase (Appendix 6.3; Fig. 4.36). The low effluent VSS suggested that very little cell biomass had been produced from the COD removed. The sCOD removal gradually deteriorated from 96.0 to 79.9% on days 76 and 131 respectively as the GAC became more exhausted (Fig. 4.40). The pH and VFA concentrations (C_3 less than 50 mg.l^{-1}) were stable and normal for a healthy reactor (Fig. 4.37 and 4.34). However, the alkalinity rose gradually from approximately 1,500 to 3,000 mg.l^{-1} (Fig. 4.37). This could be due to dissolving carbon dioxide since fermentative non-methanogenic processes were implicated in R2 (see Section 4.4.4).

First colour breakthrough was observed after 96 days of operation (at 5 EBVs). However, the effluent colour remained low at 50 CPU (96% removal) or less at this stage (Fig. 4.38 and 4.39). R2 had performed better than physical and chemical adsorption would predict. However, no firm conclusions could be drawn at this stage regarding GAC bioregeneration in terms of colour and sCOD removal.

4.4.3.1 Conclusions from R2 Phase 2 Operation

An organic loading rate of 1.47 kg tCOD.m^{-3}.d^{-1} was achieved in this phase of operation with tCOD and sCOD removals of 68.3 and 80.0% respectively. Negligible methanogenic activity was observed and the percentage sCOD removal decreased as the GAC gradually became exhausted.

First colour breakthrough was observed after 96 days of operation, equivalent to 5 EBVs of lagoon treated stillage. The effluent colour remained low at 50 CPU or at 95.6% colour removal. It appeared that R2 carbon had performed better in terms of colour removal capacity than predicted for purely physical and chemical adsorption. However, no firm conclusions regarding GAC bioregeneration (for colour and sCOD removal) can be made at this stage.

4.4.4 R2 Phase 3 Operation - The Operational Phase

This was a period of low HRT (2.5 d HRT and below) and reasonably high OLRs (above 1.5 kg tCOD.m^{-3}.d^{-1}) to R2.

Since negligible gas production had been observed to date, R2 was reseeded on day 136 with 150 ml sludge from R1 (VSS of 8.18 g.l^{-1}) to see if R2 was capable of demonstrating some methanogenic activity. Definite signs of gas production were only observed ten days later (Appendix 6.3) as shown by gas being slowly released into the gas bubbler. The percentage methane was then measured at 6.5% (Fig. 4.33). It was possible that some, but very little, methane gas had been produced before them. Due to the low gas production rate, it would take quite some time to displace air from the gas collection system before its detection. Only very slight gas production was recorded at less than 0.04 l.d^{-1} even at an OLR of about 4 kg tCOD.m^{-3}.d^{-1} (Fig. 4.33). However, low tCOD and sCOD removals were achieved at 21.5 and 17.8% respectively on day 170 (Fig. 4.40). Table 4.9 presents a summary of R2 operation at the 2.0, 1.15 and 0.95 d nominal HRTs for days 148, 161 and 170 respectively.

FIGURE 4.40:

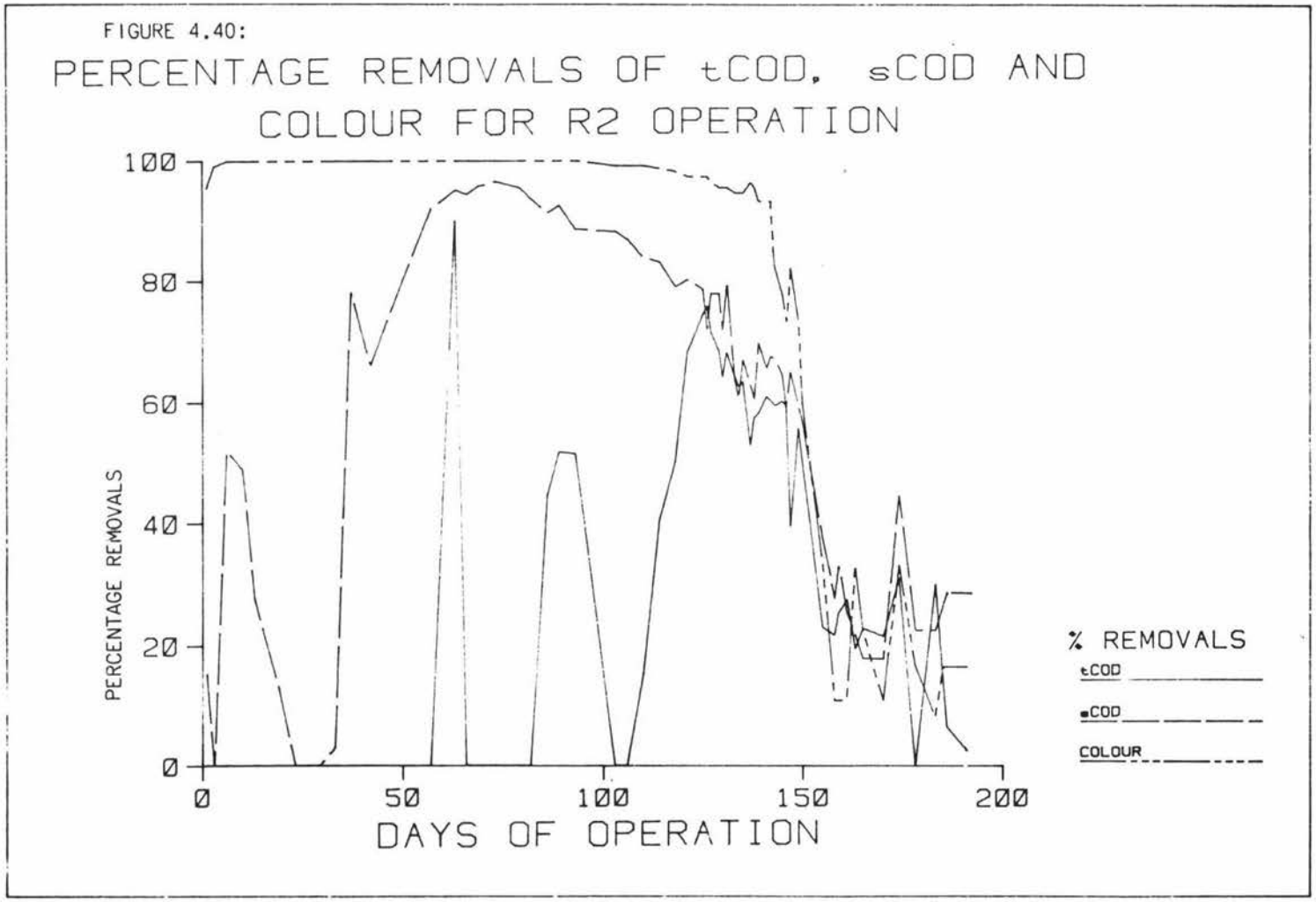

PERCENTAGE REMOVALS OF tCOD, sCOD AND COLOUR FOR R2 OPERATION
100
80
60
40
20
0
PERCENTAGE REMOVALS
0
50
100
150
200
DAYS OF OPERATION
% REMOVALS
tCOD
sCOD
COLOUR

TABLE 4.9: SUMMARY OF RESULTS FOR R2 OPERATING AT THE 2.0, 1.15 AND 0.95 NOMINAL HRTs

R2 Operating Parameters and Performance Data			
Feed	Anaerobic lagoon treated stillage		
Temperature of operation (°C)	37.0	37.0	37.0
Hydraulic retention time (d)	1.95	1.14	0.93
Organic loading rate (kg tCOD.m^{-3}.d^{-1})	1.84	3.16	3.88
pH	7.55	7.60	7.90
Alkalinity (mg.l^{-1} as $CaCo_3$)	2851	2951	2974
tCOD removal (%)	56.3	27.6	21.5
sCOD removal (%)	59.7	25.3	17.8
$tBOD_5$ removal (%)	0	50	-
Effluent tCOD (mg.l^{-1})	1575	2605	2825
Effluent sCOD (mg.l^{-1})	820	1613	1775
Effluent $tBOD_5$ (mg.l^{-1})	1308	647	-
Total solids (g.l^{-1})	6.18	-	7.30
Volatile solids (g.l^{-1})	1.69	-	2.47
Total suspended solids (g.l^{-1})	-	-	1.46
Volatile suspended solids (g.l^{-1})	0.05	-	1.28
Colour loading rate (kg Cl_2Pt.m^{-3}.d^{-1})	0.58	0.99	1.22
Colour removal (%)	77.8	11.1	11.1
Effluent colour (CPU)	250	1000	1000
Gas production (l.d^{-1})	0.02	0.03	0.02
Methane in gas (%)	9.0	10.0	6.0
Theoretical gas production (l.d^{-1})	22.7	17.2	27.5
VFA levels (mg.l^{-1}): Acetate	72	167	115
Propionate	4	25	25
Butyrate	0	0	0
Valerate	0	0	0
Nutrient addition (mg.l^{-1}): Nitrogen	240	240	240
Phosphorus	80	80	80
Feed NH_3-N (mg.l^{-1})	359	-	-
Feed RDP (mg.l^{-1})	-	5.94	-
Effluent NH_3-N (mg.l^{-1})	363	-	290
Effluent RDP (mg.l^{-1})	-	4.05	-
Soluble sulfide level (mg.l^{-1})	0	-	-
Biomass concentration (g.l^{-1})	-	-	2.38
Specific sludge activity (kg tCOD.kg VSS^{-1}.d^{-1})	-	-	0.35

An unreasonably high estimated theoretical biogas production rate was obtained (Table 4.9). This was because of the low percentage methane values used in the calculation. The theoretical gas production rate would be equal to 3.4, 2.9 and 1.0 $l.d^{-1}$ (at HRTs of 1.95, 1.14 and 0.93 respectively) for a methane composition of 60%. It thus appeared that the COD removed was not accounted for after treatment. It was suspected that fermentative processes other than methanogenesis might have occured in R2 which resulted in organic conversion to reduced fermentation end products, CO_2, water and cells. A slight negative gas production rate (e.g. -0.1 $l.d^{-1}$) was frequently observed but this had been reported as zero in Appendix 6.3. This could have been due to dissolved CO_2 in the reactor liquor creating a vacuum.

A conservative calculation based on CO_2 and water system using Henry's Law demonstrated that more than 2.8 l of CO_2 (or 5.0 g) could be dissolved in 5 l of water under R2 conditions (Appendix 7). The COD equivalence of the dissolved CO_2 was found to be 711 $mg.l^{-1}$ as compared to 995 $mg.l^{-1}$ COD removed in practice at the 1.14 d HRT stage (Appendix 7). This does not take into account of the small amount of COD conversion to methane and bacterial cells. Moreover, the dissolved CO_2 were expected to form bicarbonate (chiefly with calcium ions) in R2 thus raising the reactor alkalinity. The gradual increase in alkalinity can be seen in Fig. 4.37 thus supporting the argument that fermentative processes other than methanogenesis occured in R2.

The percentage colour removal deteriorated significantly from 77.8% at 1.95 d HRT to 11.1% at 1.14 and 0.93 d HRTs (Table 4.8). By the end of this phase of operation, approximately 39 EBVs of lagoon treated stillage had been treated, i.e. about twice the amount required to saturate the GAC without biological activity and some colour removal was still achieved (Fig. 4.8). Thus some bioregeneration of the GAC in R2 might have occured. However, the percentage colour removal dropped sharply from day 140 onwards when the colour loading rate was rapidly increased (Fig. 4.39). The fact that some chromophoric species have been removed biologically while there was evidence of only negligible methanogenic activity in R2 implied that the chromophores constitute only a small fraction of the sCOD and that non-methanogenic bacteria were those involved.

4.4.4.1 Conclusions from phase 3 R2 operation

Methanogenic activity has been demonstrated in R2 but at a very low level due to the recalcitrant nature of the anaerobic lagoon pretreated stillage feed.

Colour and sCOD removals dropped sharply to about 20% during this phase of operation. There was evidence to suggest that fermentative processes other than methanogenesis might have been involved in R2 operation to account for the COD removed as dissolved CO_2. Some bioregeneration of the GAC in terms of colour removal was believed to have occured. This was subjected to confirmation in the last phase of operation.

4.4.5 R2 Phase 4 Operation - The GAC Bioregeneration Confirmation Phase

During this period of operation, the R2 loading rate was decreased to the 2 d HRT at approximately 2.2 kg tCOD.m^{-3}.d^{-1} and a colour loading rate of approximately 0.75 kg chloroplatinate.m^{-3} .d^{-1} . This was operated for a period of 20 days.

The percentage tCOD removal deteriorated further and approached zero even with a decrease in OLR while the sCOD removal improved slightly to 30% (Fig. 4.40). The 10% improvement in sCOD removal suggests some GAC bioregeneration in term of sCOD removal. Thus some of the sCOD (approximately 30%) was easier to remove than the remainder. However, this is of little practical significance considering the concentrations of sCOD involved (less than 3600 mg.l^{-1} - Appendix 6.1).

No significant improvement in colour removal was observed when the HRT was increased to 2 d HRT (Fig. 4.39). The percentage colour removal stabilized at approximately 20% suggesting a GAC bioregeneration of 20% for R2 carbon (Fig. 4.24). Thus approximately 20% of the chromophoric material in the lagoon pretreated stillage was more readily degradable under the conditions prevailing in R2.

Carbon samples from R2 were also taken for electron microscopic scans (Fig. 4.41 and 4.42). As with R1, the R2 carbon particles were also rounded with the sharp edges being removed after 160 d in the expanded-bed (Fig. 4.41). However, the carbon surface was not as smooth as the R1 carbon (Fig. 4.21) and was covered by a thick layer of slime (Fig. 4.42). Rod-like bacteria were also evident but at much lower numbers than in R1 carbon. Using the RNH_2 biomass measurement method, approximately the same concentrations of attached biomass in R1 and R2 carbon were obtained (Table 4.6 and 4.9). It is believed that the organic matrix in R2 carbon may contain organic nitrogen and hence contributed to the relatively high estimated R2 carbon biomass.

4.4.5.1 Extent of GAC bioregeneration in R2

As with R1, the breakthrough curves for sCOD and colour removal from the lagoon pretreated stillage were determined (Fig. 4.43).

As stated ealier, the recalcitrant nature of the feed resulted in low methanogenic activities in R2 and hence ultimately very low sCOD removals. The breakthrough curves for sCOD removal with and without biological activity were not significantly different (Fig. 4.43). It appears that the sCOD removals were particularly low at HRT's less than 1.4 d where the percentage removals were within ± 15% of the sCOD breakthrough curve without biological activity (Fig. 4.43). Otherwise, the sCOD removals were generally at approximately 30% better than predicted by physical and chemical adsorption alone. Thus bioregeneration of the GAC is about 30% for sCOD removal and a treatment time of more than 1.14 d was required for its degradation.

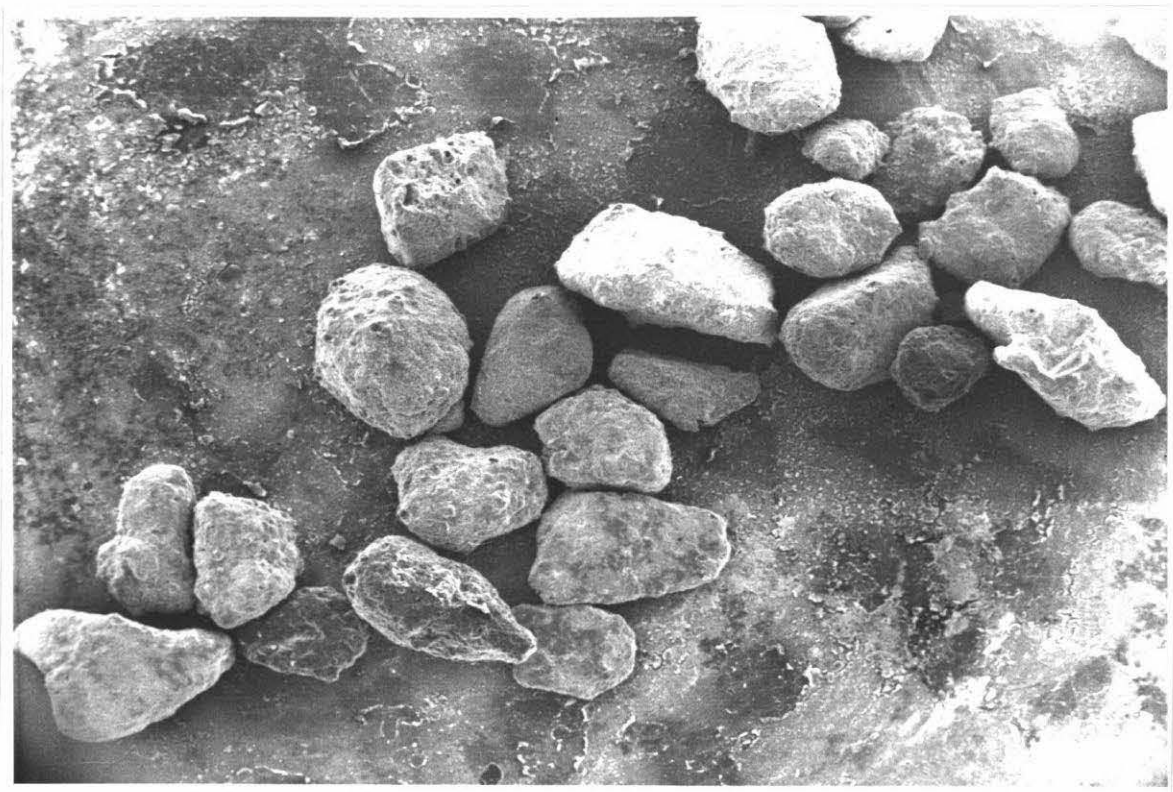
For colour removal, R2 carbon also performed better than the case without biological activity (Fig. 4.43). The R2 colour removals were generally approximately 30-70% higher before the 1.4 d HRT period and at approximately 20% better after this HRT period. This was despite the loss of approximately 1 l of carbon from R2 during the first 3 EBVs of operation. The lower rate of colour breakthrough also supported the concept of GAC bioregeneration (Fig. 4.43).

FIGURE 4.41: SEM of R2 Carbon Particles after 160 days of Operation. x 20.

The R2 carbon also appear to have a smooth surface and rounded in shape as for R1 carbon.

FIGURE 4.42: SEM of R2 carbon after 160 days of Operation. x 1250

The R2 carbon was covered by a thick layer of slime. Only a sparce bacterial population is evident in the figure and they appear to be embedded in a slime matrix.


1 mm

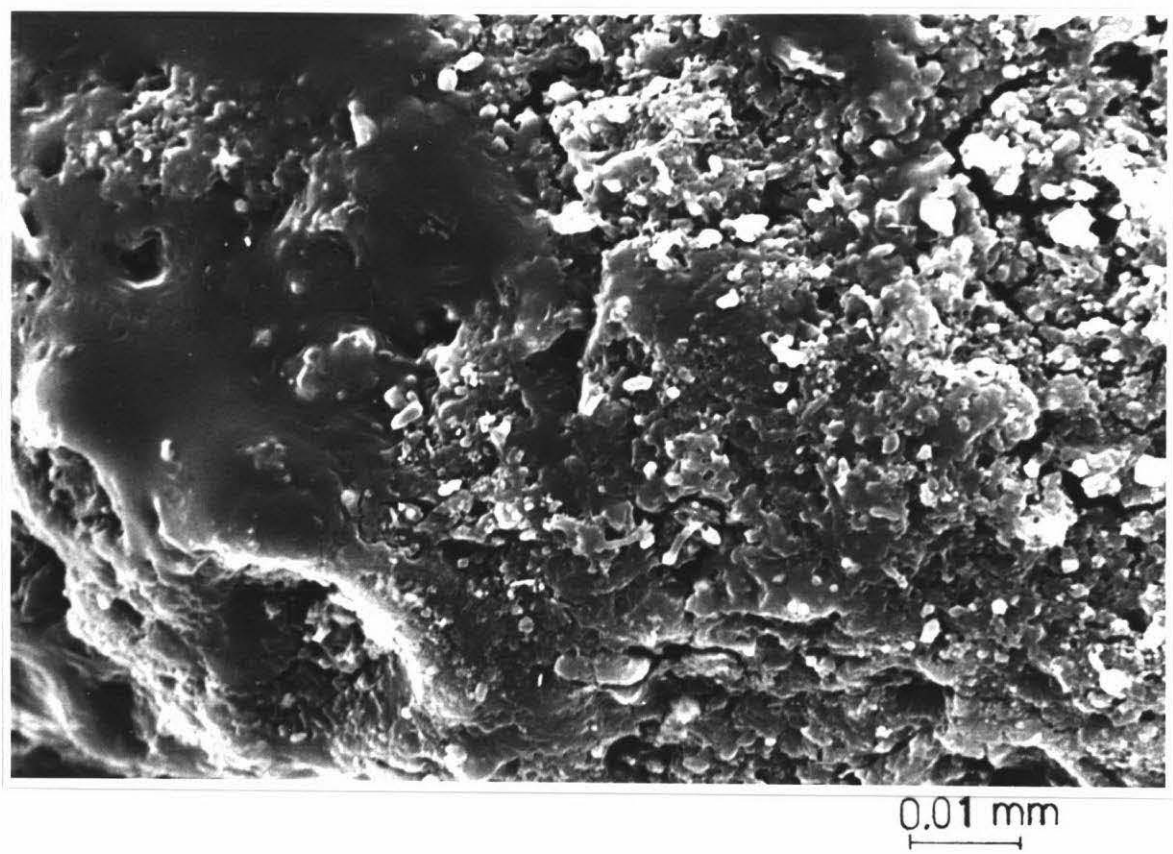
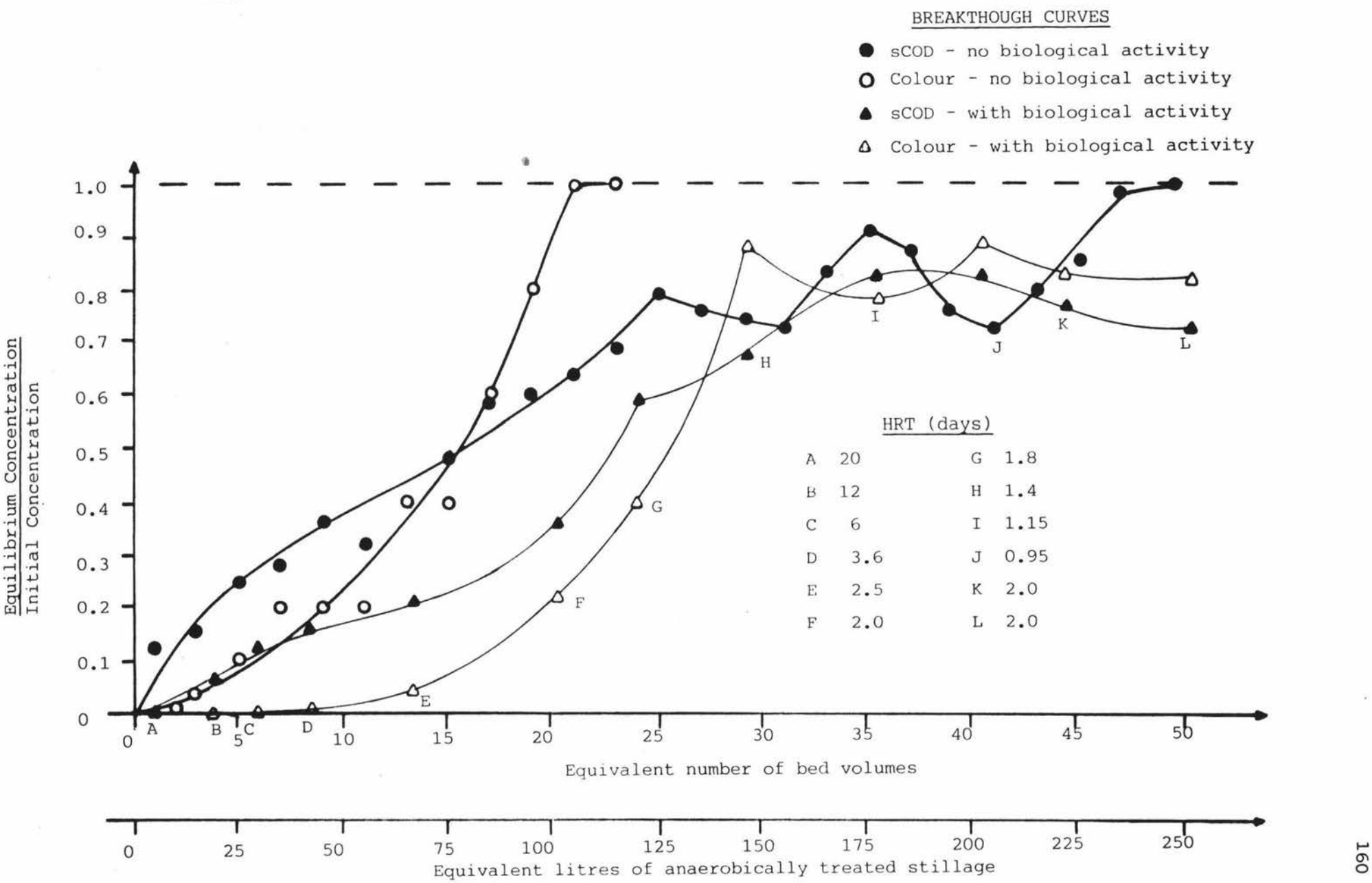

0.01 mm

FIGURE 4.43: BREAKTHROUGH CURVES FOR COLOUR AND sCOD REMOVAL FROM LAGOON TREATED STILLAGE USING GAC: RESULTS FOR ADSORPTION WITH AND WITHOUT BIOLOGICAL ACTIVITY



The GAC bioregneration in R2 was confirmed using spectrophotometric scans. The UV-visible spectra (Fig. 4.44 and 4.45) show that the R2 effluents had a lower absorbance than the lagoon treated stillage feed. The extent of UV-Visible chromophoric species that had been degraded varies according to HRT rather than the number of EBVs of feed treated (Fig. 4.44 and 4.45) which implicated microbial degradation of the UV-visible chromophoric species.

The UV-ionization spectra (Fig. 4.27) were similar to the spectra for R1 effluent at 2.0 d HRT (Fig. 4.27, Section 4.3.5.4.2). The R2 effluent at 2.0 d HRT generally had a slightly lower UV-ionization spectra than the R2 feed. This correlated well with the observation that GAC bioregeneration in R2 was only minimal.

4.4.5.2 Comparison between UV-Visible Spectra for R1 and R2 operations

The UV-spectra for R1 and R2 effluents and, the lagoon treated stillage feed were not significantly different but all had a much lower absorbance than the raw stillage (Fig. 4.46). Thus, it appeared that anaerobic digestion of raw stillage resulted in the degradation of similar UV-absorbing species. However, the rates of degradation were dissimilar with R1 being superior since it resulted in the same removal of UV-absorbing species as anaerobic lagoon treated stillage but working at a very mcuh shorter HRT (2.0 d compared to 90 d). As for R2 effluent the amount of UV-absorbing species removed was very much less than R1 treating raw stillage despite the fact that R2 effluent had gone through a two reactor treatment by the anaerobic lagoon and the expanded bed reactor.

The same conclusions were also apparent from the comparison of R1 and R2 visible spectra (Fig. 4.47). It thus appeared that there was a certain amount of UV and visible chromophoric species that were particularly recalcitrant to microbial degradation (as concluded earlier in the discussions of R1 operation). However, the fact that R1 performed at least as well than the other systems considered here could be due to the long sludge age in the reactor and the enhancement of microbial degradation by GAC carrier as was originally conceived and proposed for this study.

FIGURE 4.45: VISIBLE-SPECTRA FOR LAGOON TREATED STILLAGE (LTS) AND R2 EFFLUENTS

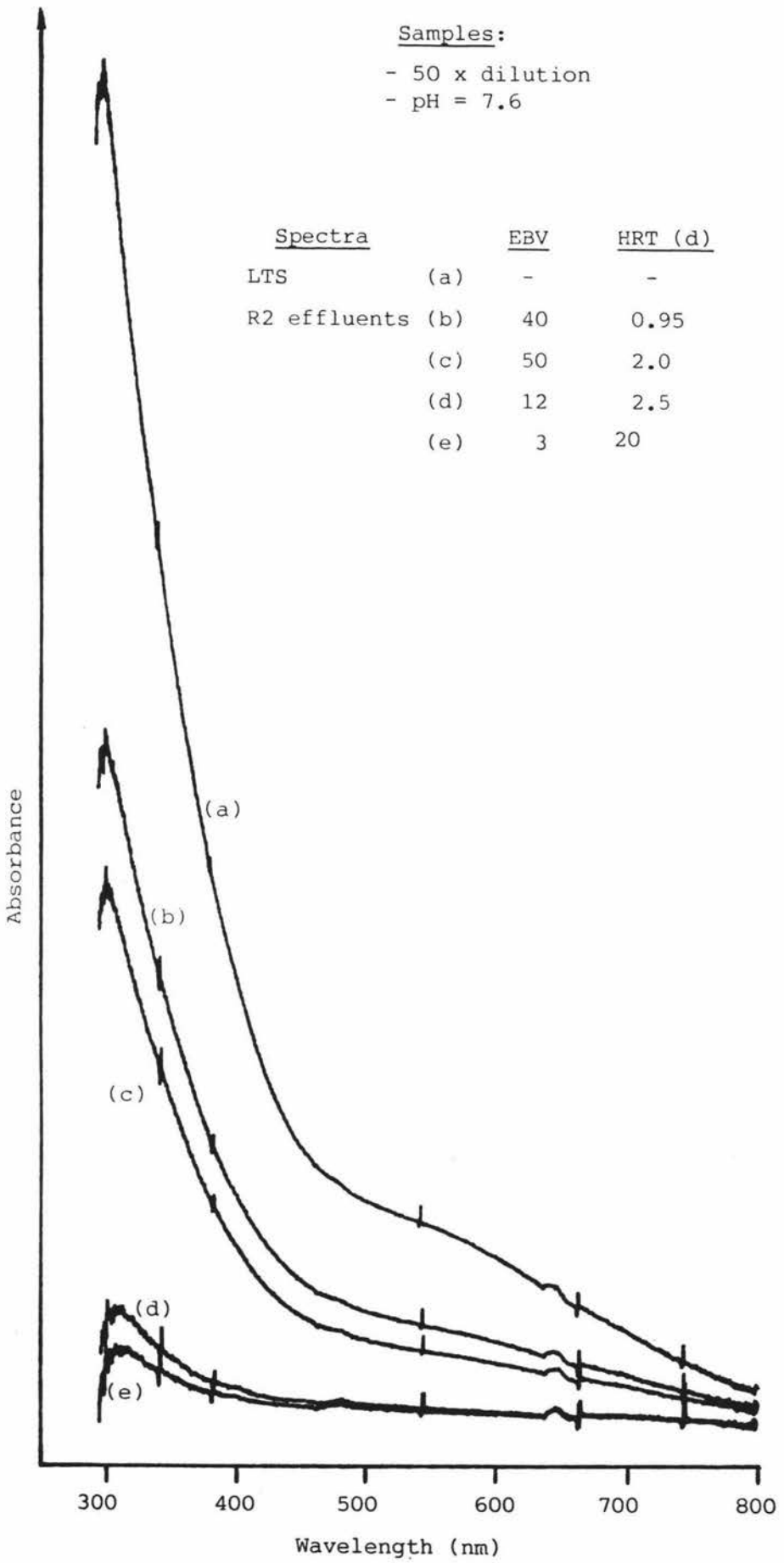


FIGURE 4.44: UV-SPECTRA FOR LAGOON TREATED STILLAGE (LTS) AND R2 EFFLUENTS

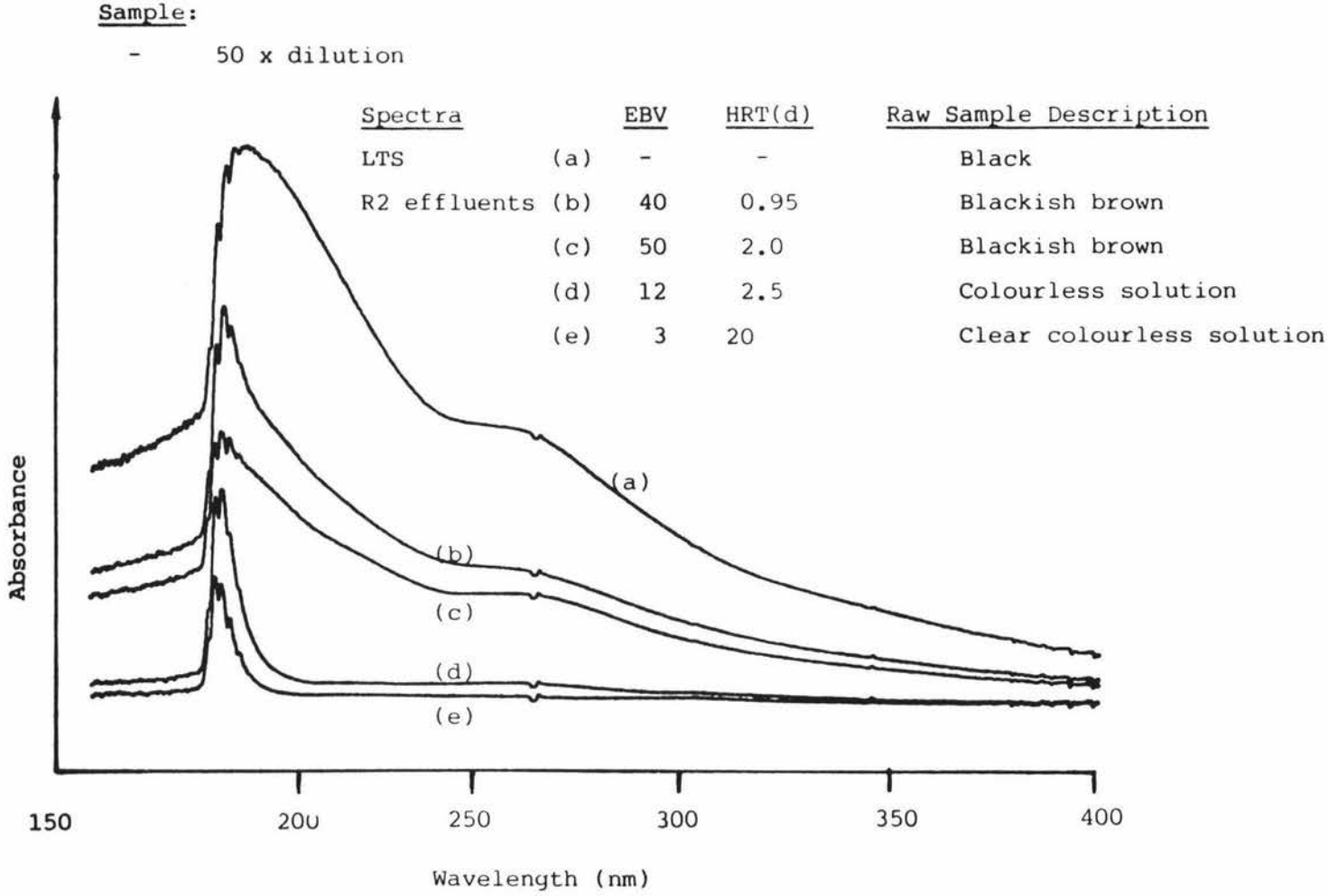


FIGURE 4.46: UV-SPECTRA FOR R1 AND R2 OPERATION - A COMPARISON

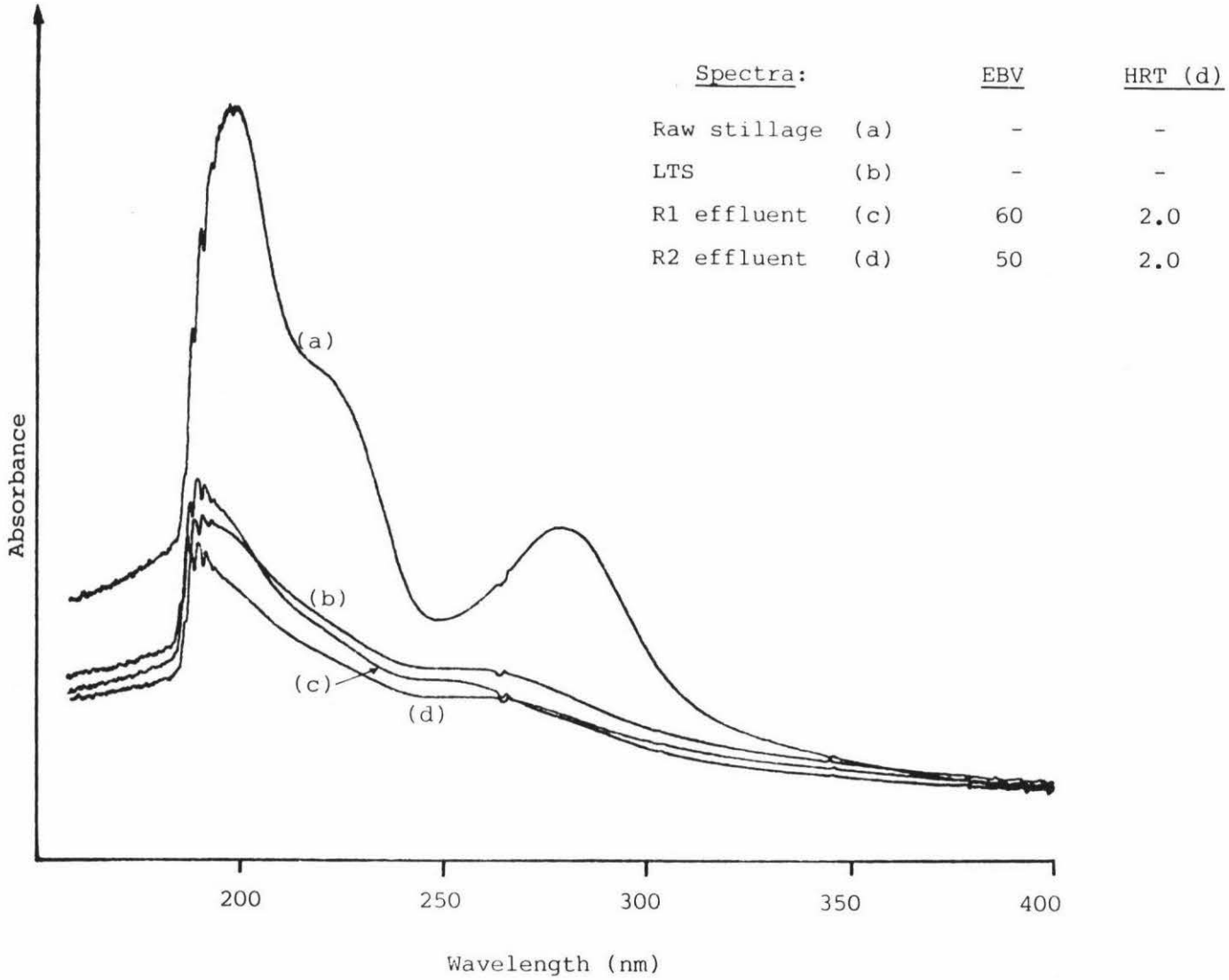
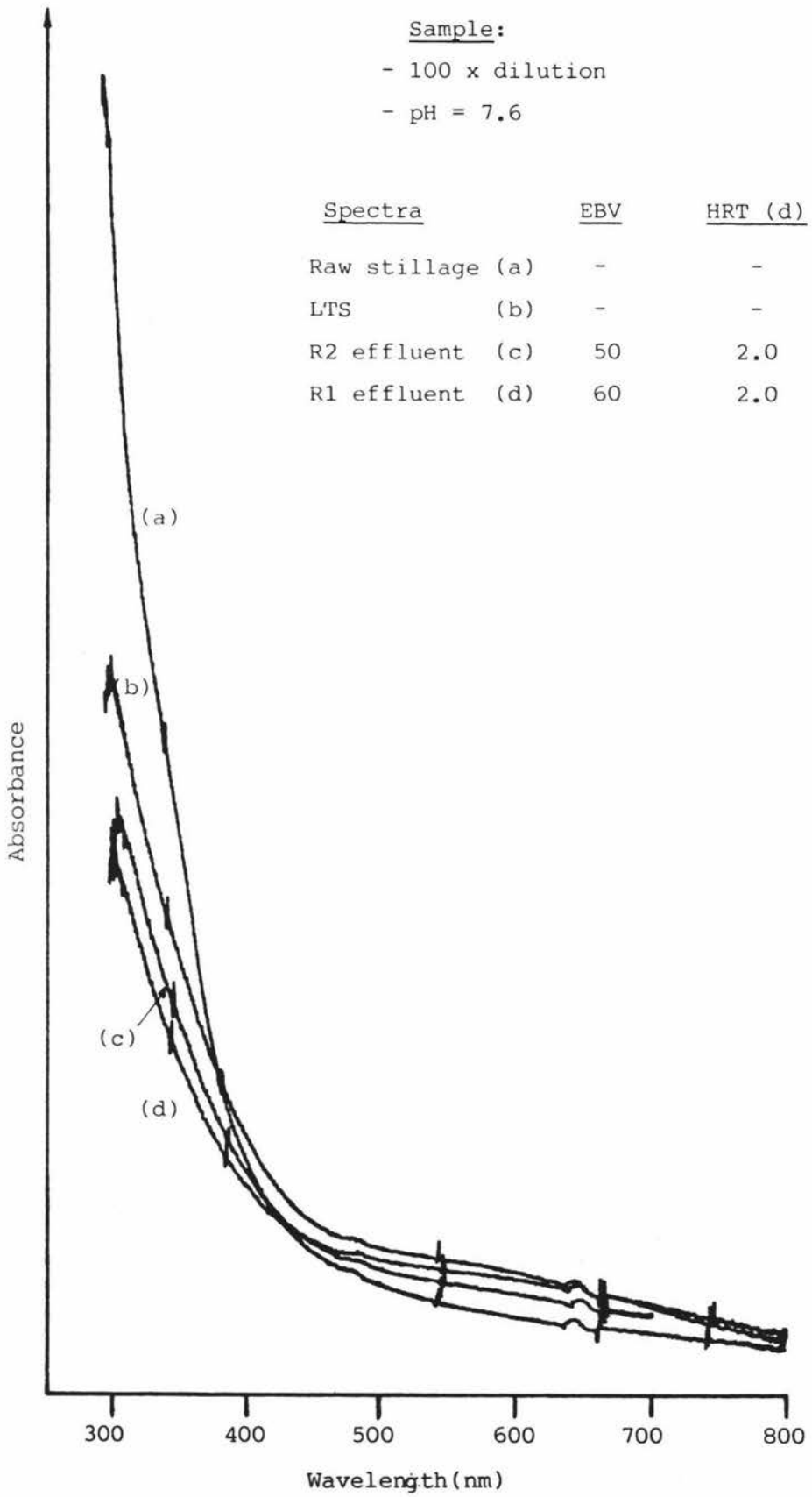


FIGURE 4.47: VISIBLE-SPECTRA FOR R1 AND R2 OPERATIONS - A COMPARISON



4.4.5.3 Conclusions from R2 Phase 4 Operation

There was negligible bioregeneration of GAC in R2 in terms of sCOD removal. As anticipated, this showed that the anaerobic lagoon treated stillage was particularly recalcitrant to further anaerobic digestion.

Approximately 20% of the colour fed to R2 was biodegradable. The occurance of GAC bioregeneration in terms of colour removal was confirmed by the UV and visible spectrophotometric scans and the breakthrough curves for GAC adsorption with and without biological activity.

The UV scans indicated that anaerobic digestion of raw stillage resulted in the degradation of similar UV-absorbing chromophoric species using the lagoon or the expanded-bed system treating raw stillage. The UV and visible scans also demonstrated than R1 was superior than the anaerobic lagoon for colour removal from raw stillage and R2 for the decolourization of the lagoon pretreated stillage.

CHAPTER 5

PRACTICAL ASPECTS OF THE GAC PACKED ANAEROBIC EXPANDED-BED PROCESS (AEB) FOR THE TREATMENT OF WOOD-ETHANOL STILLAGE

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PRACTICAL ASPECTS OF THE GAC PACKED ANAEROBIC EXPANDED-BED PROCESS (AEB) FOR THE TREATMENT OF WOOD-ETHANOL STILLAGE

This study has demonstrated that the performance of the AEB process approaches the 'ideal' reactor system for the anaerobic treatment of wood-ethanol stillage and in many ways, it is superior to all other anaerobic systems reported for wood-ethanol stillage treatment. In this chapter, the engineering significance of this AEB system and some suggestions on further improvements on reactor design are presented. The discussions are mainly based on work done on the treatment of the raw wood-ethanol stillage rather than for the decolourization of anaerobically lagoon treated stillage.

5.1 Treatment efficiency

(a) Organic loading rate and removal

The AEB system compared favourably with other treatment processes with respect to the loading rates which can be applied and the removals obtained. Comparable COD removal rates were obtained even when the reactor was loaded at more than twice that of the highest loading rate reported for treatment of similar stillage.

(b) Colour removal

To my knowledge, there is no report of significant colour removal in the anaerobic digestion of organic matter. This system studied is superior to all previous systems for the treatment of wood-ethanol stillage in terms of colour removal. The GAC enhanced the microbial degradation of the chromophoric species resulting in the continuous bioregeneration of the

GAC *in situ*. Thus significant cost savings on carbon regeneration are possible.

(c) Methane gas and sludge yields

The methane gas yield was near to that predicted by theory thus giving maximum recovery of the stillage energy. This is important since the main objective was on recovery of energy from wood and a higher energy conversion efficiency here would make the conversion process more economically feasible.

The high methane gas yield also meant a low sludge yield. Thus eliminating the needs of sludge wasting or sludge disposal cost.

(d) Reactor stability

There was no problem of biomass washout even at very high hydraulic loading rates since this is a fixed film process allowing independent control of SRT and HRT. This also meant that the reactor could withstand short term hydraulic, organic or toxic overloading. The use of GAC as the packing material also provided greater toxicity sequestering ability.

(e) Nutrient requirements

The AEB process had a very low nutrient requirement due to the long sludge age in the reactor and having a very high sludge activity for COD conversion. Only additions of N, P and an alkalinity reagent were required. There was no need for the supplement of any trace elements.

5.2 Economics of the AEB Process

Though insufficient data were available for accurate economic comparison of this AEB process to other wood-ethanol stillage treatment processes, it is clear that the AEB process is economically competitive from the following capital and operational cost savings:

(i) Only one reactor is required for effective organic and colour removals in a single step;

(ii) A comparatively much smaller reactor is required as compared to all the other previous systems thus reducing reactor cost and land requirements;

(iii) No GAC bioregeneration cost;

(iv) No sludge disposal cost;

(v) Maximum methane gas yield from the process;

(vi) Minimal nutrient and alkalinity addition cost;

(vii) Less frequency of reactor 'upsets';

(viii) Simple reactor design with no additional requirements for reactor mixing and biomass retention.

The AEB process also has some disadvantages:

(i) High initial cost for the GAC support media;

(ii) A slightly longer reactor start-up period;

(iii) Energy cost in effluent recycle.

The cost of activated carbon can possibly be reduced if New Zealand can produce its own GAC. This may be a feasible option if large quantities of lignin become available from the wood-hydrolysis process. Its conversion to GAC for the thorough treatment of hydrolysis plant wastewater has already been demonstrated (Evsyukova et al., 1981).

As discussed in Section 4.3.4.2, the superficial velocity of R1 was already lower than other AEB processes and hence is not as energy intensive as a number of others reported. However, a lower recycling energy cost is possible if smaller GAC particles were used. Also, smaller particles would provide a larger surface area to volume ratio for microbial attachment and this is expected to increase the performance of the AEB process. This would permit a higher space loadings and possibly result in faster reactor start-up.

5.3 REACTOR DESIGN

A suitable parameter for the design of the AEB reactor for ethanol stillage treatment is the organic loading rate.

For an economic scale operation of 100 tonne.d^{-1} dry wood (Melhuish, 1983), 18.5 tonne.d^{-1} of stillage at 23,000 mg.l^{-1} COD can be expected (Table 2.2). The reactor organic loading rate is given by:

$$OLR = \frac{23}{HRT} \quad kg.m^{-3}.d^{-1}$$

Suitable HRTs range from 2.5 - 0.85 d depending on treatment efficiency required (for OLRs ranging from 27.1 - 9.2 kg.m^{-3}.d^{-1}). The reactor working volume (V) can be calculated from:

$$V = \frac{18.5}{OLR} \times 1000 \times 1.2 \times \frac{7.2}{5} \; m^3$$

The '7.2/5' factor represented 5 litres reactor liquid volume and 2.2 litres GAC as packing medium. A reactor scale-up of 20% is also incorporated for head space allowance.

For an OLR of 20 kg.m^{-3}.d^{-1}, the reactor volume is approximately 1600 m^3. At this size range, a full scale plant operation would be designed with a lower height to volume ratio and would incorporate a number of feed distributors at the bottom for a more even feed distribution.

At this stage, Gist-Brocades Co., Delft, Netherlands is constructing a two-stage anaerobic treatment plant comprising two 300 m^3 fluidized sand bed reactors 20 m in height (Dunn, 1984). Only two other full-scale expanded/fluidized bed processes for treating industrial wastes have been reported to date and few design details are available (Switzenbaum, 1983).

CHAPTER 6

CONCLUSIONS

CHAPTER 6

GENERAL CONCLUSIONS

The anaerobic digestion of raw wood-ethanol stillage (R1) and anaerobic lagoon pretreated stillage (R2) has been studied using two originally designed GAC packed expanded-bed reactors.

For R1, a start-up period of about 5 months was required to build up a well balanced and acclimatized methanogenic consortium and to achieve OLRs in excess of 10 $kg.m^{-3}.d^{-1}$. Lowering the feed sulphate concentration to 500 $mg.l^{-1}$ was necessary to prevent sulfide inhibition. After operating for 227 d, it was demonstrated that this system, is superior to all the previous systems reported for the anaerobic treatment of similar stillage. A non-maximal OLR of 29.0 kg $tCOD.m^{-3}.d^{-1}$ at 0.85 d HRT with tCOD and sCOD removals of 74.5 and 83.5% respectively were achieved. Digestion stability was excellent with acetate at 160 $mg.l^{-1}$, propionate at 490 $mg.l^{-1}$ and a gas methane composition at 61.0%. The colour removal was 75% at a loading rate of 4.7 kg chloroplatinate. $m^{-3}.d^{-1}$. Higher colour removals may be obtained by operating at a longer HRT. The methane gas yield was near to that predicted by theory (99.7% at 2 d HRT) with a very low sludge yield (2.8% based on 91.8% sCOD removal). Consequently, the AEB reactor had a very low nutrient requirement for effective treatment. There was no requirement for trace metals addition. The use of GAC as the bacterial carrier provides a toxicity sequesting potential against biological inhibitors and can accomodate very high hydraulic loading rates of less than 0.85 d without problems of cell washout. Continuous bioregeneration of the GAC in R1 has also been demonstrated using sCOD and colour breakthrough curves for GAC adsorption with and without biological activity. Microbial degradation of chromophoric species has been confirmed using UV-visible spectrophotometric scans.

R2 was operated for 191 days with only N and P supplements of 240 and 80 $mg.l^{-1}$ respectively. Little methanogenic activity was observed due to the recalcitrant nature of the anaerobic lagoon pretreated stillage. There was evidence to suggest that fermentative processes other than methanogenesis had accounted for the COD removed. Only approximately 20% bioregeneration in terms of colour removal was achieved at a colour loading rate of 1.2 kg chloroplatinate.$m^{-3}.d^{-1}$. Though R2 was a two reactor system (lagoon and AEB), the performance in terms of COD and colour is inferior compared to R1 and would not be cost effective for stillage treatment.

Approximately 9% w/v of the chromophoric materials present in the stillage are particularly recalcitrant to anaerobic degradation. GC-MS analyses may be able to identify the chemical compounds which are particularly recalcitrant.

It has thus been demonstrated that the GAC packed expanded-bed reactor (R1) provides a very effective treatment of wood-ethanol stillage (including decolourization) while achieving a high stillage energy recovery of 89%. Considerable cost savings are possible for the AEB system since effective treatment can be achieved in a single step utilizing a relatively small reactor with minimal nutrient, sludge disposal and GAC regeneration/replacement costs. The only disadvantages of the system are the capital cost of the GAC, a long start-up period of approximately 5 months and a recycle energy cost to maintain an expanded bed. It is believed that they can be reduced by using a GAC carrier with a smaller particle size.

Thus, anaerobic digestion, utilizing a GAC packed expanded-bed reactor, represents a cost effective and commercially attractive option for the utilization/disposal of wood-ethanol stillage.

ABBREVIATIONS AND NOMENCLATURE

AEB	Anaerobic expanded-bed
A_{sp}	Specific surface area ($m^2.kg^{-1}$)
BOD	Biological oxygen demand ($mg.l^{-1}$)
C	Concentration of solute remaining in the solution at equilibrium ($g.l^{-1}$)
C_0	Initial concentration of contaminants ($g.l^{-1}$)
C_2	Acetic acid ($mg.l^{-1}$)
C_3	Propionic acid ($mg.l^{-1}$)
C_4	Butyric acid ($mg.l^{-1}$)
C_5	Valeric acid ($mg.l^{-1}$)
COD	Chemical oxygen demand ($mg.l^{-1}$)
CPU	Chloroplatinate unit ($mg.l^{-1}$ chloroplatinate)
CSTR	Continuous stirred tank reactor
DNA	Deoxyribo-nucleic acid
D_0	GAC dosage (w/v)
dp	Particle diameter (m)
EBV	Equivalent bed volume
FFAP	Free fatty acid phase
FRI	Forest Research Institute
g	Acceleration due to gravity ($m.S^{-2}$)
GAC	Granular activated carbon
GC-MS	Gas chromatograph-mass spectrograph
H_A	Henry's constant
HRT	Hydraulic retention time (d)
K_F	Adsorption capacity indicator (a constant)
LFTB	Liquid Fuels Trust Board
LTS	Lagoon Treated Stillage
n	Adsorption intensity indicator (a constant)
N	Nitrogen ($mg.l^{-1}$)
NH_3-N	Ammonia nitrogen ($mg.l^{-1}$)
NWASCO	National Water and Soil Conservation Authority
NZ	New Zealand
OLR	Organic loading rate (kg $COD.m^{-3}.d^{-1}$)
P	Phosphorus ($mg.l^{-1}$)

P_A	Partial Pressure (atmosphere)
P_P	Particle density ($kg.m^{-3}$)
P_O	Water density ($kg.m^{-3}$)
PAC	Powdered activated carbon
q_e	The amount of solute adsorbed per unit weight of solid adsorbate ($g.g^{-1}$)
R1	Reactor One (treating raw stillage)
R2	Reactor Two (treating anaerobic lagoon pretreated stillage)
R_{148}	Carbon samples taken after 148 days of operation
RDP	Reactive dissolved phosphorus ($mg.l^{-1}$)
RNH_2	Organic nitrogen ($mg.l^{-1}$)
SEM	Scanning electron micrograph
SRT	Biological solid retention time (d)
TDP	Total dissolved phosphorus ($mg.l^{-1}$)
TP	Total phosphorus ($mg.l^{-1}$)
TS	Total solids ($g.l^{-1}$)
TSS	Total suspended solids ($g.l^{-1}$)
UASB	Upflow anaerobic sludge blanket
UV	Ultra Violet
VFA	Volatile fatty acid ($mg.l^{-1}$)
VS	Volatile solids ($g.l^{-1}$)
VSS	Volatile suspended solids ($g.l^{-1}$)
v_t	Terminal velocity ($m.S^{-1}$)
$\bar{x}$	Mean value
x_A	Mole fraction
$Y_{COD}^{CH_4}$	Methane yield from COD removed (ml CH_4 at STP/g)
$Y_{BOD_5}^{CH_4}$	Methane yield from BOD_5 removed (ml CH_4 at STP/g)
$Y_{VS}^{CH_4}$	Methane yield from VS removed (ml CH_4 at STP/g)
$\Delta G°'$	Free energy change ($Kcal.reaction^{-1}$)
μ	Viscosity ($Ns.m^{-2}$)
σ	Standard deviation
λ	Wavelength (nm)
Φ	Sphericity
$\emptyset$	Diameter

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APPENDIX 1

SEED SLUDGE CHARACTERISTIC

Seed Sludge	Chemical Oxygen Demand (mg/l)		pH	Alkalinity (mg/l)	Solids (g.l)				Volatile Fatty Acids (mg/l)				Colour* CPU
	tCOD	sCOD			TS	VS	TSS	VSS	C_2	C_3	C_4	C_5	
Dairy waste Anaerobic lagoon			7.1		26.25	11.86			70	80	Traces	0	
Anaerobic lagoon for stillage			6.8		9.95	4.75			155	165	Traces	0	
Silage leachate			5.5		19.54	6.29			2520	210	6450	1700	
Sawdust leachate			6.0		6.48	1.60			70	80	0	0	
Seed sludge mixture**	13400	8190	6.7	2394	18.62	9.64	10.76	4.95	585	90	620	160	2000

* Visual comparison method
** Seed sludge mixture not including distilled water

APPENDIX 2

DERIVATION OF FREUNDLICH ISOTHERM PLOT

The empirical Freundlich equation can be defined as (Weber, 1972; Neely and Isacoff, 1982): -

$$q_e = K_F C^{\frac{1}{n}} \qquad - \quad (1)$$

where,

q_e = the amount of solute adsorbed per unit weight of solid adsorbent ($g.g^{-1}$)

C = concentration of solute remaining in the solution at equilibrium ($g.l^{-1}$)

K_F and n are constants

Taking the log of equation (1),

$$\log q_e = \log K_F + \frac{1}{n} \log C \qquad - \quad (2)$$

which is in the form of $Y = mx + C$

alternatively equation (2) can be written as: -

$$\log \frac{C_o - C}{D_o} = \log K_F + \frac{1}{n} \log C \qquad - \quad (3)$$

Where,

C_o = initial concentration of contaminants,

D_o = GAC dosage (weight/volume)

Thus a plot of $\log \frac{C_o - C}{D_o}$ versus log C (i.e. the adsorption isotherm) should give a straight line with slope of $\frac{1}{n}$ and intercept as $\log K_F$.

APPENDIX 3

REACTOR ONE OPERATION ON RAW STILLAGE - SUMMARY OF RAW DATA

APPENDIX 3.1: STILLAGE CHARACTERISTICS AFTER MODIFICATION - FEED TO R1

Feed Characteristics \ Feed Used from Date stated	8/6/83	1/7/83	28/7/83	30/7/83	7/8/83	27/8/83	8/9/93	12/11/83	23/12/83
Dilution	1/2	3/4	3/4	1/10**	3/4	0.87/1	None	None	None
tCOD ($mg.l^{-1}$)	10750	18625	15050	2318	15050	16450	25325	25850	24400
sCOD ($mg.l^{-1}$)	10280	16188	13925	2180	13925	15650	24450	23263	23580
$tBOD_5$ ($mg.l^{-1}$)								16171	14470
$sBOD_5$ ($mg.l^{-1}$)									13558
*pH	4.9	4.6	7.1	6.0			5.1	5.70	6.80
* Alkalinity ($mg.l^{-1}$)			1186	228			843.6	1271	1339
TS ($g.l^{-1}$)	7.77		13.97				13.05	19.02	18.95
VS ($g.l^{-1}$)	6.23		9.26				12.0	14.75	13.53
TSS ($g.l^{-1}$)	0.05		0.74					0.60	1.25
VSS ($g.l^{-1}$)	0.29							0.59	0.41
Acetate ($mg.l^{-1}$)	592		555			955	1744	1262	655
Propionate ($mg.l^{-1}$)	90		30			10	25	15	30
Butyrate ($mg.l^{-1}$)	615		20			0	0	0	0
Valerate ($mg.l^{-1}$)	145		65			48	98	45	39
Colour (CPU) Visual Comparison method pH = 5	1000						2500	4000	3000
Colour (CPU) Visual Comparison method pH = 7.6	2000					7000	3000	5000	4000
Colour (CPU) Visual Comparison method pH = 9.0	2500						7000	7000	5500
Colour (CPU) Spectrophotometric method pH = 5	2330						7250	9300	2600
Colour (CPU) Spectrophotometric method pH = 7.6	3000					15500	9200	10300	3500
Colour (CPU) Spectrophotometric method pH = 9.0	3650						14400	13200	5200
Soluble SO_4^{2-} (mg.l)	1800***				113	478	424	224	

* pH and alkalinity will vary depending on alkali dosing (see Appendix 3.2)

** To wash out barium toxicant

*** Data from NZFRI

APPENDIX 3.2: R1 FEED PREPARATIONS, NUTRIENT ANALYSES AND RELATED PARAMETERS

	Feed modifications						NUTRIENT ANALYSES ** ($mg.l^{-1}$)									
							NH_3-N levels		Phosphorus levels							
Date	Feed Strength	Added 20% NaOH ($ml.l^{-1}$ feed)	Added *** Nitrogen ($mg.l^{-1}$ feed)	Added Phosphorus ($mg.l^{-1}$ feed)***	Desulphated to 500 $mg.l^{-1}$ SO_4^{2-}	Observed HRT (days)	Feed	Effluent	Feed TP	Effluent TP	Feed TDP	Effluent TDP	Feed RDP	Effluent RDP	Soluble Sulfide Concentration ($mg.l^{-1}$)	Comments
8/ 6/83	0.5	0	240	80	No	25.0										
27/ 6/83	0.75	0	240	80	No	28.0										
23/ 7/83	0.75	0	240	80	Yes	28.0										Desulphated feed
26/ 7/83	0.75	2.5	240	80	Yes	36.7	236.3		148.2					13.9		Barium inhibitic
29/ 7/83	0.10	2.5	240	80	No	7.1		80.1		35.5			2.5	1.1		Remove toxican
7/ 8/83	0.75	2.5	240	80	Yes	33.0	234	89.6								
12/ 8/83	0.75	2.5	240	80	Yes	34.1										
23/ 8/83	0.75	2.5	240	80	Yes	28.4							6.5	0.95		Low RDP
26/ 8/83	0.875	2.5	240	120	Yes	30.3									0.029	Low soluble sulfide
7/ 9/83	1	2.5	240	359/0	Yes	31.3									0	Remove Iron with P
13/ 9/83	1	2.5	240	359/0	Yes	25.0							34.0	0.11	0	RDP still low
29/ 9/83	1	2.5	240	480/120*	Yes	14.7	163.0	128.8					16.1	0.24		
9/10/83	1	3.0	240	551/200	Yes	9.80				186.3			17.1	0.12		
18/10/83	1	3.0	240	551/200	Yes	7.04							49.5	0.31	0	
23/10/83	1	2.5	240	551/200	Yes	5.62										
28/10/83	1	2.5	240	551/200	Yes	4.05	180.6	28.4					62.3	0.26		
1/11/83	1	2.5	240	551/200	Yes	3.60							36.0	0.16		
5/11/83	1	2.5	240	551/200	Yes	3.6										
9/11/83	1	2.5	240	551/200	Yes	3.64	157.4	50.4	336	227.5	72	1.25	25.9	4.9		
12/11/83	1	2.0	240	242/200	Yes	2.55									0	
15/11/83	1	2.5	240	242/200	Yes	2.63	178.1	2.2	213	87.5	125	0	84.3	0.24		Low NH_3-N
21/11/83	1	4.5	300	242/200	Yes	2.00	148.4	9.0	329.5	122		1.5	16.0	0.45	0.45	High Alkalinity addition
29/11/83	1	4.5	350	242/200	Yes	1.92									0.27	
7/12/83	1	4.5	350	242/200	Yes	1.75	327.6	66.7	368	156.5	21.0	2.06	33.5	1.03	0.33	NH_3-N level okay
11/12/83	1	4.5	350	242/200	Yes	1.32	296.8	68.0	354	117.5	10.0	1.15	29.5	0.81	0.30	
16/12/83	1	4.5	350	242/200	Yes	1.18										
22/12/83	1	4.5	350	263/250	Yes	1.14	315.9	54.2	351	159	35.8	2.40	44.3	1.00	0.186	
24/12/83	1	4.5	350	263/250	Yes	1.04	315.9	89.9								
28/12/83	1	4.5	350	263/250	Yes	0.91	284.7	104.2							0	
31/12/83	1	4.5	350	218/200	Yes	0.91			268.5	70.0	58.0	2.82	20.8	1.13		
3/ 1/84	1	3.0	350	218/200	Yes	0.84	378.7	185.9	241	82.0	56.5	1.85	60.0	1.04		
11/ 1/84	1	2.5	350	218/200	Yes	1.85	378.7	188.2					89.0	0.48	0	

* 480/120 meaning added 480 $mg.l^{-1}$ P to the stillage. Of this, 360 $mg.l^{-1}$ P is used to precipitate the soluble iron level to 200 $mg.l^{-1}$ and 120 $mg.l^{-1}$ P is used as nutrient.

** Nutrient levels were not necessarily measured on the exact date quoted for feed preparation. However, they were analysed within the loading rate period using the corresponding feed modifications.

*** N and P were added as NH_4Cl and K_2HPO_4 respectively.

APPENDIX 6.3

R2 EFFLUENT CHARACTERISTICS

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	Visual comparison method			Spectrophotometric method					
																pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
	5/7		0																					Reactor seeded at 3 pm
	6/7		0			7.7	1550																0	
	7/7		0			7.5	1687																0	
	8/7		0	4000	440	6.7	912	5.58	3.21	1.28		125	0	0	0	50	50	50		550	510		0.59	Seed in R2 is not fluidized
	9/7		0			6.9	866																-0.04	
1	10/7			15875	190	6.55	866	14.19	10.39	11.57	9.01	145	20	0	0		25						0	Start feeding at 12 a.m.
2		22.00	0.050			6.55	866																	Use 1:2 diluted lagoon treated feed
3		38.50	0.028	22500	270	6.80	1268	20.10	15.00	18.10	14.30	70	40	0	0		5			68				Add 5g M4 on day 2
4		31.30	0.036			6.90	1459															0.0	0.00	Feed with 240 $mg.l^{-1}$N and 80mg^{-1}P added
5		38.50	0.028			7.00	2189																0.00	
6		76.00	0.014	14438	108	6.75	1414	8.49	6.23	6.55	5.58	50	0	0	0		0						0.00	Add 5g M4
7		28.90	0.038			7.20	2006																0.00	
8	17/7	29.20	0.037			7.10	1520																0.00	Granular floc formation on top of carbon bed
9		37.90	0.029			7.10	1687																0.00	
10		40.00	0.027		115	7.00	1687	13.90	10.53	12.00	10.50	85	110	180	0		0					0.0	0.00	Very clear effluent after filtration
11		34.00	0.032			7.20	1778																0.00	
12		33.30	0.033			7.30	1778																0.00	
13		38.00	0.029	37750	163	7.00	1870	11.70		10.60		100	120	55	23		0						0.00	Very high tCOD from seed sludge washout
14		33.00	0.033			7.30	1414																0.00	
15	24/7	38.00	0.028			7.30	1368																0.00	
16		23.00	0.047	30125	170	7.00	1915	13.20	9.65	10.70	9.63	90	95	45	0		0	ppt		0		0.0	0.00	Good sCOD and full colour removal due to GAC adsorption
17		30.00	0.036			7.40	1642																0.00	
18		25.00	0.044			7.10	1778																0.00	No gas produced due to recalcitrant nature of feed
19		26.30	0.041	1760	197	7.10	1406										0						0.00	
20		25.00	0.044			7.20	1406																0.00	Bed not fluidizing well
21		28.10	0.039			7.10	1915																0.00	
22	31/7	26.70	0.041			7.20	1824																0.00	
23		26.90	0.040	45000	282	7.10		43.10	36.12	40.00	35.10	100	55	35	0		0						0.00	High solids in effluent due to fluidization problem

APPENDIX 6.3 (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
24		25.00	0.044			7.30	1687															0.0	0.00	
25		25.80	0.042			7.10	1847																0.00	
26		27.80	0.039	49500	253	7.05	1642	24.70	20.40	22.70	19.50	95	105	0	0		0						0.00	High solids and tCOD due to fluid-ization problem.
27		19.50	0.056			7.25	1687																0.00	
28		14.50	0.076			7.10	1619																0.00	
29	7/8	16.60	0.066	52300	417	7.10	1778	21.50	16.90	20.30	16.90	135	40	15	0		0					0.0	0.00	Low VFA throughout R2 operation.
30		16.60	0.066			7.20	1778																0.00	
31		48.00	0.022			7.30	1733																0.00	
32		48.00	0.022			7.25	1733																0.00	
33		17.70	0.062	22375	218	7.10	1436	14.60	11.30	11.50	10.40						0						0.00	
34		20.00	0.096			7.15	1778															0.0	0.00	Use 3/4 strength feed
35		17.80	0.107			7.30	1778																0.00	
36	14/8	18.20	0.105			7.40	1847																0.00	
37		22.70	0.084	61700	175	7.10	1733	39.20	39.90	36.50	32.50	62	65	0	0		0					0.0	0.00	1.79 equivalent bed volume (EBV)
38		20.10	0.095			7.10	1801																0.00	
39		19.60	0.097			7.35	1801															0.0	0.00	
40		19.70	0.097			7.35	2010																0.00	
41		22.60	0.084																				0.00	
42		22.60	0.084	36500	270	7.10	1824	12.90	9.55	9.36	8.51	205	20	12	0		0						0.00	
43	21/8	20.00	0.096			7.15	1801																0.00	
44		21.70	0.088	64000	211	7.15	1984	66.30	59.60	63.90		205	22	10	0		0					0.0	0.00	Very high effluent TS and tCOD.
45		19.20	0.100			7.25	1961																0.00	
46		19.20	0.099			7.70	1866																0.00	
47		19.20	0.099			7.20	2006																0.00	
48		18.90	0.101	67450	325	7.12	1915	34.70	28.40	31.40	38.90	265	25	12	0		0					0.0	0.00	
49		18.20	0.178			7.35	1915					160	30	0	0								0.00	Full strength feed
50	28/8	19.20	0.169			7.95	1505																0.00	
51		18.00	0.180			8.00	1733																0.00	
52		21.80	0.149	34900	225	7.70	1762										0					0.0	0.00	
53		17.80	0.182			7.70																	0.00	

APPENDIX 6.3 (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	µil-5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
54		20.00	0.162			7.80	1824																0.00	
55		17.50	0.185			7.80	1473																0.00	
56		19.20	0.169			7.65	1596																0.00	
57	4/9	19.80	0.164	17000	188	7.50	1658	18.10	11.30	12.90	11.20	216	25	0	0		0					0.0	0.00	
58		16.60	0.195																				0.00	
59		16.60	0.195			7.70																	0.00	
60		20.40	0.159	11150	133	7.55	1579										0					0.0	0.00	
61		20.00	0.162				1349																0.00	
62		70.40	0.159			8.20	1596																0.00	
63		20.00	0.162	320	115	8.05	965					150	0	0	0		0					0.0	0.00	Bed not fluidized
64	11/9		0.000																				0.00	
65			0.000																				0.00	Feed distributor cleaned out found the tube is full of fine carbon or solids.
66		19.20	0.169	20800	133	9.30	1254					410	5	0	0		0					0.0	0.00	
67		20.00	0.162			8.85	1163																0.00	Lost about 1 l carbon from attrition. Significant scouring on bottom plate
68		20.00	0.162			8.70	1327																0.00	
69		19.60	0.165	16725	96	8.60	1230					110	0	0	0		0						0.00	
70		19.20	0.169																				0.00	Feed line blocked
71	18/9		0,000																				0.00	
72			0.000																				0.00	Change recycle pump
73		17.20	0.188	17250	82	8.30	1285	9.81	7.16	7.00	6.09	115	0	0	0		0					0.00	0.00	End of Acclimatization Phase
74		18.50	0.175			8.20	933																0.00	Start Increased Organic Loading Phase
75		16.80	0.193			8.15	1573																0.00	
76		18.30	0.178	8500	95	8.00	1756	7.88	5.69	5.44	4.38	270	22	7	0		0			30		0.0	0.00	Effluent solids back to normal when bed fluidize well
77		17.80	0.188			8.00	1505																0.00	
78	25/9	17.80	0.188			7.90	1618																0.00	
79		16.70	0.195	15150	106	7.85	1892	6.59	4.43	4.01	3.77	145	10	0	0		0						0.00	
80		14.90	0.217			7.85	1720																0.00	
81		14.30	0.227			7.85	1741																0.00	Occasional check found no gas leak
82		14.30	0.227	3750	151	7.75	1741					350	25	0	0		0						0.00	
83		12.10	0.269			7.75	1756																0.00	3.88 EBV
84		11.50	0.282			7.75	1783																0.00	

APPENDIX 6.3 (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	Visual comparison method			Spectrophotometric method					
																pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
85	2/10	11.50	0.273			7.75	2029																0.00	
86		12.20	0.266	1800	203	7.70	1894	4.25	1.05	1.81	0.78	92	0	0	0		0				0.0		0.00	R2 liquid temperature = 37°C
87		11.90	0.273			7.75	2029																0.00	
88		11.40	0.286			7.75	2029																0.00	
89		12.10	0.269	1563	175	7.75	2062	4.55	1.34	0.83	0.54	78	40	0	0		0				0.0		0.00	
90		10.50	0.308			7.70	2085																0.00	
91		10.50	0.308			7.60	2107																0.00	
92	9/10	9.09	0.357			7.55	2212																0.00	
93		10.80	0.302	1575	270	7.60	2234	5.26	1.22	0.82	0.50	195	50	10	0		0				0.0		0.00	
94		8.33	0.390			7.55	2220																0.00	
95		8.15	0.398			7.60	2243																0.00	
96		8.16	0.998	1255	214	7.50	2310	4.52	1.84			220	12	5	0		5				0.0		0.00	First colour breakthrough
97		8.07	0.403			7.55	2401																0.00	
98			0.000			7.60	2378																0.00	Feed line blocked
99	16/10	7.81	0.416			7.60	2537																0.00	Bed not fluidized
100		7.94	0.409	3813	349	7.55	2371	6.20	3.08	2.37	2.16	208	20	0	0		10				0.0		0.00	Check found gas meter not working
101		6.85	0.474			7.70	2424																0.00	Use continuous feeding
102		6.94	0.468			7.55	2446																0.00	
103			0.000	4600	280	7.50	2310	7.78	3.88	3.07	2.46	200	30	0	9		10						0.00	Low effluent colour
104		5.79	0.561			7.55	2508																0.00	Sulfide level = 0
105		5.35	0.607			7.50	3584																0.00	
106	23/10	5.88	0.552	3613	310	7.50	2582	6.88	2.62	2.60	2.05	175	18	0	0		10				0.0		0.00	6.52 EBV
107		5.10	0.637			7.50	2690																0.00	
108		4.55	0.715			7.50	2673																0.00	
109		4.97	0.653			7.50	2650																0.00	
110		4.59	0.707	2750	383	7.45	3035	6.79	2.08	0.47	0.28	160	10	0	0		10				0.0		0.00	7.32 EBV
111		4.02	0.828			7.40	2786																0.00	
112		3.76	0.864			7.45	2877																0.00	
113	30/10	4.78	0.682			7.40	2809																0.00	
114		4.78	0.682	1925	400	7.40	2809	6.32	2.00		1.56	162	16	0	0		15				0.0		0.00	

APPENDIX 6.3 (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	Visual comparison method			Spectrophotometric method					
																μl-5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
115		3.60	0.903			7.45	2899																0.00	8.55 EBV
116		3.47	0.936			7.40	2899																0.00	
117		3.92	0.830			7.50	3012																0.00	
118		3.68	0.884	1615	498	7.45	2982	6.31	1.86	1.08	0.81	265	15	0	0		20					0.0	0.00	R1 liquid at 37.5°C
119		3.83	0.848			7.40	2982																0.00	
120	6/11	3.94	0.825			7.40	2850																0.00	
121		3.80	0.85	1025	469	7.45	2945	2.63	1.59		0.23	232	10	0	0	10	30	10	98	88	78	0.0	0.00	10.5 EBV
122		3.11	1.04			7.50	2888																0.00	
123		6.29	0.98			7.50	2793																0.00	
124		3.10	1.04																				0.00	
125		3.01	1.07	825	510	7.45	3096	5.39	1.06		0.34	125	25	0	0		30						0.00	11.8 EBV. Start Operational Phase
126		2.60	1.38	868	603	7.40	2926	5.39	1.50		0.18	135	40	0	0		30						0.00	R2 effluent darkish clear
127	13/11	2.81	1.28	1025	477	7.30	2793	5.69	1.05	1.87	0.43	150	26	0	0		40						0.00	
128			0.00	1013	479	7.30	2869	5.16	0.77	0.10		50	45	0	0		40					0.0	0.00	
129		2.58	1.39	1133	476	7.35	3171	6.11	1.92	0.33		130	21	0	0		50						0.00	
130		2.44	1.47	1280	605	7.40	2945	5.82	1.65	0.13		178	32	0	0		50						0.00	Effluent colour start coming up
131		2.45	1.46	1140	235	7.40	3096	5.99	1.90	1.21		310	50	0	0		50					0.0	0.00	13.3 EBV
132		1.92	1.87	1225	578	7.35	2793	6.00	1.39	0.75	0.57	242	13	0	0		60					0.0	0.00	
133		1.94	1.85	1280	779	7.73	3096	5.59	1.18			125	15	0	0		60					0.0	0.00	
134	20/11	2.00	1.80	1340	848	7.30	2809	6.83	2.74	1.04	0.67	175	122	0	0		60					0.0	0.00	
135		1.98	1.41	1310	710	7.35	2877	6.25	1.46	0.63	0.47	92	38	0	0		60					0.0	0.00	H_2S = 0
136		2.20	1.77	1310	772	7.40	2492	4.90	0.81		0.68	175	49	0	0		60					0.0	0.00	Reseeded with 150 ml sludge from R1 (VSS = 8.18g.l^{-1})
137			0.00	1690		7.40	2945	6.20	1.89	0.93	0.34	200	55	0	0		40					0.0	0.00	
138		2.79	1.29	1525	848	7.40	2922	6.03	1.43	0.89	0.49	225	76	0	0		50					0.0	0.00	
139		2.17	1.65	1500	650	7.40	2996	6.74	1.88	0.51	0.10	185	48	0	0	63	75	100	130	150	190	0.0	0.00	17.3 EBV
140		2.05	1.75	1500	628	7.40	2883	6.18	1.86	1.69			68	0	0		75					0.0	0.00	Still no gas production
141	24/11	3.00	1.80	1400	738	7.45	2860	6.97	2.37	1.35	1.24	175	43	0	0		75					0.0	0.00	
142		2.04	1.76		697	7.40	2815	6.95	1.76	1.43	0.73	187	60	0	0		75					0.0	0.00	
143		2.03	1.77	1450	705	7.50	2838	6.57	2.39	1.44		106	15	0	0		200					0.0	0.00	Effluent sample at pH 7.6 after filtration had pinkish appearance
144		2.02	1.78	1325	760	7.55	2749	6.32	1.70	0.43		105	20	0	0		200					0.0	0.00	
145		2.01	1.79	1425	759	7.55	2769	6.76	2.40	1.03	0.67	134	21	0	0	200	250	300	460	520	670	0.0	0.00	20.2 EBV

APPENDIX 6.3 (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
146		1.85	1.94	1453	880	7.55	2815	6.38	1.82			155	55	0	0		300					0.0	0.00	
147			0.00	2178	251	7.50	2838	5.90	1.05	1.56	0.26	60	100	0	0		200					6.5	0.00	Definite sign of gas production from gas bubbler. 24.2 EBV
148	4/12	1.95	1.84	1575	820	7.55	2851	6.18	1.69		0.05	72	4	0	0		250					9.0	0.02	
149		2.09	1.72	1590	870	7.50	2877	6.21				102	40	0	0		300					8.0	0.02	
150		1.88	1.91	1805	130	7.45	2860	6.24	2.22			170	34	0	0	400	450	500	1150	1650	1650	11.5	0.00	Colour compare well with standard
151		1.81	1.99			7.50	2838																0.00	
152		1.77	2.01	1988	1280	7.50	2967	7.12	2.59	1.43	1.34	145	52	0	0		450					12.0	0.01	Gas production is evident from % CH_4 in gas
153		1.77	2.01			7.60	3042																0.01	
154		1.56	2.30			7.55	2974																0.00	
155	11/12	1.41	2.56	2775	1350	7.55	3178	8.08	2.73	2.34	1.47	122	52	0	0		750					6.0	0.00	
156		1.39	2.59			7.50	3153															15.8	0.03	
157		1.37	2.63			7.55	3133																0.01	
158		1.34	2.67	2820	1560	7.50	3087	7.66	2.25	1.52	0.92	160	35	0	0	100	1000	1250	2450	3000	3950	12.0	0.01	H_2S = 0
159		1.32	2.72	2683	1443	7.55	3087	7.25	2.45	1.00		245	60	0	0		1000					11.0	0.01	29.2 EBV
160		1.12	3.22			7.70	2951															10.0	0.03	Very low gas production
161		1.14	3.16	2605	1613	7.60	2951					167	25	0	0		1000					10.0	0.03	
162	18/12	1.12	3.22			7.70	2928																0.02	
163			0.00	2895	1685	7.69	3110	7.67	2.51	1.46	0.70	200	22	0	0		750					10.0	0.00	
164		3.13	2.75			7.85	3110																0.02	
165		1.33	2.70	2778	1773	7.80	3473	7.11	3.66	6.33	0.99	110	28	0	0	750	875	1000	2550	3250	4550	9.0	0.01	
166		1.23	2.92			7.80	2928																0.03	35.3 EBV
167		0.98	3.68			7.80	2972																0.00	
168		0.98	3.67	2500	1593	7.80	2565	7.63	3.23	1.66		126	38	0	0		875			3250		7.0	0.01	
169	25/12	0.95	3.77			7.85	2906																0.04	MERRY CHRISTMAS
170		0.93	3.88	2825	1775	7.90	2974	7.30	2.74	1.46	1.28	115	25	0	0	1000	1000	1125	3950	8000		6.0	0.02	
171		0.79	4.37			7.95	2951																0.03	Start of Bioregeneration Confirmation Phase 40.5 EBV
172		2.78	1.52			7.95	3155															6.0	0.04	
173		2.17	1.94			7.95	3065																0.03	
174		2.11	2.00	2910	1963	8.00	3178	9.72	4.24	3.47	2.22	160	53	0	0		1000					6.0	0.00	
175		2.05	2.06			8.05	3339																0.00	
176	1/1/84	2.00	2.11			8.05	3450																0.01	HAPPY NEW YEAR FOR 1984

APPENDIX 6.3 (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
177		2.38	1.77			8.05	3541																0.00	
178		1.89	2.23	5100	2750	7.75	2632	8.89	3.40	2.60	1.36	210	200	0	0	1250	1250	1500	3200	4875	5875		0.00	
179		1.89	2.12			7.70																	0.00	44.5 EBV
180		1.98	1.12			7.85	4268																0.00	
181		1.89	2.12			7.95																	0.00	
182		1.95	2.16			8.05																	0.00	
183	8/1	1.98	2.13	2950	2750	8.10	3904	9.32	4.00	3.21	1.52	275	260	0	0	1750	1375	1500	5300	5000	2450	14.0	0.00	
184		2.03	2.07			8.05																	0.00	
185		1.92	2.19			8.10										1500	1250	1750	5325	6325			0.00	
186		1.97	2.13	3950	2525	8.25	3587	7.41	3.24	1.31	0.61	12	0	0	0								0.00	
187		1.96	2.15			8.25																	0.00	
188		1.95	2.16			8.20																	0.00	
189		1.95	2.16			8.20	3768	7.19	2.22	1.28	0.06	0	5	0	0	1500	1250	1750	3520	4925	6500	16.9	0.00	
190	15/1	2.27	1.85			8.20																	0.00	
191		1.92	2.19	4115	2503	8.25	3814	7.50	2.78	1.76	0.94	22	5	0	0	1500	1250	1750	3200	4325	6025		0.00	50.5 EBV

APPENDIX 4

GAC PARTICLE SIZE ANALYSIS: THE DETERMINATION OF CARBON EXTERNAL SPECIFIC SURFACE AREA

(a) Introduction

Some particle size measurement techniques (e.g. using a micrometer) could not be used since irregular particles were involved. This dilemma was resolved by considering the particle to be equivalent in size to that of a spherical particle with respect to some parameter like equivalent volume, equivalent surface or equivalent settling velocity (Scarlett, 1977). In this study, the equivalent settling velocity parameter was used to determine the carbon particle size by the sedimentation technique.

(b) Method

The time for the wetted carbon samples to fall a fixed height (24.9 cm using a 500 ml measuring cylinder) were measured. Care was taken to make sure that timing was only done after the falling particle had reached a terminal velocity (by allowing a certain initial height of fall). The particle equivalent diameter (dp) was then computed using Stokes Law (Scarlett, 1977).

$$v_t = \frac{(Pp - Po)\, dp^2\, g}{18\, \mu}$$

Where: v_t = particle settling terminal velocity ($m.s^{-1}$)

P_p = Wetted carbon particle density
= 1.45 $kg.m^{-3}$ (for Filtrasorb 200, CALGON Corporation)

P_o = density of water
= 998 $kg.m^{-3}$ at 20°C (Tennent, 1971)

d_p = carbon particle diameter to be determined (m)

g = acceleration due to gravity ($m.s^{-2}$)

μ = water viscosity
= 1×10^{-3} $NS.m^{-2}$ at 20°C (Tennent, 1971)

The Stokes equation is only valid in the laminar flow region when Reynolds Number is less than 0.2. Using water and with GAC density of less than 2×10^3 $kg.m^{-3}$, the R_e criterion was satisfied for particle size of less than 71 μm (Scarlett, 1977). Thus the sedimentation technique is valid for this study.

Since a range of particle sizes are involved, the average settling time has to be used. This can be best determined from the settling time distribution curves (Fig. 4.25, Section 4.3.5.1) and the log normal probability plots (Fig. A1). Log normal plots were used because the settling time distribution curves were found to be log normally distributed. The log normal probability plots can then be used to estimate the average settling time. This is taken as the time that corresponds to the 50 percentile scale (Fig. A1).

(c) Particle Size Determination

From Fig. A1 the average carbon particle settling times were:

R1 Carbon, 206 days operation ($R1_{206}$) = 6.6 S

R1 Carbon, 148 days operation ($R1_{148}$) = 8.0 S

Virgin carbon ($R1_o$) = 8.2 S

Using Stokes Law, the d_p were found to be:

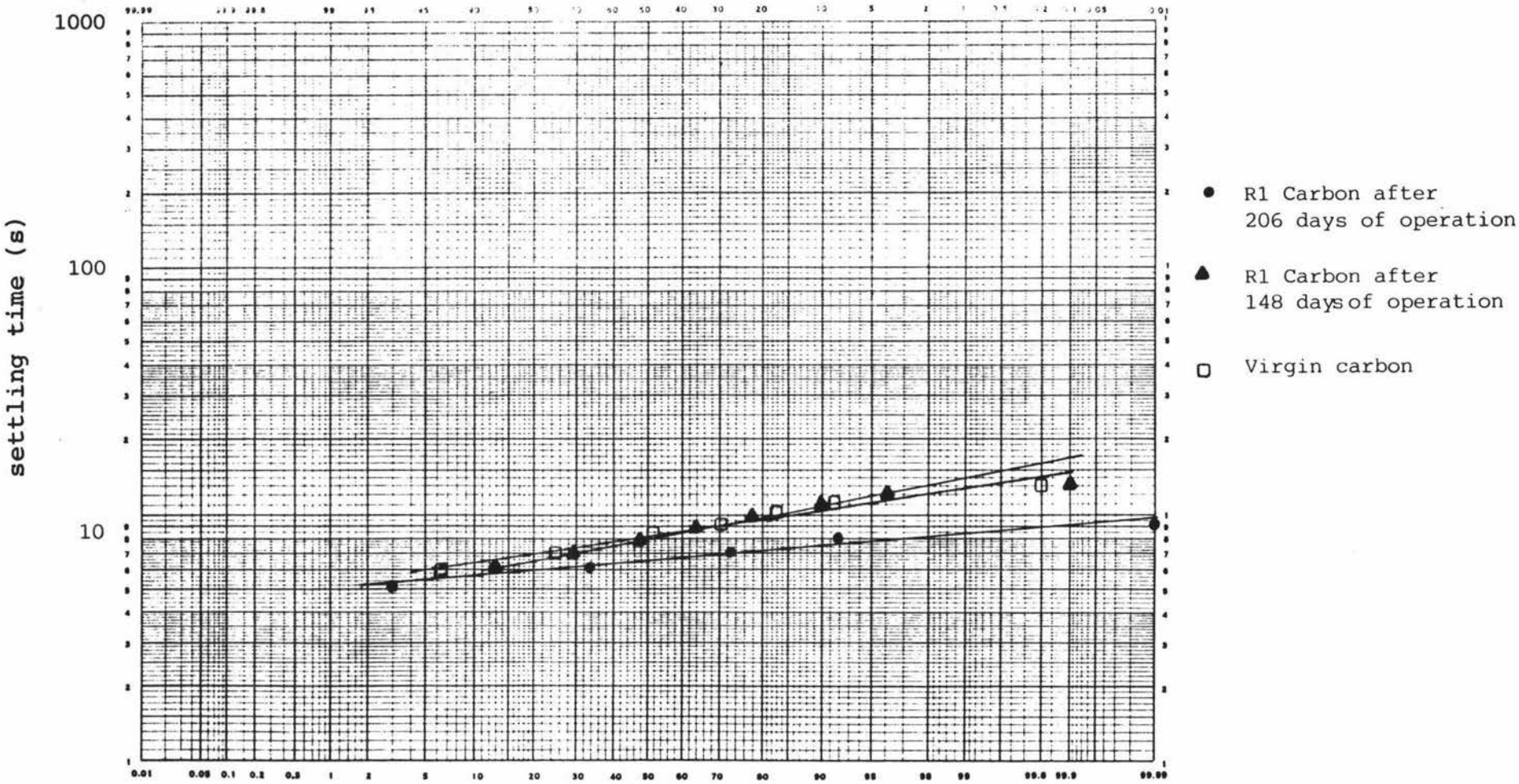
$R1_{206} = 3.92 \times 10^{-4}$ m (or 0.392 mm)

$R1_{148} = 3.23 \times 10^{-4}$ m (or 0.323 mm)

$R1_0 = 3.15 \times 10^{-4}$ m (or 0.315 mm)

Thus $R1_{206}$ carbon had a larger particle size than $R1_{148}$ and $R1_0$! The lower settling time for $R1_{206}$ (results in larger d_p) is believed to be due to the more rounded and smoother surface of $R1_{206}$ carbon having a smaller drag coefficient. The difference in real density of R1 carbon and the virgin carbon was found to minimal with R1 carbon (sampled at end of Phase 4 operation) at 11.3% lower than the virgin carbon. Thus effect of density difference could not have resulted in the lower settling time for R1

FIGURE A1: LOG-NORMAL PROBABILITY PLOTS TO DETERMINE AVERAGE CARBON PARTICLE SETTLING TIMES



carbon. The virgin carbon used to pack R1 had a size range from 0.420 mm to 0.850 mm. The results were thus considered to be reasonable.

(d) Specific Surface Area Determination (Asp)

The specific surface area can be determined from (McCabe and Smith, 1976):

$$Asp = \frac{6}{\Phi_s P_p d_p}$$

Where Φ_2 = sphericity = $\frac{\text{surface area of a sphere of same volume}}{\text{surface area of particle}}$

Sphericity is an expression to describe the shape of a particle. Since this data is not available, a suitable approximation would be round sand for $R1_{206}$ carbon particle (Φ_s = 0.83, McCabe and Smith, 1976) and angular sand for virgin carbon (Φ_s = 0.73). The shape of $R1_{148}$ can be taken as in between the two (Φ_s = 0.78). Note that P_p is for dry particle density (i.e. 1.2 x 10^3 kg.m^{-3}).

Thus the Asp can be estimated as:

$R1_{206}$ = 15.4 $m^3.kg^{-1}$ (or 18.5 x 10^3 $m^2.m^{-3}$)

$R1_{148}$ = 19.9 $m^3.kg^{-1}$ (or 23.9 x 10^3 $m^2.m^{-3}$)

$R1_0$ = 21.7 $m^3.kg^{-1}$ (or 26.0 x 10^3 $m^2.m^{-3}$)

Thus no new carbon surface areas were generated from an analysis of the R1 and the virgin particle size. There is in fact a very significant reduction in R1 carbon surface area (32%) after 206 days of operation. However, even then, there is still a very large surface area for film development.

APPENDIX 5

COD BALANCES

Note: Data are from the average values for the operation of R1 at 2 d HRT over a period of 13 days from day 164 to 176 (Appendix 3.3).

(a) Case for tCOD Removal

tCOD removal rate = (25.850 - 4.343) kg tCOD.$m^{-3}.d^{-1}$

= 21.51 kg tCOD.$m^{-3}.d^{-1}$

As 0.348 m^3 of CH_4 is produced at STP from each kg of COD consumed and since the volumetric feed rate of raw stillage was 2.55 $l.d^{-1}$, the theoretical maximum amount of biogas (at 62.3 ± 1.6% CH_4) produced at 37°C and 1 atmosphere

$$= 0.348 \times 21.51 \times 2.55 \times \frac{1}{0.623} \times \frac{310}{273}\ l.d^{-1}$$

= 34.8 $l.d^{-1}$ (based on tCOD removed).

The average gas production rate observed was 33.5 $l.d^{-1}$. Therefore, methane gas yield based on tCOD removed

$$= \frac{33.5}{34.8} \times 100\%$$

$$= 96.3\%$$

Thus only 3.7% of the tCOD fed to the reactor goes to cell growth or is adsorbed onto the GAC at the 2 days HRT loading period.

(b) Case for sCOD Removal

sCOD removal rate = (23.263 - 1.909)

= 21.35 kg sCOD.$m^{-3}.d^{-1}$

i.e. not much difference from tCOD removal rate.

Theoretical maximum amount of biogas produced at 37°C and 1 atmosphere

$$= 0.348 \times 21.35 \times 2.55 \times \frac{1}{0.623} \times \frac{310}{273}\ l.d^{-1}$$

$$= 34.54\ l.d^{-1} \text{ (based on sCOD removed)}$$

Thus methane yield based on sCOD removed

$$= \frac{33.5}{34.54} \times 100\%$$

$$= 97.0\%$$

(c) <u>% CH_4 Gas and Cell Yields Based on sCOD Loading</u>

Stillage sCOD = 23.263 kg $sCOD.m^{-3}$.

The theoretical maximum amount of biogas produced at 37°C based on sCOD loading

$$= 0.348 \times 23.263 \times 2.55 \times \frac{1}{0.623} \times \frac{310}{273}\ l.d^{-1}$$

$$= 37.63\ l.d^{-1}$$

Thus, CH_4 gas yield from sCOD loading

$$= \frac{33.5}{37.63} \times 100\%$$

$$= 89.0\%$$

know % sCOD removal = 91.8%

Therefore, sludge yield or organics adsorbed on the GAC based on 91.8% sCOD removed = (91.8 - 89.0)%

= 2.8%

cf: CSTR reactor : sludge yield 12%
methane yield 78%

Values based on sCOD removal of 90% (Callander et al., 1983)

APPENDIX 6

REACTOR TWO OPERATION ON ANAEROBICALLY TREATED STILLAGE

APPENDIX 6.1: ANAEROBIC LAGOON TREATED STILLAGE CHARACTERISTICS AFTER MODIFICATION

Feed used from date stated / Feed Parameter	10/7/83	12/8/83	27/8/83	12/11/83	27/12/83
Dilution	1/2	3/4	None	None	None
tCOD ($mg.l^{-1}$)	1100	1920	3250	3600	4225
sCOD ($mg.l^{-1}$)	225	700	2375	2160	3550
$tBOD_5$ ($mg.l^{-1}$)				1099	1296
$sBOD_5$ ($mg.l^{-1}$)					196
pH	7.6	7.80	7.90		7.45
Alkalinity ($mg.l^{-1}$)	1322	2189	2804		5221
TS ($g.l^{-1}$)	3.1	6.24	8.17	8.88	9.91
VS ($g.l^{-1}$)	1.44	2.05	3.62	3.87	4.89
TSS ($g.l^{-1}$)	2.21	1.15	2.60	2.62	3.36
VSS ($g.l^{-1}$)	1.33	0.64	1.06	1.29	2.18
Acetate ($mg.l^{-1}$)	35	112	76		125
Propionate ($mg.l^{-1}$)	45	25	0		120
Butyrate ($mg.l^{-1}$)	0	0	0		0
Valerate ($mg.l^{-1}$)	0	0	0		0
Colour (CPU) / Visual Comparison method / pH = 5	500	750	1000		1250
Colour (CPU) / Visual Comparison method / pH = 7.6	560	840	1125		1500
Colour (CPU) / Visual Comparison method / pH = 9.0	880	1310	1750		1750
Colour (CPU) / Spectrophotometric method / pH = 5	2000	3000	4000		2250
Colour (CPU) / Spectrophotometric method / pH = 7.6	3657	5485	7313		3300
Colour (CPU) / Spectrophotometric method / pH = 9.0	4000	6000	8000		4550
$SO_4^{=}$ ($mg.l^{-1}$)		18		280	

APPENDIX 6.2: R2 FEED PREPARATIONS, NUTRIENT ANALYSES AND RELATED PARAMETERS

Date	Feed modifications: Feed Strength	Added Nitrogen ($mg.l^{-1}$ feed)	Added Phosphorus ($mg.l^{-1}$ feed)	Nutrient Analysis ($mg.l^{-1}$): NH_3-N Levels: Feed	NH_3-N Levels: Effluent	Phosphorus Levels: Feed TP	Phosphorus Levels: Effluent TP	Phosphorus Levels: Feed TDP	Phosphorus Levels: Effluent TDP	Phosphorus Levels: Feed RDP	Phosphorus Levels: Effluent RDP	Soluble sulfide Concentration ($mg.l^{-1}$)	Comments
10/ 7/83	0.5												
12/ 8/83	0.75			291	196	107	71				7.0		
23/ 8/83	0.75									8.5	15.2		
27/ 8/83	1											0	
13/ 9/83	1									15.9	1.25		
29/ 9/83	1			276	106					6.08	1.52		
9/10/83	1					227	113			2.05	2.40		
18/10/83	1									10.1	3.50	0	
24/10/83	1									16.1	4.15		
28/10/83	1			310	298								No sign of nutrient deficiency
1/11/83	1									8.25	2.56		
5/11/83	1	240	80	346	328								
9/11/83	1					133	43.8	14.8	8	8.39	6.52		
11/11/83	1												
15/11/83	1			293	304	185	80	27.0	47.5	15.6	3.68	0	High NH_3-N for feed and effluent
21/11/83	1			360	346	82	181		4.31		3.01	0	
29/11/83	1					94	50.3	40	1.75	7.20	1.75	0	H_2S not detected for R2 operation
7/12/83	1			354	368		83.0		15.9		4.90	0	
13/12/83	1			359	378		105		17.0	4.4	1.11	0	Low RDP even with 80 $mg.l^{-1}$ P addition
17/12/83	1				363								
23/12/83	1					107	98	7.85	5.95	5.94	4.05		
26/12/83	1				290								
11/ 1/84	1												

APPENDIX 3.3: R1 EFFLUENT CHARACTERISTICS

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	Visual comparison method			Spectrophotometric method					
																pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
	3/6/83		0																					Reactor seeded 12 a.m.
	4/6		0			7.6																		
	5/6		0			7.3																		
	6/6		0			6.85																		
	7/6		0	7810	250	8.3	1094					65	0	0	0					170				
1	8/6			5900	261	8.2	958	6.55	4.29	4.95	3.17	240	0	0	5	20	20	20	15	18	15		0.00	Start feeding at 5 p.m.
2		25.00	0.430			7.8																	0.18	Use 1:2 diluted feed
3		16.00	0.666	2900	176	7.1	547					120	0	0	0		15		32	32	33		0.40	
4		23.00	0.466			6.4	364																0.76	Add 3gm M4
5		28.20	0.380			6.5	501																0.28	Add 3gm M4
6		26.30	0.408	6000	250	6.5	684					130	0	0	0		5		0	22			0.04	Add 9gm M4
7		22.70	0.473			7.6	1870																0.22	pH normal
8	15/6	26.00	0.408			7.15	1276																0.24	
9		26.00	0.408	3875	425	7.0	1185	4.66	3.30			165	0	0	0		0						0.23	8% bed expansion
10		24.40	0.494			7.0	1185																0.00	
11		26.50	0.404			6.9	1368																0.20	
12		22.60	0.430	6025	600	6.75	1185	5.69	3.96			410	20	0	0		0						0.13	Bed expanded well
13		20.80	0.516			6.9	1140																0.07	
14		24.50	0.438	5525	1095	6.7	1140	5.97	3.64	2.62	1.77	340	25	0	0		0						0.16	Add 4gm M4
15	22/6	23.00	0.466			7.2	1596																0.28	
16		21.70	0.494			6.9	1687																0.30	5% bed expansion
17		33.0	0.322	5338	1235	6.9	1049					690	50	0	0		0						0.13	
18		27.70	0.387			6.9	1322																0.25	1st progress report
19		25.00	0.430			7.0	1642																0.18	R1 content 'clears'
20		28.00	0.384	5075	1175	6.9	1596	5.86	2.75	2.50	1.73	220	100	0	0								0.32	
21						7.1	1596																0.20	pump stopped
22	29/6	26.90	0.707			7.0	1550																0.47	use 3:4 diluted feed
23		29.40	0.633			6,9	1505																0.77	
24		26.30	0.707	3850	1205	7.0	1550	5.64	3.05	2.07	1.65	90	0	80	0								0.90	

APPENDIX 3.3: (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
25		27.40	0.677			7.15	1733																1.06	Add 4gm M4
26		20.00	0.931			6.95	1550																1.05	
27		27.50	0.677	4500	1100	6.9	1550	5.37	2.13	2.22	1.64	630	95	0	0		0						0.71	
28		25.80	0.722			7.0	1505																	gas leak
29	6/7	26.80	0.696			7.0	1550																1.60	
30		17.70	1.050			7.2	1459																1.84	
31		20.00	0.931	1938	825	6.95	1915	3.70	1.31	1.55	0.92	285	555	0	0	0	0	ppt	23	0	ppt		2.02	HIgh C_3 level
32		20.00	0.931			7.3	1368																1.97	Flocs on carbon top
33		28.50	0.651			7.0	1505																1.52	
34		18.50	1.016			7.0	1505																1.53	
35		22.70	0.819	2500	740	7.0	1414	4.22	1.62	1.16	1.03	160	730	0	0	0	0			175			1.28	effluent slightly milky white
36	13/7	22.50	0.826			7.2	1414															62.0	1.39	C_3 high stop loading
37						7.0	1544					70	590	0	0								0.56	Start loading
38		37.50	0.495	1875	675	6.9	1459	2.10	1.53			220	590	0	0		0						0.00	Use long HRT
39		30.60	0.607			6.9	1459																0.21	
40		38.80	0.480			7.0	1550																0.69	
41		56.00	0.335			7.0	1414																0.54	
42	19/7	42.00	0.447	1975	730	6.9	1642	3.50	1.23	1.05		150	600	80	120							57.0	0.50	C_3 still high
43		45.80	0.406			7.2	1444																0.65	
44		38.50	0.484			7.3	1414																0.75	
45		43.00	0.432	3500	1060	7.0	1277	3.98	1.56	2.42	0.92	104	580	90	0		0						0.84	C_3 high. stop feed
46						7.2	1505																0.30	Start with desulphated feed
47		28.00	0.535			7.1	1368																0.38	gas rate dropping
48		45.80	0.328	2100	890	6.9	1520	3.82	1.09	0.78	0.54	90	690	0	0							66.0	0.14	
49	26/7	36.70	0.409			7.0	1368																0.00	Barium toxicity
50		38.50	0.391	2050	725	6.9	1368					45	575	0	0								0.00	Reactor upset
51		38.20	0.394	1875	788	7.0	1414	4.00	1.91	0.88	0.38	75	570	20	0								0.00	Stop feeding
52				2345	777	7.1	1254																0.00	Use 1:10 diluted feed
53		7.10	0.324			7.1	1368																0.00	Add 2.5ml 10% NaOH per litre feed
54		7.04	0.329			7.0	1327																0.00	
55		6.85	0.338	1818	349		1277	4.31	1.85	0.78	0.65	28	245	0	0		0							gas leak

APPENDIX 3.3 (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
56	2/8	6.00	0.387			7.0	1140															35.1	0.71	gas production resumed
57		6.38	0.363			7.0	1049																0.44	% CH_4 low
58		6.63	0.349	1763	533	6.9	1003	3.16	1.54	0.60		105	265	0	0		0						0.57	
59		5.80	0.399			7.05	1117																0.59	
60		5.80	0.399			7.0	866																0.55	Barium 'washout'
61		7.34	0.314	1025	140	6.95	821	1.98	0.29		0.15	140	282	40	55		0					59.5	0.47	Use 3:4 diluted feed
62		33.00	0.451			6.95	556																0.13	
63	9/8	47.00	0.319			7.1	821																0.26	
64		47.00	0.319			6.9	1117																0.30	Add 500ml lagoon seed
65		21.60	0.695	1765	593	6.95	1273	3.76	1.66	0.68	0.61	149	255	55	46		0						1.25	
66		33.10	0.454			7.1	1235																0.74	Bed not fluidized
67		34.10	0.439			6.9	1194																0.59	Reactor side leaks
68		32.10	0.469			7.4	1140																0.75	
69		39.30	0.382	1163	300	6.85	1117	3.16	1.05	0.60	0.26	55	150	0	0		0					36.0	0.59	% CH_4 low
70	16/8	33.10	0.454			7.0	1094																0.73	
71		33.30	0.451			6.9	1078															45.0	0.75	
72		33.10	0.454			7.0	1072																0.78	
73		35.20	0.427																				1.07	
74		35.20	0.427	940	413	6.8	1094	3.45	1.44	0.64	0.27	128	150	0	0		0						1.07	Add 500ml UASB seed
75						6.95	2394																0.82	Stop feed
76				3255	310	6.85	2531	5.90	2.08	4.00		130	105	0	0		10					46.8	0.69	colour appearing due to reactor reseeding
77	23/8	28.40	0.529			6.9	2189																1.29	start feed
78		30.90	0.487			6.9	2280																	gas leak
79		30.30	0.496			5.95	2143																1.32	
80		30.30	0.496	1925	316	5.95	2143	7.14	2.59	4.25	2.13	150	65	12	0		0					54.0	1.45	Reactor fully recovered
81		32.30	0.509			7.10	1870																1.34	Use 0.87 strength feed
82		32.90	0.500			7.20	1733																1.39	120$mg.l^{-1}$P
83		29.80	0.552			7.0	2052																1.82	2.77 equivalent bed volume (EBV)
84	30/8	33.10	0.496	2455	314	7.0	1870	4.78	2.17	2.62	1.38	175	75	8	0		0					62.5	0.96	
85		31.60	0.519			7.05	1824																0.00	
86		32.60	0.503	1713	379	6.95	1801					175	40	0	0		0					48.0	0.61	

APPENDIX 3.3: (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
87		28.60	0.526			7.0	1619																0.09	
88		28.80	0.523			7.05	1948																0.01	
89		30.20	0.505	1333	241	6.95	1801	4.64	1.12	1.71	1.10	185	35	0	0		0							2nd Progress Report
90		39.40	0.382																					gas leaks
91	6/9	39.40	0.382			7.0	1720																	
92		31.30	0.481	1113	241	7.0	1733					42	0	0	0		0					39.0	0.59	photo taken
93		28.60	0.886			7.4	1666																0.89	Use undiluted stillage
94		30.30	0.835			7.2	1615																1.00	
95		63.30	0.962	1120	260	6.9	1679	4.73	1.68	0.99	0.50	65	15	0	0		0					53.5	1.40	End of Acclimatization Phase
96		23.30	1.08			7.0	1756																1.68	Begin increased organic loading phase
97		24.40	1.03			7.0	1710																1.65	
98	13/9	25.00	1.01	1225	318	6.85	1778	4.18	1.91	1.20	0.77	312	92	0	0		0					63.0	1.77	Stable operation
99		25.00	1.01			6.95	1824																1.77	
100		25.60	0.98			7.1	1870																1.50	
101		23.80	1.06	1085	260	6.9	1948					111	20	0	0		5					61.5	1.73	
102		22.20	1.13			7.0	1866																2.30	
103		23.30	1.08			7.05	1824																1.71	3.36 EBV
104		21.70	1.16			7.0	1847																1.77	
105	20/9	22.20	1.13	1200	283	6.85	1907	4.86	1.37	1.01		136	10	0	0		10					57.8	2.40	1st colour breakthrough
106		18.50	1.36			7.1	2280										10						2.01	
107		18.10	1.30			6.95	2029										10						2.47	Reactor load is coming up fast
108		18.60	1.36	1200	296	6.85	2006	4.61	1.49	1.25	0.29	315	35	0	0		10			153		62.5	2.45	
109		17.50	1.44			7.0	1915																	
110		17.50	1.44			6.80	2029																2.29	
111		17.50	1.44	898	290	6.80	1961	4.76	1.88	1.42	0.63	97	0	0	0		10					65.8		gas leaks
112	27/9	14.70	1.72			6.9	2143										10						2.32	
113		14.70	1.72			7.0	2029																	
114		14.70	1.72	1765	310	6.80	2143					255	40	0	0		10					61.0	2.85	Stable operation
115		11.90	2.12			6.9	2217																3.63	
116		11.20	2.25			7.0	2326																4.93	3ml 20% NaOH/l feed
117		11.50	2.20			7.05	2326																4.55	
118		11.90	2.12	1888	393	6.95	2401	5.47	1.48	1.15	0.67	335	155	0	0		10					67.0	4.36	R1 at 38°C

APPENDIX 3.3: (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
119	4/10	13.50	1.87			7.0	2401																3.36	
120		11.40	2.22			7.2	2401																3.65	
121		11.40	2.22	2250	373	7.0	2627	6.52	2.53	2.06	1.76	260	25	0	0		10					61.6	3.52	
122		10.10	2.50			7.0	2554																	
123		10.30	2.45			7.05	2582																4.68	
124		9.80	2.58			7.0	2762																5.77	Crack 5 $l.d^{-1}$ gas production
125		10.20	2.48	2300	455	7.0	2673	7.40	2.07	1.99	0.99	240	63	0	0		10					65.3	5.63	
126	11/10	8.15	3.10			7.05	2786																7.12	200mg.l^{-1} P
127		8.40	3.01			7.05	2831																6.97	
128		8.48	2.98	2050	418	7.0	2763	7.27	2.72	2.74	1.64	203	70	5	5		10					64.0	6.83	
129		8.07	3.14			7.1	2763																6.87	
130		8.20	3.08			7.05	2877																7.21	
131		7.87	3.21			7.05	2945																7.41	
132		8.20	3.08	2455	568	7.0	3035	7.77	2.82	2.71	1.52	185	55	0	0		10					62.5	6.80	H_2S = 0 in gas
133	18/10	7.04	3.59			7.05	3080																8.13	Continuous feeding now
134		8.07	3.14			7.0	3148																6.47	5% bed expansion
135		6.94	3.64	2575	663	7.0	3126	8.96	2.96	3.38	1.43	255	80	0	0		10						8.59	Effluent turbid even after filtration
136		6.67	3.79			7.0	3194																8.30	
137		5.59	4.53			7.1	3398																12.20	
138		5.62	4.50	3125	738	7.0	3443	10.20	4.04	4.49	2.33	155	62	0	0		10					62.0	10.30	6.71 EBV
139		5.00	5.06			7.0	3375																11.30	2.5ml 20% NaOH/l feed
140	15/10	4.95	5.11			7.0	3443																11.80	
141		5.00	5.06			7.05	3443																11.70	Continuous feeding results in - low VFA's
142		5.11	4.95	3513	1024	7.0	3330	9.94	2.97	5.07	2.47	350	85	0	0		10					63.0	12.00	
143		4.05	6.25			7.0	3975																14.10	
144		4.19	6.04			7.0	3375																14.60	more foam. lots of gas
145		3.89	6.50			7.0	3080																14.00	
146		3.89	6.50	3104	970	7.0	3070	9.16	2.62	2.83	1.13	212	28	0	0		20					62.0	14.00	8.74 EBV, Effluent pinkish turbid at pH=7.6 after filtration
147	1/11	3.60	7.04			7.0	3148																17.30	R1 at 37.5°C
148		3.79	6.68			7.0	3148																14.20	
149		16.30	1.54			7.1	3284																6.80	

APPENDIX 3.3: (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
																Visual comparison method			Spectrophotometric method					
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	µi-5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
150		3.40	7.44	3929	1458	7.0	3274	10.00	4.18	2.54	1.83	232	95	0	0		20					62.5	16.4	Feedline blocks
151		25.00	1.01			7.0	3274																4.1	
152		3.82	6.63			7.0	3322																14.4	
153		3.85	6.58	3400	1107	7.0	3080	9.40	3.30	3.34	1.41	192	70	0	0	20	20		820	1100	1200	64.5	14.9	
154	8/11		0.00			7.0	3245																2.1	
155		3.64	6.96			7.0	3096																14.3	
156		3.06	8.28			7.0	3096																17.9	End of INC. organic loading phase
157		2.82	8.99	4175	1450	7.0	2945	11.20	4.68		3.43	180	98	0	0		20						19.7	12.7 EBV
158		2.55	9.92	4915	1265	7.0	3020	10.90	4.62	4.22	2.09	253	164	0	0		20						11.6	New batch of feed 2.0ml 20% - NaOH/l feed
159		2.59	9.97	5040	1663	6.85	2907	10.40	4.53	3.57	2.13	232	169	0	0		20						21.4	
160		2.12	12.20	4950	1800	6.75	2718	10.20	4.80	3.82	2.93	350	185	0	0		30					62.5	20.5	R1 at 37°C
161	15/11	2.63	9.82	4220	1613	6.75	2356	8.47	3.78	1.88	1.12	240	138	0	0		30							2.5ml 20% NaOH/l feed
162		2.81	9.20	3800	1608	6.8	2416	7.98	3.04	1.93	1.33	235	150	0	0		30						23.4	Effluent milky white!
163		2.43	10.65	3825	1638	6.8	2356	8.57	4.22	2.82	2.71	235	135	0	0		75					64.5	25.6	High loading rate
164		1.84	14.06	4020	1750	6.65	2227	8.14	3.09	1.41	0.86	287	252	0	0		75					60.0	33.4	3.5ml 20% NaOH/l feed
165		1.95	13.23	4490	2000	6.75	2227	8.13	3.06	1.54	1.22	328	268	0	0		75					60.5	34.5	4.5ml 20% NaOH/l feed
166		1.94	13.33	3975	2008	6.8	2356	8.26	4.07	1.79	0.42	280	238	0	0		75					61.0	35.2	
167		2.00	12.92	3850	1668	6.8	2378	8.64	3.40	1.58	1.10	322	220	0	0		75					62.0	33.1	300mg/l N
168	22/11	2.02	12.78	4160	1708	6.85	2782	6.31	1.39			172	155	0	0		75					60.0	33.3	18.8 EBV
169		2.26	11.96	4550	1750	6.9	2582	9.35	4.68	3.12	2.29	255	151	0	0		75					64.5	31.2	Recycle flow measurement
170		2.08	12.40	4100	1735	6.85	2514	9.21	4.83	2.59	2.46	340	230	0	0		75					65.0	30.6	
171		1.95	13.23	4530	1980	6.85	2452	9.28	4.76		1.34	310	195	0	0	200	250	400	1500	2150	2600	62.5	33.4	Very stable operation at 2 d HRT
172		1.92	13.49	4550	1285	6.8	2633	8.52	3.15	2.11	1.50	142	155	0	0		250						35.0	
173		1.87	13.85	4490	1928	6.82	2633	10.00	4.45	3.37	2.71	225	144	0	0		300					63.0	35.1	
174		1.93	13.39	4950	2030	6.82	2588	10.20	4.57	3.77	2.64	250	122	0	0		300					63.0	34.0	Colour is coming up
175	29/11	1.92	13.44	4450	2050	6.85	2906	10.30	4.77	4.25	2.47	280	130	0	0		300					63.0	33.7	
176		1.95	13.28		2030	6.9	2815	11.50	5.58	4.39	2.61	190	140	0	0		300					62.5	32.4	
177		1.87	13.85	5710	2325	6.9	2724	10.30	4.95	2.87	2.43	158	145	0	0	350	400	500	2300	2430	3800	61.0	34.3	Reactor top plate cleaned out
178		1.68	15.35	5500	2190	6.9	2701	11.00	5.44	2.82	2.11	234	115	0	0		400					56.0	39.7	
179		1.68	15.35	5640	2525	6.85	2740	10.40	4.36	2.84	1.05	60	100	0	0		400					56.5	35.3	
180		1.68	15.35	5600	2510	6.84	2724	10.70	5.05	3.14	1.15	142	160	0	0		400					57.5	38.3	

APPENDIX 3.3: (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate (KgtCOD.m^{-3}.d^{-1})	Effluent tCOD (mg.l^{-1})	Effluent sCOD (mg.l^{-1})	pH	Alkalinity (mg.l^{-1})	Effluent Solids (g.l^{-1})				Volatile Fatty Acids (mg.l^{-1})				Effluent Colour (CPU)						CH_4 composition (%)	Gas production (l.d^{-1})	Notes
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	Visual comparison method			Spectrophotometric method					
																µil-5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
181		1.73	14.94	5975	2925	6.85	2724	10.10		2.30		87	103	0	0		625					61.5	38.4	
182	6/12	1.68	15.35	5960	2750	6.8	2792	10.60	5.42	4.46	2.63	175	110	0	0	750	875	1250	4750	5750	6575	57.5	39.1	
183		1.75	15.19			6.8	2701																37.0	
184		1.71	15.09	6325	2700	6.85	2838	10.90	4.95	3.07	2.04	160	88	0	0		875	875				61.5	37.0	26.5 EBV
185		1.39	18.61			6.9	2701																44.5	
186		1.36	19.02			6.9	2701																45.3	
187		1.32	19.54	6330	3360	6.82	2679	10.00	4.79	2.38	1.84	210	170	0	0		1000					60.5	43.7	
188		1.50	17.21			6.88	2679										1000					60.5		
189	13/12	1.31	19.74			6.85	2724										1000							gas meter 'stucked'
190		1.32	19.54	6575	3305	6.8	2679	10.20	5.02	2.22	2.10	228	310	0	0	1500	1250	1000	6000	6400	8650	60.5	50.8	31.5 EBV
191		1.31	19.74	5915	3430	6.82	2724	10.30	5.03	2.33	2.14	240	222	23	8		1250					60.5	51.5	
192		1.18	21.92			6.95	2565																58.2	Samples for GC/MS and electron -
193		1.14	22.64	6400	3525	6.9	2633					240	366	0	0		1250					61.0	58.5	microscope scanning
194		1.14	22.64			6.95	2633										1250						58.8	
195		1.14	22.64	6075	3500	6.95	2497	9.78	5.33		2.35	250	358	0	0		1250					61.0	61.20	250mg.l^{-1} P
196	20/12	1.14	22.64			7.0	2565																59.30	Still very stable operation -
197		1.14	22.69	6950	3675	7.0	2679	11.20	5.88	2.80	2.78	258	342	0	0	1250	1250	1500	3900	5400	7900	62.0	62.0	at these high loading rates!
198		1.14	22.64			7.0	2656																59.7	37.5 EBV
199		0.93	26.20			7.0	2724																67.3	New batch of feed
200		1.04	23.47	7320	3445	6.95	2633	11.70	6.78	3.92		225	305	0	0		1000					62.0	62.8	
201		0.98	24.88			6.95	2474																64.3	MERRY X'MAS!
202		0.93	26.15	6640	3315	6.95	2384	9.80	5.42		1.52	275	442	6	0	1000	1000	1250	3450	8100	9500	62.0	68.1	
203	27/12	0.96	25.37			7.0	2360																67.9	6% increased bed Vol.
204		0.91	26.79	6050	3750	7.0	2429	10.30	4.50	1.96	1.61	395	550	6	0		1000					62.5	68.8	9% bed expansion
205		0.91	26.84			7.05	2452																68.7	Well DONE, R1
206		0.94	26.01	5500	3205	7.0	2406					318	460	0	0		1000			2750		61.0	68.6	47.0 EBV
207		0.91	26.74	5150	3483	7.05	2406					372	488	0	0							61.0	68.2	200mg.l^{-1} P
208		0.85	28.79	5815	3354	7.05	2488					225	597	0	0		1000					61.0	74.2	NEW YEAR. 1984
209		0.85	28.79	6175	4075	7.0	2565	10.30	4.94	2.39	0.96	250	708	0	0		1000			3000		61.0	74.3	High C_3
210	3/1	0.85	28.88	5940	4008	7.0	2542	9.57	4.52	1.49	1.17	162	480	0	0	1250	1000	2000	3000	4500	7600	61.0	75.8	C_3 coming down imply stable -
211		0.84	28.98	6220	3843	7.0	2588	10.20	5.94	2.66		160	490	0	0		1000					61.5	74.2	operation End of reactor operational phase 51.7 EBV

APPENDIX 3.3: (CONTINUED)

Days of Operation	Date	Observed HRT (days)	Loading Rate ($KgtCOD.m^{-3}.d^{-1}$)	Effluent tCOD ($mg.l^{-1}$)	Effluent sCOD ($mg.l^{-1}$)	pH	Alkalinity ($mg.l^{-1}$)	Effluent Solids ($g.l^{-1}$)				Volatile Fatty Acids ($mg.l^{-1}$)				Effluent Colour (CPU)						CH_4 composition (%)	Gas production ($l.d^{-1}$)	Notes
								TS	VS	TSS	VSS	Acetate	Propionate	Butyrate	Valerate	Visual comparison method			Spectrophotometric method					
																pH=5.0	pH=7.6	pH=9.0	pH=5.0	pH=7.6	pH=9.0			
212	3/1	2.08	11.7	5900	3390	7.20	2611					110	165	0	0		1000			2800		62.0	36.6	Phase 4: Bioregeneration confirmatic phase
213		2.19	11.1	5260	2938	7.30	2588					100	130	0	0		750			5625		62.0	28.5	VFA level dropped within 24 hours at 2d HRT
214		2.00	12.2			7.30	2611																32.4	
215		1.95	12.5	5290	2885	7.35	2662	9.19	5.06	1.60	0.80	152	60	0	0	875	625	1250	2875	4200	7500	62.0	32.7	Very stable reactor operation
216		2.03	12.0			7.30	2656																31.0	
217	10/1	1.95	12.4			7.30	3110																31.0	
218		1.85	13.1	6800	2900	7.22	3369	12.10	7.00	4.88	3.35	190	82	0	0	750	500	1000	4125	6375		60.0	31.2	Colour coming down fast
219		1.92	12.6			7.30	3110																29.0	
220		1.97	12.3			7.28	2951																31.8	
221		1.85	13.1	6520	3038	7.25	2682	10.50	5.29	3.05	2.29	23	15	0	0	750	375	1000	4050	5625	7500	62.5	36.8	
222		2.01	12.1			7.26																	31.3	
223		2.08	11.7	5930	2863	7.15	2453	9.51	4.33	2.50	1.63	142	40	0	0	750	375	1000	3250	4775	7200	61.0	29.6	
224	17/1	1.91	12.7			7.25	2225																32.0	
225		1.95	12.5	5275	2505	7.22	2270	8.70	4.60	1.45	1.25	162	100	0	0	750	375	1000		3938	7188	61.0	32.0	low effluent sCOD
226		1.85	13.1	4275	2258	7.25	2247	8.53	3.54	1.85							375			4875			32.4	Colour stabilize at 375 CPU at pH 7.
227		1.97	12.3	3980	2215	7.20	2134					170	93	0	0	750	375	1000		4700		60.0	30.5	60 EBV

APPENDIX 7

R2 COD REMOVAL-ACCOUNTABILITY

(a) Solubility of CO_2 in Water

From Henry's Law:

$$x_a = \frac{P_A}{H_A}$$

where: x_a = mole fraction of CO_2 in solution

P_A = partial pressure of CO_2
= 0.9 atmosphere (assuming 10% CH_4)

H_A = Henry's Law constant
= 2.19×10^{-3} at 37°C (Perry and Chilton, 1963)

Thus,

$$x_a = \frac{0.9}{2.19 \times 10^{-3}}$$

$$= 4.11 \times 10^{-4}$$

Volume of liquid = 5 l
= 5.0 kg
= 0.2777 kg moles

$$\text{from } x_a = \frac{\text{kg moles of } CO_2}{\text{Total kg moles of water}}$$

$\therefore$ maximum CO_2 that can be dissolved in 5 l of water at 37°C

= 4.11×10^{-4} x 0.2777 kg moles

= 0.114 kg moles

= 5.03×10^{-3} kg

know density of CO_2 = 1.741 $kg.m^{-3}$ (Tennent, 1971)

$\therefore$ volume of 5.03×10^{-3} kg CO_2

$= 2.87 \times 10^{-3}\ m^3$

$= 2.87\ l$

The maximum amount of CO_2 that can be dissolved in R2 is expected to be higher because R2 liquor had pH greater than 7.0 and contained dissolving species (e.g. calcium ions) that can react with CO_2.

Working at 1.14 d HRT, the maximum amount of CO_2 that can be removed $= \frac{2.87}{1.14}\ l.d^{-1}$

$= 2.52\ l.d^{-1}$ at 37°C

$= 2.52 \times \frac{273}{310}\ .d.^{-1}$ at STP

$= 2.22\ l.d^{-1}$ (STP)

(b) COD Equivalence of CO_2

Assume COD removed consists of $C_6H_{12}O_6$. Stoichiometrically,

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6\ CO_2 + 6H_2O$$

i.e. 1 mole $CO_2 \equiv$ 1 mole O_2

know 2.22 $l.d^{-1}$ CO_2 (at STP) can be removed per day

$= 0.0973$ mole CO_2

Thus the COD equivalence per day

$= 0.0973$ mole $O_2 \times 32\ g.mole^{-1}$

$= 3.12$ g COD removed

or $= 3.12 \times 10^3$ mg $COD.d^{-1} \times \frac{1.14}{5}\ d.l^{-1}$

$= \underline{711\ mg.l^{-1}}$ COD removed

In practice:

$$\text{tCOD removed} = 3600 - 2605 \text{ mg.l}^{-1}$$

(Appendix 6.1; Table 4.9)

$$= \underline{995 \text{ mg.l}^{-1} \text{ COD}}$$

This does not take into account of the small amount of COD conversion to methane, bacterial cells and other fermentative products (if any). Thus, the CO_2 - COD balance suggests that fermentative processes other than methanogenesis were implicated in R2 operation.

Similar results were also obtained if the calculations were done for the 0.93 d HRT period (i.e. estimated 580 $mg.l^{-1}$ COD removed from CO_2 consideration as compared to observed COD removal of 775 $mg.l^{-1}$).