APPLICATION OF BIOGRANULES IN THE ANAEROBIC TREATMENT OF DISTILLERY EFFLUENTS

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

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ABSTRACT

The distillery industry produces large volumes of waste water with a high organic content throughout the year. These effluents must be treated in some manner before being discharged or recycled in the factory. Several treatment options are in use presently, but they all have disadvantages of some nature, such as long retention times, bad odours or the need for large areas of land. Considerable interest has been shown in the application of anaerobic digestion, especially the UASB design (upflow anaerobic sludge blanket), to treat this high strength waste water. Thus, the aim of this study was to investigate the efficiency of an upflow anaerobic sludge blanket (UASB) bioreactor using full-strength distillery effluent. The activity of the bacteria in the biogranules was also evaluated by developing an easy and reliable activity method to estimate the general biogas and methanogenic activity and to calibrate this method using different anaerobic granules from different sources.

The influence of high strength distillery effluent on the anaerobic digestion process was investigated using a mesophilic lab-scale UASB bioreactor. During the experimental study, the organic loading rate (OLR) was gradually increased from 2.01 to 30.00 $kgCOD.m^{-3}.d^{-1}$, and simultaneously, the substrate pH was gradually lowered from 7.0 to 4.7. It was found that at an OLR of 30.00 $kgCOD.m^{-3}.d^{-1}$, the pH, alkalinity and biogas production stabilised to average values of 7.8, 6 000 $mg.l^{-1}$ and 18.5 $l.d^{-1}$, respectively. An average COD removal > 90% was found indicating excellent bioreactor stability. The low substrate pH holds considerable implications in terms of operational costs, as neutralisation of the biorector substrate is no longer necessary. The accumulation of fine solids present in the distillery substrate was found at the higher OLR's and resulted in the granular bed increasing with subsequent biomass washout and a lowering in efficiency parameters. However, a possible pre-treatment filtration of these fine solids would eliminate this problem.

The success of the upflow anaerobic sludge bed (UASB) process is mainly due to the capability of retaining the active biomass in the reactor. Over the years, several methods have been developed to characterise and quantify sludge activity but each has advantages and disadvantages. There is thus an increasing need for

a rapid method to evaluate the activity of the granular biomass. The activity method of Owen *et al.* (1979) as adapted by Lamb (1995), was thus evaluated in terms of efficiency and applicability in determining the activity of granular samples. The method was found to be inaccurate as well as time consuming and it was thus modified. Results obtained with the modified assay method were found to be more accurate and the impact of the different test substrates (glucose, lactate, acetate and formate) on activity, was more evident. The activity of seven different anaerobic granules, was subsequently evaluated. Biogas (S_B) and methanogenic (S_M) activity was not measured in volume of gas produced per unit COD converted or volatile suspended solids (VSS), but as tempo of gas production ($ml.h^{-1}$) in a standardised basic growth medium. The activity data obtained were also displayed as bar charts and "calibration scales". This illustrative depiction of activity data gave valuable information about population dynamics as well as possible substrate inhibition.

The "calibration scales" can also be used to group the general biogas (S_B) and methanogenic activities (S_M) of any new biogranule relative to active (O-type) and inactive (W-type) anaerobic granules, providing that the same method of activity testing is used. The "calibration scales" can thus be used to give a fast indication of how the activity value of one sample relates to the activity values of other granules, even when using different test substrates.

UITTREKSEL

Die stokery industrie produseer groot hoeveelhede afvalwater, wat hoë ladings van organiese materiaal gedurede die hele jaar bevat. Hierdie afvalwater moet op een of ander manier behandel word voordat dit gestort of vir hergebruik aangewend kan word. Daar is tans verskeie behandelingsmetodes wat gebruik kan word, maar elk het sy eie tekortkominge soos bv. lang retensie tye, onaangename reuke of die behoefte aan groot stukke oop grond. Groot belangstelling is getoon vir die gebruik van anaerobiese vertering, en meer spesifiek die "uflow anaerobic sludge blanket" UASB bioreaktor vir die behandeling van stokery uitvloeisels. Die doel van die studie was dus om die algehele effektiwiteit van 'n UASB bioreaktor, wat onverdunde stokery uitvloeisel behandel, te evalueer. Die methanogene- en algehele aktiwiteit van die bakterië in die biogranules was ook ge-evalueer deurdat 'n maklike en betroubare aktiwiteitsmetode omtwikkel is, waarna hierdie metode ook toegepas was op 'n reeks van verskillende tipe biogranules.

Die invloed van volsterkte stokery uitvloeisel op die anaerobiese verteringsprosesse was ondersoek met die gebruik van 'n mesofiele laboratoriumskaal UASB bioreaktor. Gedurende die eksperimentele studie, was die organiese ladingstempo (OLT) verhoog van 2.01 na 30.00 $kgCSB.m^{-3}.d^{-1}$ (CSB = chemiese suurstof behoefte) met die gelyktydige verlaging in die pH van die bioreaktorsubstraat van 7.0 na 4.7. Dit was vasgestel dat met 'n OLT van 30.00 $kgCSB.m^{-3}.d^{-1}$, die pH, alkaliniteit en biogas geproduseer, gestabiliseer het na gemiddelde waardes van 7.8, 6 000 $mg.l^{-1}$ en 18.5 $l.d^{-1}$, respektiewelik, sowel as 'n gemiddelde CSB verwydering van > 90%. Al hierdie waardes dui uitstekende bioreaktor stabiliteit aan. Die lae bioreaktorsubstraat pH kan van groot waarde wees vir die industrie, aangesien neutralisering van die uitvloeisel nie meer nodig is nie en kan sodoende die operasionele koste van die proses verlaag. Die konsentrering van fyn opgeloste soliedes in die bioreaktor by hoë OLT's, kan egter problematies raak, aangesien dit die granule-bed kan vergroot en veroorsaak dat van die biomassa uitspoel en kan verlore gaan. Die verlies van aktiewe biomassa kan die effektiwiteitsparameters negatief beinvloed, maar die plasing van 'n filterings stap voor die verterings stap, behoort hierdie probleem op te los.

The sukses van die UASB-stelsel rus op die versekering dat die aktiewe biomassa in die reaktor behoue bly. Oor die jare was daar 'n verskeidenheid van aktiwiteitstoetsings-metodes ontwikkel, elk met sy eie nadele. Daar bestaan dus nogsteeds 'n groot behoefte vir die daarstelling van 'n aktiwiteitstoetsings-metode wat vinnig en maklik is om uittevoer. Die aktiwiteitstoetsings-metode van Owen *et al.* (1979) wat deur Lamb (1995) aangepas is, was in terme van sy effektiwiteit en toepaslikheid ten opsigte van die gebruik daarvan vir aktiwiteitstoetsing vir biogranules, ge-evalueer. Dit is bevind dat die metode onakkuraat sowel as tydsrowend was en gevolglik dus aangepas. Die aangepaste metode het meer akkurate resultate gelewer en die impak van die verskillende toetssubstrate (glukose, laktaat, asetaat en formaat) op die granules het ook meer duidelik na vore gekom. Gevolglik was die aktiwiteit van sewe verskillende anaerobiese biogranules ondersoek. Die eenheid waarin atiwiteitsresultate aangegee is, was nie in volume gas geproduseer per eenheid CSB verwyder of per hoeveelheid gesuspendeerde vlugtige vetsure in die biomassa nie, maar as tempo van biogas (S_B)- of metaan (S_M) produksie ($ml.h^{-1}$). Die data wat op hierdie wyse bekom was, is gebruik om staafdiagramme sowel as "kalibrasie skale" daar te stel. Hierdie illustrerende wyse om aktiwiteitsdata uit te beeld verskaf waardevolle informasie ten opsigte van die interaksies tussen die verskillende populasies in die granule en kan ook die aanwesigheid van moontlike substraat inhibisie aandui. Die "Kalibrasie skale" kan ook gebruik word om die algehele (S_B) en methanogene (S_M) aktiwiteite van einge nuwe biogranule vinnig te klassifiseer ten op sigte van 'n aktiewe (O-tipe) en 'n minder aktiewe (W-tipe) anaerobiese granules, mits dieselfde metode gebruik word om die aktiwiteits data te bekom.

dedicated to my parents

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CONTENTS

Language and style in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology.* This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some redundancy between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

Water is South Africa's biggest resource problem and national projections indicate that the water demand will overtake the supply by between 2020 and 2030, although absolute water shortages already occur on a regional basis (Pubys, 1999). Environmental pressures and the resultant financial and legal implications are forcing the industries to make strenuous efforts to minimise the pollution level of wastes remaining after industrial manufacturing processes such as distillation (Frostell, 1981; De Bazúa & Cabrero, 1991). Distilleries, as part of the fermentation industry, generate large volumes of effluent that need to be treated, but the high pollution levels associated with these types of effluents as well as the original substrate composition variations, limits the treatment options.

The direct land irrigation might be a satisfactory solution in principal, as it allows the recycling of nutrients. In temperate climates, however, severe pollution problems arise because of waste drainage into ground waters, rivers and streams. Evaporation followed by incineration or the use of feed stock is also an option, but only if the increasing energy cost can be absorbed by the marketability of the evaporated product (Frostell, 1981; Maiorella *et al.*, 1983). Biological treatment appears to be the most promising treatment technique, since distillery effluent consists mainly of organic matter. It has been generally excepted that anaerobic methanogenic fermentation is better suited to the treatment of high strength effluents than aerobic biological treatments. In the case of distillery wastes, it has been shown that this technology can reduce high concentrations of organic pollutants while producing biogas which could cover a part of the energy requirements (Ehlinger *et al.*, 1992).

The use of anaerobic digestion for the treatment of industrial effluents was pioneered in the fifties by Stander & Syders (1950) and by Schroepefer *et al.* (1955). This concept has grown and has become popular in Europe and in other parts of the world like China, Japan and Brazil (Schmidt & Ahring, 1996). Anaerobic treatment is essentially a conversion process in which 60 - 80% of the chemical energy available is converted to methane gas (Ross, 1991). Only recently, high-rate anaerobic upflow designs have been applied, with the subsequent development of the highly successful upflow anaerobic sludge blanket (UASB) reactor (MacLeod *et al.*, 1990).

The formation of microbial aggregates into granular sludge permits the retention

of a greater amount of active biomass, compared to the other anaerobic reactors. These UASB systems can, therefore, maintain much higher organic loading rates than other similar systems and this leads to the more effective treatment of high strength distillery effluents (MacLeod *et al.*, 1990). The good settling properties of these granules also limit the amount of biomass washout, which is a serious problem in other reactor designs. The UASB reactors are easy to operate as well as economical, because of the lower maintenance costs. The production of biogas which moves to the upper section of the reactor, besides being used for combustion, causes a natural flow of nutrients throughout the reactor, reducing the cost of manual mixing devices and other energy related costs. With the granular sludge, the specific surface to which the substrate is subjected to ensures extremely good contact between the waste water and the biomass, favouring the effective removal of organic pollutants (Hanaoka *et al.*, 1994). The compact nature of these granules also ensures that they can withstand the high hydraulic shear rate caused by the upward flow of effluent (Quarmby & Foster, 1995). Because of the long generation times of the methanogens, extensively long start-up periods of between four and eight months are needed (Lettinga, 1995). The successful industrial operation of the UASB reactors thus relies on the presence of these suspended bacterial aggregates, as well as the activity of the different microbial populations within the granules.

Consortium activity in an anaerobic digester is defined as the substrate dependent biogas/methane production rate per unit mass of volatile solids of biomass (Sørensen & Ahring, 1993). In general, activity testing involves the addition of specific substrates to either a continuous or a batch biomass system, followed by the measurement of the biogas produced (Sørensen & Ahring, 1993; Lamb, 1995; Angelidaki *et al.*, 1999). Sludge or granule activity measurements can be either an overall measurement, giving an indication of the total activity of the process, or a measurement of each basic stage in the digestion process. The total activity measurement can be used to assist in the selection of a suitable inoculum for an anaerobic digester. In contrast, the individual population activity determination can shed light on potential unbalanced situations between the different bacterial populations (Soto *et al.*, 1993). The methanogenic population's specific activity is of great importance since they are the final electron acceptors in the degradation processes. The failure of methanogens to produce methane or even their absence or inhibition can result in the accumulation of high concentrations of volatile fatty acids, which will lead to the lowering of the digester system pH and subsequent system failure. Monitoring the

activity performance of the granular biomass in terms of specifically the methanogenic activity is thus essential to prevent digester failure that is directly caused by unfavourable environmental conditions (Meyer & Oellerman, 1994).

There are a few well-established methodologies that have been used to monitor the activity of methanogenic biomass and include measuring either the volume of biogas, type and concentration of the volatile fatty acids produced or the rate at which the substrate is utilised. These methods are often inaccurate and time-consuming (Dolfing & Mulder, 1985) and some also require expensive equipment (James *et al.*, 1990). Modifications of the original tests have also been developed and currently researchers are investigating the use of co-enzymes, biosensors and ATP for assessing the activity of anaerobic biomass (Pause & Switzerbaum, 1984; Chung & Neethling, 1988; and Yamaguchi *et al.*, 1991; Angelidaki *et al.*, 1998).

The main objectives of this study were firstly, to evaluate the feasibility of applying anaerobic digestion technology, in particular the UASB design, in treating high strength effluent from wine distilleries. Secondly, to develop an easy to use and reliable method to determine the methanogenic activity of anaerobic UASB granules and then to apply this method to determine the activity level of different types of anaerobic granules. This will be done to establish a calibration activity range whereby the relative activity of the methanogenic population of an anaerobic granular sample, can be estimated.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

South Africa is a water scarce country where the average rainfall per year is just over half of that of the world's rainfall (Pybus, 1999). Extended and severe droughts occur regularly and the rainfall is unevenly distributed between the coastal and mountainous regions. Furthermore, only 15% of South Africa's water supply is derived from groundwater, with the remaining 85% coming from surface-water, which is subjected to high evaporation rates. Projections have indicated that the water demand will overtake the supply between 2020 and 2030, but absolute water shortages already occur on a regional basis and this has necessitated the construction of major inter-basin transfer schemes (Pybus, 1999).

Industrial use of water accounts for around one quarter of the world's water demand. This has led to the increasingly stringent environmental legislation and associated escalating costs of water supply and discharge. With this in mind, it is not surprising that industrial wastewater recycling has become of great significance. Recycling of water usually involves the treatment at municipal water works, with some industries including a pre-treatment process (Judd, 1999).

Distilleries, as part of the fermentation industry, generate large volumes of effluent that need to be treated. However, the high pollution levels associated with the effluent as well as substrate composition diversity, limit treatment options.

In 1993, the specific water intake of the spirit distillation industry was reported to be between 1.8 to 6.2 litre's of water per litre of product produced (Water Research Commission, 1993). Furthermore, the water used is also soiled with a specific pollution load between 95 to 145 kg COD per hecto-litre of product produced. Wine distillery effluent contains residual organic acids, soluble proteins, carbohydrates and various inorganic compounds. The composition of distillery wastewater varies considerably from one plant to another according to the origin of fermentation materials and the distillation process (Water Research Commission, 1987; Harada *et al.*, 1996).

In the last two decades, much research has been done on evaluating suitable methods to treat distillery effluents (Maiorella *et al.*, 1983; Romero *et al.*, 1988; Shin *et*

al., 1992; Garcia-Calderon *et al.*, 1998). Most procedures are based on treating the effluent to remove and/or concentrate the organic compounds and include methods like evaporation and feed yeast production. The disposal of distillery waste by means of evaporation or drying is only feasible and economical if the product produced can be successfully marketed. The use of membrane technology also seems plausible, but more extensive research needs to be done to combat the rapid fouling of some of these systems (Burton *et al.*, 1999). Reverse osmosis has also been used in the treatment of distillery effluent. The lack of understanding, however, has limited the efficiency of this method. Advances in this area have shown that this treatment option can be successfully implemented, particularly for cane molasses distillery wastewater (Water Research Commission, 1987).

The production of biogas through anaerobic digestion and aerobic wastewater treatment has also been considered (Braun & Huss, 1982). Anaerobic digestion has been shown to be an efficient and economical treatment option for wine distillery and other distillery effluents (Ross, 1991). Various anaerobic digester designs have successfully been used (Braun & Huss, 1982). Furthermore, digesters treating wine distillery effluent develop high alkalinity levels of up to 6 000 $mg.l^{-1}$. This is due to the high potassium bi-tartrate concentration and subsequent release of potassium ions. These potassium ions then aid in buffering the system during digestion (Ross, 1991).

One digestion design that has been used is the upflow anaerobic sludge blanket (UASB) bioreactor. Previously the use of the UASB process was restricted to low and medium strength effluents and it was believed that for distillery slops, with concentrations of up to 120 g total solids per litre effluent, the UASB would not be very suitable. The use of the anaerobic contact process as well as clarigesters were reported to be more successful (Ross, 1991). The use of anaerobic filters and plug flow reactors have also been used and showed excellent results in treating molasses, maize and potato distillery slops (Braun & Huss, 1982). The latest technology in anaerobic granular digestion is the Biobed EGSB (expanded granular sludge bed) system, which combines UASB technology with the fluidised bed process. Granular sludge can be grown and upheld under higher liquid flow rates (10 $m.h^{-1}$) and gas velocities (7 $m.h^{-1}$). The ESGB reactor is especially suited to treat effluents that contain compounds that are toxic in high concentrations and, therefore, ideally suited for treating distillery effluent which has high concentrations of phenolic compounds (Britz *et al.*, 1992; Shin *et al.*, 1992). In the EGSB reactor, the ultra high loading rates of the fluidised bed process can be achieved with the UASB's ability to obtain a settled granular biomass. Already

more than 20 full-scale Biobed EGSB reactors are in operation world-wide (Zoutberg & de Been, 1997).

A further advantage of the anaerobic digestion of distillery effluent lies in the utilisation of the biogas produced during the anaerobic process. The biogas can be used for heating purposes in the distillery, thus lowering energy costs, which have been estimated to be in the range of 63 to 100% (Braun & Huss, 1982). Low operational costs and low residence times are also of great importance. Low residence time enables the system to treat greater volumes of effluent in a shorter time, and means that more effluent can be treated in a shorter time enhancing the economical efficiency.

B. DISTILLERY TREATMENT OPTIONS

Over the last decade, research has been conducted in several countries in an attempt to find effective methods to handle distillery vinasses (Racault, 1990; Shin *et al.* 1992; Kida *et al.*, 1994; García-Bernet *et al.,* 1998). Environmental pressures and the resultant financial and legal implications are forcing the industries to make even more strenuous efforts to minimise the pollution level of the wastes remaining after distillation (Frostell, 1981; De Bazúa & Cabrero, 1991). When considering a treatment option for a specific distillery plant, the type of raw material used for fermentation and the resulting type of waste, the regulations for waste disposal as well as the energy profile of the plant must always be taken into consideration. These conditions vary considerably from distillery to distillery and subsequently a broad variety of treatment solutions may be found, each with it's own drawbacks and advantages (Frostell, 1981). The different distillery waste characteristics of different raw products are reported and summarised in Table 1.

One solution for the disposal of distillery effluent (stillage) is by land application. The direct irrigation on land might be a satisfactory solution in principal as it allows the recycling of nutrients. In temperate climates, however, severe pollution problems may arise because of waste drainage into the ground waters, rivers and streams. The high potassium content (0.8% of the total solids) applied to the soil over an extended time period may affect the sodium adsorption ratio, which is important in controlling soil permeability (Springer & Goissis, 1988). The land irrigation of stillage will also increase soil acidification and change the nature of the microbial population present (Springer & Goissis, 1988). Ross *et al.* (1938) set the maximum broad irrigation rate of stillage at

Table 1. Distillery waste characteristics of different raw materials used (Sheehan & Greenfield, 1980).

Characteristics ($g.l^{-1}$)	Distillery type					
	Molasses		Grain		Wine	
	Range	Average	Range	Average	Range	Average
pH	3.5-5.7	4.2	3.8-7.5	5.4	3.9-4.5	4.1
Temperature (ºC)	80-105	94	42-95	73	-	-
Total solids	21-140	78.5	20.5-47.3	33.8	24-125	62
Volatile solids	40-100	58.9	24-36	29.5	-	29.5
Suspended solids	1-13	5.1	-	11.4	0.2-0.9	0.55
Dissolved solids	25-110	56.9	-	-	-	22
Crude Fibre	-	-	-	10	-	-
Ash	16-40	28.9	-	3.6	-	-
Volatile fatty acids(acetic)	0.7-5.5	2.18	1.8-2.4	2.10	-	0.75
Reducing sugars	14-45.0	26.50	10.9-30.5	24.0	-	-
Fats and oils	-	-	-	2.9	-	-
Total nitrogen	0.6-8.9	1.78	0.2-1.9	0.98	0.4-1.0	0.69
Organic nitrogen	0.6-8.7	1.94	1.4-2.1	1.73	-	-
Ammoniacal nitrogen	0.04-0.89	0.26	0.01-0.09	0.05	0.01-0.05	0.03
Sodium (Na_2O)	0.13-2.51	1.04	-	-	--	1.34
Potassium ($K_2O_)$	4.80-22.59	10.73	-	-	-	16.46
Calcium (CaO)	1.26-6.70	3.52	-	-	-	1.34
Magnesium (MgO)	0.66-2.35	1.63	-	-	-	2.35
Phosphorus (P^{5+})	0.026-0.326	0.168	0.039-0.087	0.063	-	1.17
Silicate (SiO_2)	-	1.51	-	-	-	0.51
Chlorate (Cl^{-1})	0.68-7.39	3.79	-	-	-	1.34
Sulphates (SO_4^{2-})	1.56-6.60	4.36	-	-	-	3.64
Total Iron (Fe^{3+})	0.001-0.120	0.690	-	-	-	-
Copper (Cu^{2+})	0.004-0.030	0.014	-	-	-	-
Zinc (Zn^{2+})	0.027-0.225	0.115	-	-	-	-
COD	15-176.0	77.7	-	-	-	-
BOD_5	7-95.0	35.7	15-340	22.2	-	12.3
					-	

92.9 $m^3.ha^{-1}.d^{-1}$, but stated that odours and putrefaction may cause problems. Likewise, Sastry & Mohanrao (1964) did not consider land treatment economical if odour problems are not well controlled. Studies by Benke *et al.* (1999), where stillage irrigation levels ranging from 0 to 1 376 $m^3.ha^{-1}$ were simulated, concluded that continuous application of high doses of stillage to land may saturate adsorptive sites within the ground. This would increase possible leaching of dissolved organic carbon and the possibility of contaminating ground waters. In contrast, Cunha *et al.* (1987), who also investigated the effect of stillage irrigation on sugar-cane plantations, found that at an irrigation rate of 800 $m^3.ha^{-1}$, little or no leaching of potassium and nitrate occurred. The soil retained part of the applied potassium and the sugar-cane plants absorbed a large part of the nitrogen and potassium that had been applied to the soil. This study did not, however, consider the influence of prolonged irrigation on the soil character. Land irrigation of distillery effluent can be considered but only after extensive research has been conducted on the effects thereof on the soil. Springer (1985) found that land irrigation of stillage was effective for only five years after which most plants experienced soil-plugging problems and odour development became a serious problem.

The use of ponds has also been considered and it is reported that it can achieve any required degree of purification at very low costs, with minimum maintenance of unskilled operators. Maintenance includes regular cutting of grass embankments, removal of floating scum from the pond surface and dredging the pool every 5 - 10 years to remove accumulated solids in order to maintain detention times in the proper range (Springer & Goissis, 1988). Disposing of these solids could, however, influence the applicability of ponds for the treatment of stillage. For strong wastes (COD > 17 000 $mg.l^{-1}$) such as stillage it has been found to be advisable to use a combination of anaerobic and aerobic ponds in series. Too many ponds after each other, could cause plug flow conditions to be reached, which would make the system sensitive to shock loads. Experiments done by Sringer & Goissis (1988) showed that a reduction in BOD (biological oxygen demand) from 5 750 to 3 500 $mg.l^{-1}$ in an anaerobic lagoon within 24 d, followed by a further reduction to 2 500 $mg.l^{-1}$ in the aerobic lagoon within 20 d, could be obtained. Rao (1972) also did research on two lagoons in series with the anaerobic lagoon obtaining BOD removals ranging from 95 to 55% after 66 - 38 d followed by the aerobic finishing lagoon for 43 - 24 d to give overall removal ranging from 92 to 83%.

The bottom of ponds or lagoons should be impermeable to prevent stillage leaching to the ground water level. This can be done by sealing with polythene sheeting placed between two 100 mm layers of selected fills such as clay, bitument or asphalt.

The impermeability of the clay and its capacity to absorb organics makes it a very effective liner (Büchler, 1987; Sringer & Goissis, 1988). The treatment of stillage by means of ponds or lagoons is environmentally favourable but the possible development of odour problems must always be taken into consideration. The availability of a large area of suitable land adjacent to the distillery in a low to medium rainfall area is also a restricting factor for both ponds and land irrigation as pipeline costs, when such land is not readily available, could also prevent extensive land application (Sheehan & Greenfield, 1980).

It has also been reported that stillage could to be evaporated to provide animal feed, fertiliser, or to undergo incineration with possible recovery of the potash (Sheehan & Greenfield, 1980). Montanani (1954) reported on a system in which the stillage is neutralised with lime, evaporated in 10 cm shallow containers and used for fertiliser. In France, the stillage is concentrated to 60% and then applied as fertiliser at a rate of 2.5 - 3 ton.ha^{-1} (Lewiki, 1978). Specific chemicals with fertiliser value, such as gypsum ($CaSO_4.2H_2O$), potassium (K_2SO_4), can also be extracted from stillage (Zabrodskii *et al.*, 1970). Potash recovery also yields a product containing ca. 40% K_2O and could be further refined to provide 83% K_2SO_4 and 9% KCl for use in fertilisers (Dubey, 1974). As with direct land application, the economics of evaporation and/or incineration rely heavily on the fertiliser value of the resultant product. The product may be in a much more convenient form for handling and transport, but the energy inputs must always be taken into account (Sheehan & Greenfield, 1980). This option can, therefore, only be feasible if the high energy cost can be neutralised by the marketability of the evaporated product (Frostell, 1981; Maiorella *et al.*, 1983).

The production of value-added biochemicals or the production of biomass, where stillage is utilised as raw material, is also becoming increasingly more attractive as the price of oil-based competitors becomes less favourable. The most important of these is the use of stillage for the aerobic production of fodder yeast. This reduces the carbohydrate content and hence the BOD of the stillage. Some studies (Tomczynska, 1971) showed an initial BOD of 23 000 mg.l^{-1} reduced by 40 - 50% after yeast growth on the stillage. Yeasts such as *Candida utilis, Candida tropicalis* and *Candida scottii* can be used and have received the most attention. Little research, however, has been done on the cultivation of fungi and algae on stillage. Okuba *et al.* (1967) grew algae such as *Chlorella vulgaris* and *Chlorella pyrenoidosa* on stillage at 30° - 32°C with an aeration rate of 360 volumes air per volume liquid per hour ($v_a.v_l^{-1}.h^{-1}$). Although substantial reductions in BOD and COD were obtained, the addition of 10 mg.l^{-1}

chloramphenicaol was necessary to prevent contamination in an open tank. Various fungi can also be grown on sterilised stillage to reduce the BOD and COD as was done with the growth of *Penicillium* on wine stillage at 20°C that had been supplemented with $(NH_4)_2HPO_4$ (Magny *et al.*, 1977). After stillage has been used as a substrate for fodder yeast growth, the remaining liquor still had a high BOD since all the easily removable organics had been utilised. Further biological treatment thereof could thus be more troublesome than treating just raw stillage (Kujala *et al.*, 1976).

Distillery effluent in its dry form can also be used as fodder yeast. Daily weight gains of cattle used in a trial done by Shcherbak *et al.* (1967) were 50 to 80 g higher when distillery effluent (1 kg of molasses per day) was used. Dairy cows fed with 4 $kg.d^{-1}$ of 91% straw and 9% stillage supplemented with protein gave 1 kg extra milk per kg of stillage fed. The distillery effluent does, however, also tend to be laxative in cattle (Dubey, 1974) and the amount used in feeds must be restricted (0.5 - 1 $kg.aminal^{-1}$) due to the high level of potassium.

Physical-chemical treatment of distillery effluent include the precipitation of dissolved solids from the stillage, followed by the removal of the precipitated solids with physical treatments such as sedimentation, centrifugation and filtration (Sheehan & Greenfield, 1980; Sales *et al.*, 1986). Sales *et al.* (1986) reported that the use of $Ca(OH)_2$ gave better results in the precipitation of tartaric acid, total nitrogen, phosphates and polyphenols than NaOH. Their study also showed that filtration is the most effective means of removing the precipitate, but the problem of filter obstruction swings the balance in favour of centrifugation, which is quicker and has good technical performance. It must be kept in mind, however, that these processes only reduce the BOD level of the stillage and further treatment of the effluent is still necessary before final effluent discharge (Sheehan & Greenfield, 1980).

Recent developments in distillery effluent treatment include the use of electrolysis and advanced oxidation technologies (AOTs). AOTs include the use of ozone, hydrogen peroxide and UV radiation to generate hydroxyl radicals, which act as server oxidative agents (Beltrán *et al.*, 1997a; Beltrán *et al.,* 1997b; Benitez *et al.,* 1997). Belrán *et al.* (1997a and b) showed that UV radiation alone had no significant effect on distillery effluent, but when combined with ozone, a synergistic effect occurred. The COD and total organic carbon (TOC) conversions obtained with ozone combined with UV radiation were 1.87, that is, the oxidation rates are increased with 87%. When hydrogen peroxide is used simultaneously with ozone the improved rate of oxidation is negligible compared to the direct reactions of ozone and the dissolved components

within the stillage. This research also showed that ozone efficiency (which was most effective in reducing the pollution content) increased from 30 – 40% at a pH of 4 and 95% at a pH of 9 - 11 (Beltrán *et al.*, 1997b; Benitez *et al.*, 1997). These studies did not, however, include the cost of lamps, maintenance and energy consumption as part of its effectiveness and the economic implications thereof should first be determined before any large-scale application is implemented.

The electrochemical treatment of vinasse entails the recirculating of the effluent through an electrolytic cell consisting of a stainless steel cylindrical cathode and a titanium alloy anode (covered by platinum alloy foil) located in the centre of the cylinder (Vlyssides *et al.*, 1997). In acid solutions, chlorine is the main oxidative agent, but in alkaline solutions a cycle of chloride-chlorine-chloride takes place, which produces OCl^-, ClO_3^- and free hydroxyl radicals that lead to the oxidisation of the organic matter. These radicals are very strong oxidative agents and their effectivity increases with an increase in pH. Vlyssides *et al.* (1997) found that the most effective oxidation conditions were at a pH of 9.5 and at a feed rate of 30 $ml.min^{-1}$ or 2.16 g $COD.min^{-1}$, which resulted in a COD reduction from 72 000 to 8 000 $mg.l^{-1}$. It is thus evident that this method holds promise for the treatment of distillery effluent, but further studies, including the economical implications and the effect of up-scaling of the process, need to be done.

When considering the total criteria necessary for stillage utilisation, biological treatment offers the only real means of disposal while keeping in mind that with most of these methods the liquor that remains still needs to be treated further before discharge (Sheehan & Greenfield, 1980; Maiorella *et al.*, 1983; Sales *et al.*, 1986). Biological treatment is considered to be the most promising treatment technique, since the vinasses organic matter is highly biodegradable.

Aerobic treatment can be used to deal with waste effluent as aerobic microbial communities have several specific advantages. They have large free energy potentials, which enables them to operate different biochemical mechanisms simultaneously. They are, therefore, capable of handling low substrate levels and variations in environmental conditions. Their capabilities, such as nitrification, denitrification, phosphate accumulation, lignase radical oxidation etc. makes them very attractive for waste treatment (Verstraete & Schowanek, 1987). However, direct aerobic treatment has become less favourable, due to the high energy consumption and the production of vast amounts of excess biomass in the form of sludge (1 kg of BOD can lead to the production of 60 to 70 kg of waste biological sludge) (Munters, 1984). The nutrient requirements of the aerobic process are also five times more than the anaerobic

process, which is an additional cost that must be added to the energy expense. Burnett (1973) reported that a COD reduction of only 1.3 % was achieved after 37 d of treating a 25% mixture of rum distillery waste and domestic sewage. With only 10% stillage, better results were obtained with a reduction of 24% within 9 h with a loading rate of 64.4 $kgCOD.m^{-3}.d^{-1}$. The optimum retention time for aerobic treatment of vinasse is 8 d (Sales *et al.,* 1987). With this the final effluents have COD and BOD removals of 78 – 88%, pH values between 6.5 and 8.0 and dissolved oxygen contents of over 1 $mg.l^{-1}$ (Sales *et al.,* 1987).

Anaerobic treatment has also been used to treat distillery effluent. During the years 1982 and 1986, the treatment of concentrated effluent by anaerobic fermentation underwent considerable development in France, with distilleries being the first of the agro-foodstuffs industries to apply this technology (Racault, 1990).

The advantages of the anaerobic treatment of distillery effluent are a low energy consumption of an already high energy consumption process, a very limited production of mineralised sludge, low nutrient requirements and the generation of methane gas that may be used directly to reduce the energy consumption in the factory. Anaerobic microbial communities are specifically advantageous at high temperatures and high concentrations both of soluble but particularly of insoluble organic matter, which is true for distillery effluent (Verstraete & Schownek, 1987). However, anaerobic systems alone are normally not capable of dealing with a complete removal of organic matter. The final effluent of these systems can be treated by an aerobic polishing step to minimise the dissolved organic matter still present in the anaerobic system discharge (De Bazúa & Cabrero, 1991). The by-products of both systems can be used to combat the operational costs of the combined systems, for example, the biogas from the anaerobic process may be used as an alternative energy source and biomass from the aerobic process as a potential feed for mono- and poly-gastric animals. Frostell (1981) and De Bazúa & Cabrero. (1991) both investigated the efficiency of a combined anaerobic–aerobic system for the treatment of distillery effluent. Frostell achieved COD reductions exceeding 80% with an organic load of 2.5 – 3.0 $kgCOD.m^{-3}.d^{-1}$ with the major part of the COD (70%) removed in the anaerobic tank. De Bazúa & Cabrero (1991) also obtained 70% COD removal during the anaerobic phase with loading rates as high as 34 $kgCOD.m^{-3}.d^{-1}$ at hydraulic retention times of as low as two days. A further reduction in COD of 95% was obtained with the anaerobic effluent in the aerobic tank.

Different anaerobic designs exist for the treatment of high strength effluents such

as distillery effluent. Ehlinger *et al.* (1992) used a mesophilic completely mixed fluidised bed bioreactor (37°C) to treat lees (red wine) vinasse and obtained a 70% COD removal at an organic loading rate of 15 kgCOD.m^{-3}.d^{-1}. The high tannin content of lees vinasse, however, showed a slight inhibitory effect on methanogenic activity when the tannin concentration exceed 500 mg.l^{-1}. Thermophilic bioreactors (55°C) have also been used in distillery effluent treatment, but possible instability due to temperature fluctuations are one of the factors hampering proper implementation (Yeoh, 1997). Yeoh (1997) and Souza *et al.* (1992), however, used a thermophilic two-phase anaerobic system (stirred tank design) and an upflow anaerobic sludge bed (UASB) bioreactor, respectively, to treat cane-molasses. Souza *et al.* (1992) obtained a COD removal of up to 72% with organic loads of 25 - 30 kgCOD.m^{-3}.d^{-1} where the two-phase system was less effective with a 66% COD removal at organic loads of 14 - 20 kgCOD.m^{-3}.d^{-1}. The mesophilic UASB bioreactor used by Shin *et al.* (1992) obtained a COD removal of 80% with loading rates of up to 44 kg.COD.m^{-3}.d^{-1}. The fact that thermophilically grown sludge possesses intrinsically much higher methanogenic activity than mesophilic sludge (Wiegant & de Man, 1986) does thus not mean that thermophilic anaerobic systems are more effective than their mesophilic counterparts.

The reactor design is not the ultimate restrictive factor influencing the efficiency of the treatment process, although it does play a very important role. The ultimate deciding factor in the treatment of a distillery effluent is the biodegradability of that specific effluent. Biodegradability studies done by Harada *et al.* (1996) showed that only 10% of the COD initially imposed were converted to methane when cane-molasses was used, whereas 60% was converted to methane with malt-vinasse. This indicates that the biodegradability of the cane-molasses vinasse is much lower compared to the malt-vinasse. The nature of the raw product, which dictates the composition of the distillery effluent eventually obtained, should, therefore, be considered when deciding which treatment option is most suitable.

C. DIGESTION AND GRANULES

Anaerobic digestion is one of the biotechnological processes that has found great application as a method to treat industrial waste waters and organic residues both in developing and developed countries (Lettinga *et al.*, 1997a; Diaz, 1998; Riggle, 1998). According to an overview of the anaerobic digestion industry published by the International Energy Agency's (IEA) Anaerobic Digestion Activity Group (Riggle, 1998),

the amount of anaerobic digestion systems operating or under construction throughout the world increased from 600 to over a 1 000 in 1997. This does not include smaller farm-scale digesters, which are known to be much more numerous. The success of the anaerobic digestion technology is the way in which anaerobic biomass is retained in the reactor system (Frankin *et al.*, 1992). The most widely applied methods of biomass retention in high loaded anaerobic systems are the immobilisation of the biomass on a fixed or mobile carrier material, e.g. fixed-bed or fluidised-bed systems (Soto *et al.*, 1992; García–Bernet *et al.*, 1998), or by the spontaneous granulation system as applied in the UASB and ESBG systems (Lettinga *et al.*, 1997a; Syutsubo *et al.*, 1998).

The direct treatment of wastes was greatly stimulated by the development of these types of reactors and their successful full-scale usage. Both the UASB and ESGB as well as the new SMPA (staged 'multi-phase' anaerobic) reactor system, contain granular sludge and thus permit high space loading rates (30 $kgCOD.m^{-3}$) at low hydraulic retention times (6 – 24 h) (Lettinga & Hulshoff Pol, 1991; Lettinga *et al.*, 1997b). Although it was previously believed that anaerobic treatment was not suitable for treating difficult degradable waste waters as well as those containing potentially toxic compounds such as penta-chlorophenols, it has now been shown that these digesters designs can successfully be used to treat these effluents (Hendriksen & Ahring, 1993; Lettinga, 1995). The anaerobic process is also an useful source of energy as biogas is produced during the degradation cycle (Lettinga, 1995). However, in the future the driving force for the use of anaerobic digestion will probably shift from energy production to its application in organic stabilisation, pathogen reduction and the production of a high quality soil-improver, especially in developing countries (Riggle, 1998). One of the main problems still remaining in the application of the UASB and similar processes, is the extensively long start-up periods and the sludge sensitivity to different substrates (Wang *et al.*, 1999). The availability of large quantities of suitable, highly active anaerobic granular sludge from existing full-scale reactor systems reduces the problem of extensive start-up periods as these granules can be used so that the start-up can be made within a few days (Lettinga, 1995). But in countries where granular sludge is not easily available, the application of these digester designs is limited (Britz *et al.*, 1999).

Anaerobic digestion is essentially a conversion process in which 60 to 80% of the chemical energy in the form of complex organic compounds is anaerobically converted to methane gas, carbon dioxide and water (Ross, 1991). It also involves the metabolic activity of a wide range of symbiotic micro-organisms (Ditchfield, 1986). Some members of the granule consortium that have been identified include typical

methanogens like members of the genera *Methanobrevibacter*, *Methanosaeta,* (former *Methanothrix*) and *Methanosarcina*. Syntrophic bacteria of the genera *Syntrophomonas* and *Pelobacter* as well as the sulphate reducing bacteria are also present, e.g. *Desulfovibrio* and *Desulfobulbus* (Schmidt & Ahring, 1996).

It has been postulated (Schmidt & Ahring, 1996) that the initial development of a granule consists of a few physical steps before multiplication of cells and subsequent granulation can take place. Although the strength of adsorption depends on different physiochemical forces like ionic, dipolar, hydrogen bonds or hydrophobic interactions, irreversible adhesion is established by means of strong bonds via bacterial fimbria, polymers and other bonding type structures (Schmidt & Ahring, 1996).

The naturally produced polymers bond by means of electrostatics and physical forces and form bridges for the bacterial cells to adhere to as floc aggregates (Shen *et al.*, 1993). The apparent importance of these extracellular polymers (ECP) for the formation of granules is widely accepted. In the granules it has been found that the ECP content varies between 0.6 and 20% of the granule volatile suspended solids (VSS) and the ECP concentration and composition in granules is affected by the composition of the wastewater. The granular ECP in granular sludge grown on simple acetogenic and methanogenic substrates was found to be significantly lower than those grown on more complex effluents. This could be due to the complexity of the cell walls of primarily the methanogens, limiting the excretion of the ECP, or any other complex polymers, because of the energy consumption involved (Schmidt & Ahring, 1994). Temperature also plays a role in the production of ECP's. Granules grown under mesophilic (30° - 35°C) conditions had a higher amount of ECP's than granules grown under thermophilic (50° - 70°C) conditions. This could either mean that ECP's degrade more rapidly under thermophilic conditions or that ECP production could be limited for thermophilic acetogens and methanogens (Schmidt & Ahring, 1994).

The process of granulation has been under constant research for many years and the exact mechanism that triggers the granulation is still under debate (Dolfing *et al.*, 1985; MacLeod *et al.,* 1990; Fang *et al.,* 1995). The most popular hypothesis is based on the formation of a layered structure. The presence of *Methanosaeta*-like cells in the inner core of the granule points to the notion that a loose network of *Methanoseata* filaments provides an excellent adhesion site to be colonised by a succession of other bacteria. Some of the first colonising bacteria would be those providing the *Methanosaeta* group with the necessary substrates (MacLeod *et al.*, 1990). The formation of a second layer around the core would include H_2-producing acetogens and

H_2-consuming organisms. These two groups work together in a syntrophic association. H_2-producing acetogens degrade the volatile fatty acids produced by the fermentative bacteria to acetate. The degradation of VFA's, like propionate and butyrate, by this group are unfortunately inhibited by high H_2 concentrations. The H_2-utilising bacteria, therefore, enable these bacteria to degrade these substrates. By means of Gibbs free energy studies it was shown that only within the granular structure environments with H_2 levels low enough exist to permit the degradation of propionate (Macleod *et al.*, 1990).

The adhesion of fermentative bacteria to the aggregates to form the exterior layer of the granule would prove an ideal situation between this group and the exterior substrate of complex organic compounds. The outer layer also includes many other syntrophic micro-colonies of various bacteria as well as H_2-consuming methanogens (Fang *et al.*, 1994). These H_2-consuming organisms could consume any free hydrogen before it moved through to the second layer. The hydrogen-using organisms in this layer can then use any H_2 produced by the acetogens, producing a high level of metabolic activity by the acetogens.

The aerobic and facultative anaerobic bacteria in the exterior layers will lead to an O_2 gradient, so that the strict anaerobes can multiply within the deeper layers of the granule. This layered structure provides a very complete and stable metabolic arrangement that creates an optimal environment for all its members and, therefore, resulting in high levels of metabolic activity (MacLeod *et al.*, 1990).

Not all granules exhibit a layered structure (Fang *et al.*, 1995). If the initial step of degradation is faster than the degradation of the intermediates, bacteria near the surface of the granule convert most of the substrate. This means that the concentration of the intermediates will accumulate, causing the formation of a concentration gradient. Diffusion of the intermediates towards the biogranule interior will cause the granule to develop a layered structure. When the initial step of degradation is slow relative to the degradation of intermediates, a much bigger fraction of substrate can diffuse towards the interior before it is degraded. This results in the formation of a granule with a uniform structure as is summarised in Fig. 1. In this case the initial degradation is faster than the intermediate degradation of complex carbohydrate substrates compared to simpler substrates like formate, acetate and peptone (Fang *et al.*, 1995).

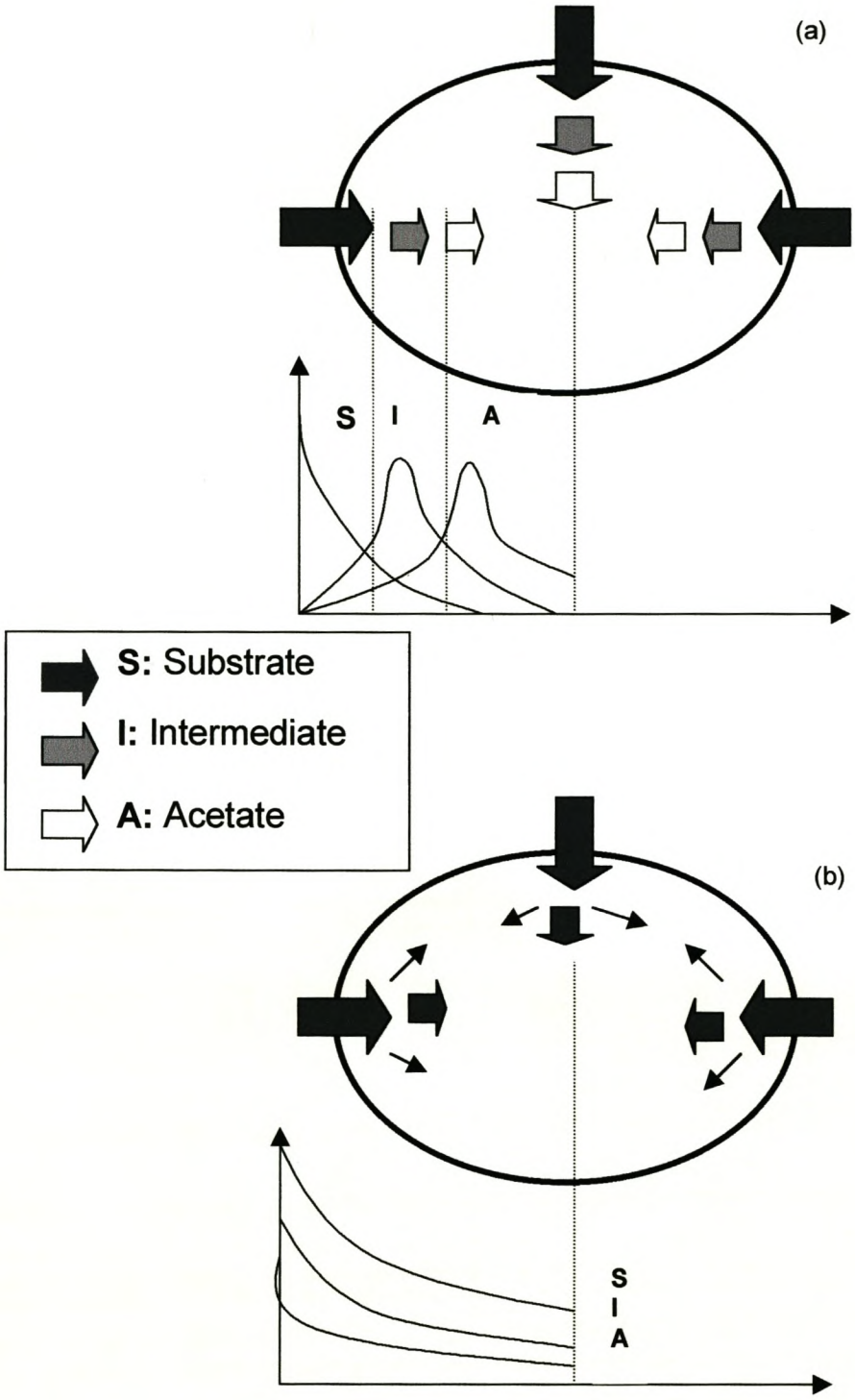

Figure 1. Diffusion and concentration profiles of biogranules with (a) layered microstructure, and (b) uniform microstructure (Fang *et al.*, 1995).

Granular sludge can be cultivated by using several inoculums such as anaerobic digested sewage sludge, wasted aerobic activated sludge and even cow manure. As the popularity of UASB reactors increases, more granular sludge will be needed as inoculum, which makes the mass cultivating and storage of granular sludge a necessity (Wu *et al.*, 1995).

Of late, the thought of engineered granules is becoming more and more attractive. Granules can possibly be produced with a higher resistance to the normal variations seen during the treatment of effluents (Schmidt & Ahring, 1996). One example of tailor-made granules are those engineered by inoculating the UASB granules with a pure culture of *Desulfomonile tiedjei*, which enables the granules to de-chlorinate substances like 3-chlorobenzoate (3-CB). This can be used for bioremediation of contaminated ground water containing xenobiotics (Verstraete & Vandevivere, 1997).

D. ACTIVITY TESTING OF ANAEROBIC DIGESTION PROCESSES

In the anaerobic digestion process, organic material is converted to stable end products, which will not cause further environmental hazards. One of the most important indicators of operational performance is the rate of biogas production (Petrozzi *et al.*, 1992). As methane production is the last step in the process, biogas rates depend on the specific activity, quality and quantity of the biomass involved. Activity testing of the biomass is thus crucial for the effectiveness of the wastewater treatment.

Activity is defined as the substrate dependent biogas/methane production rate per unit mass of volatile solids of biomass. In general, activity testing involves the addition of specific substrates to either a continuous or a batch biomass system, followed by the measurement of the biogas produced (Sørensen & Ahring, 1993; Lamb, 1995;). Sludge activity measurements can be either an overall measurement, giving information about the total activity of the process, or a measurement of each basic stage in the digestion process. The total activity measurement can thus be used to assist in the selection of a suitable inoculum for an anaerobic digester. In contrast, the individual activity determination can shed light on potential unbalanced situations between the different bacterial populations. It also shows the relative importance of the different steps in the anaerobic digestion process (Soto *et al.*, 1993). Activity tests can also be used to give valuable information on assessing the biomass loading rate for start-up as well as optimum substrate loading concentration. The specific methane yield can be

determined by means of preliminary methanogenic activity tests (Zábranská *et al.*, 1994). Activity testing also facilitates the testing of potential toxicity of specific substances on the anaerobic sludge (Soto *et al.*, 1993). Activity measurements are thus of value as they can be used to characterise the microbial composition, detect individual population activity in methanogenic environments and help optimise the start-up of a new digester (Dolfing & Bloemen, 1985).

Two main differences exist between the determination of methanogenic and non-menthanogenic (fermentative/ hydrolytic or acidogenic) activities. The first, is that the variation in substrate concentrations is of major importance with non-methanogenic activity tests as variations lead to the evaluation of one specific group only and not of all the consortium members. Secondly, the methanogenesis step rate is also lower than the non-methanogenesis steps, which necessitates the use of more accurate means of measuring biogas production. This obviously influences the methodology of the tests as well as the quality of the data generated (Soto *et al.*, 1993). Although the non-methanogenic activity tests are important in understanding the dynamics of the digestion process, emphasis should rather be put on the methanogenic activity tests. If the methanogens, being the terminal acceptor in the digestion chain, do not function properly, the efficiency of all the other bacteria are influenced.

Methanogenic activity bioassays

The methanogenic activity test involves the measuring of the amount of methane produced by either the sludge or the granules. The methanogenic activity of biomass depends on the carbon source with which the granules are acclimatised and cultivated. High activities are measured when the test substrate is identical to the growth substrate (Schmidt & Ahring, 1996). Factors like high salt and volatile fatty acid concentrations may affect methane production. Dolfing & Bloemen (1985) showed that methanogenesis was inhibited by 50% with a 150 mM NaCl containing solution. They also showed that the methanogenic activities of digested sewage sludge were low compared to those from UASB reactors activated on soluble substrates.

There are a few well-established methodologies that have been used to monitor the activity of methanogenic biomass, including measuring the amount of biogas or volatile fatty acids produced or the rate at which the substrate is utilised. These methods are often inaccurate and time-consuming (Dolfing & Mulder, 1985) and some also require expensive equipment (James *et al.*, 1990). Modifications of the original tests were also developed and currently researchers are investigating the use of co-

enzymes, bio-sensors and ATP for assessing the activity of anaerobic biomass (Pause & Switzerbaum, 1984; Chung & Neethling, 1988; Yamaguchi *et al.*, 1991).

Specific Methanogenic Activity (SMA)

The failure of methanogens to produce methane can result in the accumulation of high concentrations of volatile fatty acids, which subsequently lead to the lowering of the digester system pH. Monitoring the performance of biomass, in terms of methanogenic activity, is thus essential to prevent digester failure due to a low pH environment (Meyer & Oellerman, 1994).

The specific methanogenic activity (SMA) test involves the incubation of biomass with an excess of methanogenic substrate e.g. acetate (Sørensen & Ahring, 1993). The specific activity is measured as the chemical oxygen demand (COD) conversion rate per unit of sludge volatile suspended solids (VSS). The activity reading can thus help predict the maximal space loading rate during digester start-up as well as serving as a process control indicator (Meyer & Oellerman, 1994). Specific activity readings for good seeding sludge would be in the order of 0.2 kgCOD.kg $VSS^{-1}.d^{-1}$ (Schimdt & Ahring, 1996).

Modifications to the general SMA test were made because of the relative inaccuracy of the test as well as the difficulty in performing it (Dolfing & Mulder, 1985). One important limitation that had to be overcome was the difficulty in accurately measuring the amount of biogas produced, especially if done by means of water displacement. Relatively small volumes of sludge samples are used in the test and, with low specific methanogenic activity, little biogas is produced and accurate biogas measurements are then hampered (James *et al.*, 1990).

To simplify biogas measurements and increase accuracy, James *et al.* (1990) combined capillary manometers with an adapted Warburg respirometer. The respirometer has the advantage of being able to test many samples at the same time, giving increased accuracy through replication. The only adaptation was the addition of a special flask with a side arm. This adaptation made the sampling of the biogas through the side arm possible (James *et al.*, 1990). Chernicharo & Campos (1991) also modified the system by taking small amounts of sludge and diluting it with a mineral stock solution so as to prepare a known range of VSS concentrations (2.5 $g.l^{-1}$). The mineral solution contained buffers as well as nutrients and the flask was flushed with nitrogen to avoid air contamination and to maintain an anaerobic atmosphere. It was advised that the substrate used was only then added and the biogas production

monitored over a period of time in a shaking waterbath. The shaker must only be switched on after the addition of the substrate to avoid any "contamination " of the sample with oxygen during the acclimatisation period due to the mixing, as the manometer tap was still open and the headspace had not been made anaerobic. With the use of these modifications the specific methanogenic activity was calculated after determining the volume of methane produced per unit of time according to the method of James *et al.* (1990).

Further methodologies in determining the SMA were described by De Zeeuw & Lettinga (1980) who made use of enzymes like co-factor F_{420} in determining methanogenic activity, as methanogens are the only known organisms containing this co-factor. The number of methanogens present could also be quantified and the co-factor was therefore used as an indication of the total amount of methanogens present (Dolfing, 1986). The amount of F_{420} in each sample was determined using a Fluorometer (Schoeffel Instrument) at 420 nm, with pure F_{420} as standard (Pause & Switzerbaum, 1984). De Zeeuw & Lettinga (1980) reported a correlation of 0.80 between F_{420} concentration and the methanogenic activity of the biomass from nine different reactors determined with the conventional method. Further research with different substrates, such as hydrogen, formate, acetate and ethanol, showed a correlation of almost zero between the F_{420} and the methanogenic activity (Dolfing & Mulder, 1985). The test is, therefore, not substrate specific and the correlation between the amount of co-factor and methanogens present is restricted to certain species of methanogens and their co-factors.

A correlation of distinct types of co-factor F_{420}, e.g. F_{420}-2, which is present in hydrogenotrophic species and F_{420}-4 and F_{420}-5, which are present in acetotrophic species, was also undertaken (James *et al.*, 1990). This was done to establish a method to evaluate the different trophic groups present in the sample as well as to determine the accuracy of the test in comparison to the conventional method. Evaluation of the different co-factors was done by means of high performance liquid chromatography. A high correlation was found between the methanogenic activity of the hydrogenotropic species and their co-factors as well as for the methanogenic activity of the acetotrophic methanogenic species and their co-factors. Unfortunately, this type of assay is expensive and very time consuming and requires sophisticated equipment (James *et al.*, 1990).

The determination of the SMA can prove useful in: monitoring the behaviour of the sludge in the presence of potentially inhibitory compounds; establishing degradability

degrees for various substrates; following the changes in sludge activities caused by possible build-up of inert materials (like metals) after long periods of operation; determining maximum applicable loading rates to shorten start-up; and to evaluate batch kinetic parameters. Fang *et al.* (1994) successfully used the method to determine and confirm the microbial structure of UASB granules. Meyer & Oellerman (1994) used the method to determine sludge activity in order to monitor optimum organic loading rates, but the SMA tests are not often used because they are laborious and very time consuming.

Biochemical Methane Potential (BMP)

Biochemical methane potential (BMP) is a measurement of biomass biodegradability under anaerobic conditions (Owen *et al.*, 1979). The most acceptable way of referring to the BMP is in terms of sample organic content (m^3 CH_4.kg COD^{-1}). This permit the direct transfer of organic matter (%) converted into methane (%) by using the theoretical 0.350 m^3 CH_4 at STP per kg COD converted. Other references are according to sample volume (m^3 $CH_4.m^{-3}$ sample) or sample mass (m^3 $CH_4.kg^{-1}$ sample) (McCarty, 1964). The BMP method was applied by subtracting the background from the methane contributions resulting from sample decomposition. The gas samples extracted from serum bottles with a precision gas-syringe were monitored by means of gas chromatography.

In the BMP, proper sample size and liquid-to-volume ratios are important as they influence the precision and the accuracy of the results (Owen *et al.*, 1979). Other valuable guidelines to follow are insuring that nutrients are not limiting and to eliminate possible substrate toxicity. Total liquid volumes of up to 200 ml can be used so as to decrease the void-volume and improve the accuracy of methane determinations, when low gas production is expected. Excess gas must also be wasted to ensure that no leakage due to excessive pressure build-up, occurs. The typical incubation period is 30 days, after which most or all of the biodegradable organic compounds have been decomposed. This procedure eliminates variations due to differing metabolic rates. Longer periods for acclimatisation may be required for some organisms (Owen *et al.*, 1979). In this method the lengthy incubation time of 30 days limits the use of the method.

Anaerobic Toxcicity Assay (ATA)

Anaerobic toxicity is defined as the adverse effect of a substrate on the methanogens present in a sludge/granular sludge or granule sample. As with the BMP assay, the assay bottles are prepared with defined media, seed inocula as well as samples, which contain the substrate being tested for possible inhibitory effect. A 'spike' consisting of for example, acetate and propionate, is also added to each bottle. The total gas production is monitored by means of a gas-lock syringe to assess the metabolic rate of the acetate-propionate spike. Inhibition due to sample addition is monitored as a decreased rate of gas production relative to an active control (Ross, 1991).

In testing a substance, varying concentrations are used to provide a range from non-inhibitory to highly toxic. Gas production is monitored critically over a period of a few weeks, after which the maximum rate of gas production is determined. This is done by computing the ratio between the respective rates for samples and the average of the controls. This ratio represents the "Maximum Rate Ratio" (MRR). A MRR value of less than 0.95 indicates possible inhibition due to the sample added, while MRR values of less than 0.9 reflect significant inhibition (Owen *et al.*, 1979).

Data interpretation can be complicated by gas production resulting from sample decomposition as well as varying ratios of produced CO_2 and CH_4. The test itself is reasonably easy to perform and not very expensive (Owen *et al.*, 1979), but the test evaluation period is very long (up to 30 days).

Fatty acid degradation

The degradation of a mixture of different fatty acids can provide valuable information about the level of inhibition and, therefore, activity of the different trophic groups involved in anaerobic digestion (Wu *et al.*, 1990). The main advantage of this assay lies in its ability to examine the level to which the different syntrophic acetogens are inhibited by different substances (Dolfing & Bloemen, 1985; Wu *et al.*, 1990).

A medium containing the volatile fatty acids, sodium sulphide and sodium salts of chlorinated phenol (or any other inhibitory substance) are placed in serum bottles. The medium also contains a buffer and nutrient solutions (Wu *et al.*, 1990). These bottles are placed in a shaking water bath at 35°C and inoculated with either the sludge/granular sludge or granules. Liquid and gas samples are taken at 8 and 24 h intervals for 5 - 7 days (Wu *et al.*, 1990). The specific degradation rates of the fatty acids can be calculated according to kinetic data described by Wu *et al.* (1995).

The information obtained on the relatively inhibitory effects of the substances on the different trophic groups is of great importance. By using this method Wu *et al.* (1990) showed that acetotrophic methanogens are more sensitive to chlorophenol inhibition than syntrophic propionate and butyrate degraders, but more specific information on the other trophic groups is not reliable.

ATP measurements

Adenosine tri-phosphate (ATP) is a compound associated with the biochemical breakdown of different substrates and can, therefore, be used to calculate the activity of anaerobic sludges (Chung & Neethling, 1988). ATP measurements can subsequently be seen as an indication of total digester activity. It cannot, however, distinguish between the activity of various groups within the biochemical system (Chung & Neethling, 1988).

The amount of ATP produced is measured by means of commercially available luciferase/luciferin regents. A Luminometer is used for light measurement. ATP produces light when it reacts with luciferin (LH_2) and the enzyme luciferase (E) in the following reactions:

$$LH_2 + ATP + E \xrightarrow{Mg^{2+}} E\text{-}LH_2\text{-}AMP + PP \qquad (1)$$

$$E\text{-}LH_2\text{-}AMP + O_2 \rightarrow E + Product + CO_2 + AMP + Light \qquad (2)$$

Before the extraction of ATP, anaerobic sludges are diluted and, therefore, a less sensitive enzyme product can be used, thus lowering the cost of this method. ATP has a very short survival time after cell death, and the ATP concentration can reflect the viable organism content of the sludge. The ATP concentration also responds rapidly to changes in digester operation, making it a useful operational parameter (Chung & Neethling, 1988). However, this method only reflects the total activity of the consortium present in the digester.

Dyhydrogenase activity (DHA) as an indicator of biomass activity

Dyhydrogenase activity (DHA), similar to ATP concentrations, is also associated with the metabolic pathways of anaerobic micro-organisms and is, therefore, applicable in measuring the activity of anaerobic biomass. Furthermore, the DHA method is able to distinguish between active and inactive biomass. In contrast to the ATP method,

which can only reflect total activity of the digester, DHA is substrate specific and can possibly be used to distinguish between the activity of specific trophic groups. The activity of the acetotrophic bacteria, for example, could be measured by adding acetic acid as substrate during DHA measurements (Chung & Neethling, 1989).

DHA changes are measured by adding a tetrazolium salt e.g. triphenyltetrazoliumchloride (TTC) or 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazoliumchloride (INT) to the biological system. The salt is used by the bacteria as a terminal hydrogen acceptor:

$$\text{substrate } (RH_2) + \text{Dehydrogenase} \rightarrow \text{oxidised substrate } ® + 2H \quad (1)$$

$$2H + \text{Tetrazolium salts (colourless TTC/INT)} \downarrow \text{HCL} + \text{Triphenylformazan (red TF)} \quad (2)$$

The red TF crystals are extracted using either acetone or ethanol as organic solvents. The intensity of the red colour is then measured spectrophotometerically (Chung & Neethling, 1989).

This method is easy to perform and very sensitive because of a greater magnitude of changes in DHA content than in direct measurements during non-steady state conditions. This method can be used as an early warning tool of changes in the metabolic activity (Chung & Neethling, 1989), but does not really indicate the activity level of the different populations present in granular sludge.

E. RECOMMENDATION

Distilleries produce large volumes of wastes known as vinasses. They are acidic in nature with a high organic content and must be treated before being discharged as they can cause considerable environmental problems (Sales *et al.*, 1987; Borja *et al.*, 1993). Biological waste treatment methods such as anaerobic digestion (UASB-technology) have been reported to be suitable for these types of high strength wastes and have already been successfully implemented on large scale (Romero *et al.,* 1988; Ehlinger *et al.*, 1992; García-Bernet *et al.*, 1998; Garcia-Calderon *et al.,* 1998). However, the formation, amount and specific activity of the anaerobic granules present in these high-rate systems dictate the operational efficiency and performance of the UASB systems

(Lettinga *et al.,* 1997a). The major difficulty is to get suitable and active granules rapidly incorporated in a granular bed. If not, long start-up periods are experienced, granules are lost due to wash-out resulting in an overall loss of activity. Monitoring the activity of the anaerobic granules is, therefore, vital throughout the start-up and general operation of the anaerobic system (Chernicharo & Campos, 1991). The composition of distillery wastewater varies considerably from one plant to another according to the origin of fermentation materials and the distillation process (Water Research Commission, 1987; Harada *et al.*, 1996;). Estimating the adaptability of the anaerobic granules to the different substrates is thus possible through activity tests.

From the previous section, it is evident that a variety of activity tests can be used in evaluating the biomass activity. When accessing the potential methanogenic activity of anaerobic sludge, relatively inexpensive tests like SMA and BMP can be used. These methods are relatively easy to perform but lack accuracy and are often very time consuming. The modified tests using the adapted Wurburg respirometer, is more accurate and gives more reproducible data but is still time consuming.

Tests like the fatty acid degradation and SMA coupled with gas chromatography can be used to evaluate the specific activities of different trophic groups involved in anaerobic digestion. Again, these methods may not be that accurate because of background production of methane resulting from cell decomposition.

The use of cofactor F_{420}, as well as the use of DHA give values that are more accurate and respond to sensitive changes in the dynamics of the microbial environment. These methods do tend to be more expensive and require specialised technology. In the end, the effectiveness and efficiency of the digestion process are largely determined by the composition of the wastewater being treated.

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CHAPTER 3

OPERATIONAL OPTIMISATION OF AN UASB BIOREACTOR TREATING DISTILLERY EFFLUENT

Summary

A mesophilic laboratory-scale upflow anaerobic sludge bed bioreactor was used in the anaerobic digestion of high strength distillery effluent (COD = 2.01 to 30.00 $g.l^{-1}$). The reactor was inoculated with 600 g of anaerobic granules and the hydraulic retention time set at 24 h after a short stabilising period. During the experimental study the organic loading rate (OLR) was gradually increased. An average COD removal over the study period of 90%, was obtained. At an OLR of 30.00 $kgCOD.m^{-3}.d^{-1}$, the pH, alkalinity and biogas yield stabilised to average values of 7.8, 6 000 $mg.l^{-1}$ and 18.5 $l.d^{-1}$ respectively. This was taken as an indication of excellent bioreactor stability. The substrate pH was also lowered from 7.0 to 4.7 and no negative effects were observed and the reactor parameters soon stabilised after each successive lowering in pH. The accumulation of fine solids present in the bioreactor substrate was found at the higher OLR's and resulted in the reactor granular bed increasing with subsequent biomass washout and a lowering in the efficiency parameters. This necessitated the removal of 400 g of granules from the granular bed. After a short period of adaptation the efficiency parameters stabilised, but future work on solids accumulation is necessary.

Introduction

Distilleries have been placed in a critical situation as a consequence of the high energy consumption of the distillation process and expenses rising from the need to treat both solid and liquid wastes (Romero *et al.*, 1988; Borja *et al.*, 1993; Leal *et al.*, 1998). Distillery wastes (or vinasses) have an organic content varying between 25 and 40 $kgCOD.m^{-3}$ (Lele *et al.*, 1989; Ehlinger *et al.*, 1992; García-Bernet *et al.*, 1998) and typically have high concentrations of potassium, calcium, chloride and sulphate ions (Water Research Commission, 1987). High biodegradable dissolved solids (up to 50% present as reducing sugars), high ash content, high temperature and a low pH, makes the effluent more difficult

to treat. Distillery wastewater has a low pH (< 4) resulting from the abundance of organic acids present in the effluent (Shin *et al.*, 1992; Garcia-Calderon *et al.*, 1998) and thus treatment necessitates the neutralisation to an optimum biological pH range. The subsequent cost of neutralising agents also contributes to the treatment costs.

The use of the upflow anaerobic sludge blanket (UASB) bioreactor design in treating high strength distillery effluents has in the past been successfully implemented (Lettinga & Hulshoff Pol, 1991). The nature and strength of the distillery wastewaters provide more favourable conditions for anaerobic digestion than those for aerobic treatment technologies. The main advantage of the UASB system lies in the high biomass retainment in the form of granules despite the relatively high upflow velocities and short hydraulic retention times. The overall efficiency of the UASB system depends on the formation and activity of the granules (MacLeod *et al.*, 1990; Trovnec & Britz, 1998). These granules generally have good settling properties and vary in size from 1 to 5 mm, depending on the type of waste being treated.

The aim of this study was to evaluate the use of a mesophylic UASB system as an option in the treatment of high strength distillery effluent, and to optimise the efficiency of the bioreactor in terms of the organic loading rate and a lower substrate pH so as to minimise neutralisation costs.

Materials and methods

Bioreactor

A 2.3 litre laboratory-scale upflow anaerobic sludge blanket (UASB) bioreactor with an upflow velocity of 1 $m.h^{-1}$, was used (Fig. 1). The operational temperature was maintained at 35°C with a heating tape and an electronic control unit (Meyer *et al.*, 1985). The substrate was pulse-fed from the bottom of the reactor with a peristaltic pump (Watson-Marlow 101U) and the biogas exited at the top of an open gas/solid separator. The volume of biogas produced was determined using a manometric unit fitted with a gas-tight valve and an electronically controlled counter. Necessary corrections for standard temperature and pressure were made.

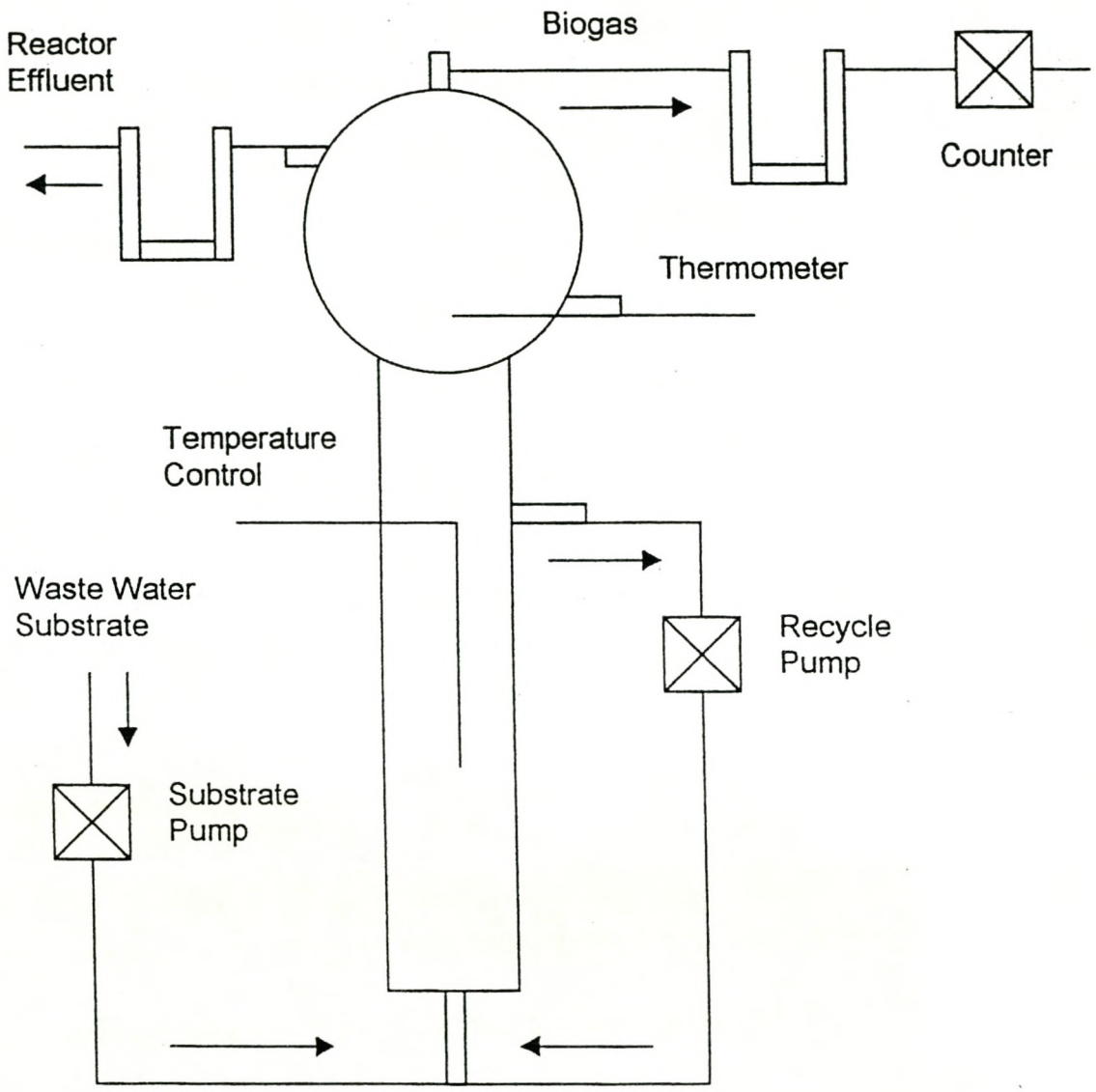

Figure 1. The experimental set-up of the laboratory-scale UASB bioreactor.

Substrate

The distillery effluent used in this study was obtained from Distillers Corporation Ltd. in Worcester during the period of April 1998 to March 1999. The average composition of the raw distillery effluent is summarised in Table 1. The substrate batches were kept at -18°C until required. The effluent was brown in colour and very murky due to the presence of high concentrations of suspended solids (on average 13 $g.l^{-1}$). The distillery effluent, when used as bioreactor substrate, was supplemented with 250 $mg.l^{-1}$ urea and 250 $mg.l^{-1}$ KH_2PO_4 so as to prevent N and P limitations and the pH adjusted stepwise as required for each study (7.5 to 4.7) with 1 M sodium hydroxide. The bioreactor substrate was also enriched with 1 ml per 1000 ml substrate per 14 retention times of a trace element solution (Nel *et al.*, 1985).

Bioreactor start-up

The bioreactor was inoculated with 600 g of water drained anaerobic granules from a laboratory scale reactor and an industrial bioreactor (Ronquest, 1999). The granular bed was allowed to settle to a bed height of 45 cm. The reactor was allowed to stabilise for 24 h for the bacterial community to acclimatise. It was then fed with a substrate containing winery effluent, diluted distillery effluent (2 000 $mg.l^{-1}$ COD) and UASB effluent (Ronquest, 1999) at a ratio of 1:1:1 (v/v) for 4 d at a hydraulic retention time (HRT) of 48 h. The HRT was then reduced from 48 to 24 h over a period of 14 d. Thereafter, the organic loading rate (OLR) was increased stepwise from 2 000 to 30 000 $kgCOD.m^{-3}.d^{-1}$, accompanied by a decrease in the percentage UASB effluent used in the reactor substrate. The substrate pH was later stepwise decreased from 7.5 to 4.7. After each adjustment the bioreactor was allowed to stabilise. Stable state was defined as a state, which can be maintained indefinitely without system failure (Cobb & Hill, 1990), during which the variation in bioreactor performance parameters is less than 10%. System failure occurs when the system environment changes to such an extent as to adversely effect the activity and proper functioning of the anaerobic bacteria present in the system.

Analytical methods

The following operational parameters were monitored according to Standard Methods (APHA, 1992): pH; alkalinity; total solids (TS); total volatile solids (TVS);

Table 1. Typical composition of wine distillery effluent.

Parameters	Concentration ($mg.l^{-1}$)		
	Study substrate (Average of 24 batches)	According to Ross, 1991	According to Ehlinger *et al.*, 1992
pH	4.2	4.3	3.5
COD	30 000	35 000	17 000
TS	19 000	8 000	n/d
SS	13 000	n/d	1 700
Alkalinity	6 000	n/d	n/d
Kjeldahl-N	220	350	300
Ammonia-N	n/d	10	n/d
Nitrate-N	n/d	1	n/d
Nitrite-N	n/d	1	n/d
Total-P	n/d	150	n/d
Ortho-P	280	130	300
K	n/d	3 000	4 100
Na	n/d	75	20
Ca	n/d	410	1 800
Mg	n/d	160	100
Fe	n/d	15	4
Cu	n/d	2	0.5
Al	n/d	3	n/d
Cl	n/d	160	540
SO_4	n/d	15	n/d
Co	n/d	2	<0.01
Ni	n/d	3	<0.01
Sr	n/d	0.6	n/d
Mn	n/d	0.4	0.3

N/d = not determined

total non-volatile solids (TNVS); Chemical Oxygen Demand (COD); orthophosphate phosphorus; and total Kjeldahl nitrogen (TKN).

Results and discussion

Raw effluent

The composition of the full strength distillery effluent used in this study is given in Table 1. The composition of the effluent used in this study was also compared to the composition of distillery effluent as reported by Ross (1991) and Ehlinger *et al.* (1992). It was evident from Table 1 that the composition of distillery effluent varies widely, and is dependant on the original raw product used for the distillation as well as the tempo at which the distillation was performed. This large variability in composition that is found in effluents from different types of distilleries must always be taken into consideration when designing and planning a biological treatment system and what is suitable for one situation might not be suitable for another.

UASB bioreactor efficiency

The influence of increases in the OLR and reduction of the substrate pH on the digester's efficiency parameters is summarised in Table 2. After the bioreactor start-up, the HRT was kept constant at 24 h and the OLR increased stepwise from 2.01 to 30.00 $kgCOD.m^{-3}.d^{-1}$ (Steps 1 to 18). A final OLR of 30.0 $kgCOD.m^{-3}.d^{-1}$ (Step 18) was thus obtained using the raw, full strength distillery effluent without any dilution. The data clearly showed (Fig. 2) that the UASB can be fed directly with the raw effluent and that, once the microbial community had acclimatised to the distillery effluent, no dilution was required. This is of importance, as any additional storage capacity required by the distillery industry for discharged effluent will be minimised. This, combined with the ability to treat the effluent at a HRT of 24 h without prior dilution will thus lower treatment expenditures in terms of the distillery's total water consumption, treatment time as well as expenditures in terms of additional storage facilities.

Table 2. The operational conditions and UASB bioreactor efficiencies obtained while treating the distillery effluent during increases in organic loading rate and reductions in the bioreactor substrate pH.

	Steps								
Parameters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
HRT (h)	24	24	24	24	24	24	24	24	24
Substrate COD ($g.l^{-1}$)	2.01	2.99	4.03	5.02	5.97	6.96	8.00	8.99	9.94
OLR ($kgCOD.m^{-3}.d^{-1}$)	2.01	2.99	4.03	5.02	5.97	6.96	8.00	8.99	9.94
CODremoval rate ($kgCOD.m^{-3}.d^{-1}$)	1.87	2.84	3.87	4.72	5.55	6.61	7.52	8.72	9.14
COD removal (%)	93	95	96	94	93	95	94	97	92
Bioreactor effluent COD ($g.l^{-1}$)	0.14	0.15	0.16	0.30	0.42	0.35	0.48	0.27	0.80
Alkalinity	1 700	1 750	1 500	1 900	2 100	2 750	3 103	3 625	4 100
Substrate pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.3
Bioreactor effluent pH	7.5	7.2	7.4	7.4	7.4	7.7	7.5	7.6	7.7
Biogas production ($l.d^{-1}$)	2.41	2.44	2.93	3.68	4.53	5.31	6.57	7.11	8.00

	Steps								
Parameters	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
HRT (h)	24	24	24	24	24	24	24	24	24
Substrate COD ($g.l^{-1}$)	10.85	11.58	14.84	17.90	19.92	22.84	24.85	27.89	30.00
OLR ($kgCOD.m^{-3}.d^{-1}$)	10.85	11.58	14.84	17.90	19.92	22.84	24.85	27.89	30.00
CODremoval rate ($kgCOD.m^{-3}.d^{-1}$)	9.33	9.26	12.91	16.11	17.93	21.01	22.61	25.94	27.60
COD removal (%)	86	80	87	90	90	92	91	93	92
Bioreactor effluent COD ($g.l^{-1}$)	1.52	2.32	1.93	1.79	1.99	1.83	2.24	1.95	2.40
Alkalinity	4 500	4 080	4 000	3 880	3 800	4 100	4 900	5 670	6 000
Substrate pH	7.0	6.8	6.6	6.4	6.3	6.0	5.6	5.0	4.7
Bioreactor effluent pH	7.4	7.4	7.6	7.6	7.6	7.6	7.7	7.8	7.8
Biogas production ($l.d^{-1}$)	8.45	8.58	9.15	10.77	12.55	12.72	13.77	15.20	18.50

The UASB bioreactor operational efficiency in terms of COD removal, organic removal rate (R) and OLR, is illustrated in Fig. 2A and 2B. The data clearly showed that, as the OLR was increased from 2 to 30 $kgCOD.m^{-3}.d^{-1}$ (Steps 1 to 18), the rate at which COD was removed (R-rate) (Fig. 2B) steadily increased. At the end of the study, the R-rate, even though the OLR was continually increased, had not reached a clear plateau or a decrease, indicating the maximum loading rate had not yet been reached for this specific reactor under these operational parameters. It was concluded that further increases in OLR would still be possible without influencing the reactor's efficiency. Thus, it is possible that the HRT could have been further shortened which would have led to a corresponding increase in the OLR. This could subsequently lead to a shorting of the treatment time, further contributing to the economical viability of this treatment option. However, this further shorting of the HRT was not done as in this study difficulties were, at times, experienced with the accumulation of partially degraded suspended solids in the granular bed. These very fine solid particles, often present in the raw distillery effluent, had to be removed by purging (during step 10), when the bed became to high in the reactor and this clearly negatively influenced the granule bed size and the efficiency of the bioreactor. It is known (Harada *et al.*, 1996) that accumulation of suspended solids within the bioreactor granule bed hampers the conversion of COD to methane. This slower conversion of COD was contributed to the low biodegradability of the solids present in the distillery effluent. In this study, this became more evident at the higher OLR's. It was concluded that the microbial population was thus not able to degrade these particles effectively and fast enough at the HRT used in the study. For partially soluble wastewaters such as distillery effluents, it might be advisable to separate the very fine suspended solids prior to introduction to the treatment system. One method would be the use of filtration technologies or even a direct precipitation method. Effluent recycling (up-front dilution) could also represent an attractive solution (Harada *et al.,* 1996; Lettinga *et al.,* 1997) but such options will have to be evaluated in future studies.

From the start (Step 1) of the stepwise increase in OLR to Step 8, the substrate pH was kept constant at 7.5 by neutralising with 1 M NaOH. The data showed that the COD removal remained consistently above 90%, with the best removal being 98% at an OLR of 3.98 $kgCOD.m^{-3}.d^{-1}$ (Fig. 2A). This was a clear indication of the good bioreactor efficiency obtained during Steps 1 to 8 (Table 2).

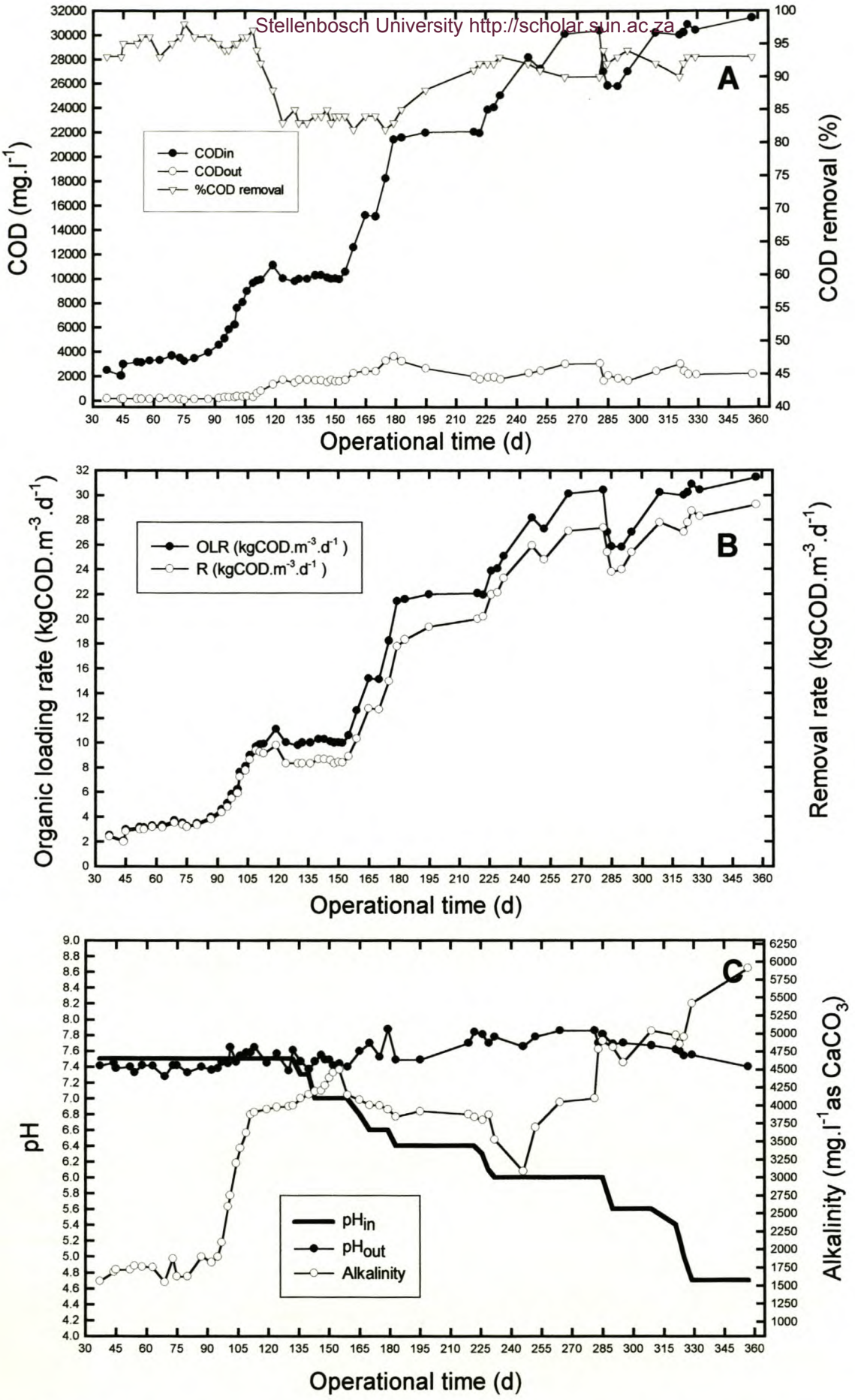

Figure 2. Reactor performance in terms of COD removal (A), Organic loading rate and removal rate (B) and reactor effluent pH and alkalinity (C), over an operational period of 360 days.

During Steps 9 to 18 (Table 2) the substrate pH was reduced from 7.5 to 4.7 together with a simultaneous increase in OLR. Therefore, the changes in efficiency parameters such as COD removal, alkalinity and biogas production are difficult to ascribe to either a reduction in substrate pH or an increase in OLR. The combined effects on the bioreactor efficiency, however, are considered. From the data it was clear that, as the substrate pH was reduced during the study from 7.5 to 7.0 (Steps 9 and 10), the alkalinity increased from 4 100 to 4 500 (Fig 2C), which was considered an indication of good bioreactor stability and efficiency. Thereafter, a reduction in alkalinity from 4 500 to 3 800 $mg.l^{-1}$, was found. This could either have resulted from a too rapid further reduction in pH from 7.0 to 6.3 (Steps 11 to 14) or from the simultaneous increase in OLR from 11 to 19.92 $kgCOD.m^{-3}.d^{-1}$, an increase in solids in the granular bed, or a combination of the three. The relatively sharp increase in OLR is believed to be the major contributor towards this reduction in alkalinity, and was ascribed to the accumulation of VFA's.

During step 10, the efficiency levels, especially the COD removal, at which the reactor had been operating decreased. The bioreactor effluent as well as the sludge blanket of the bioreactor was found to be murkier and the granular bed had clearly expanded and washout of the granule bed became evident. As a result it was deemed necessary to purge the bioreactor by removing 400 ml of granules from the bottom of the bed so as to lower the granular bed. Within 24 h a further decrease in COD removal efficiency to 80% was experienced (Step 11) (Table 2). This drop in efficiency was ascribed to disturbances in the microbial community caused by the biomass removal. Lettinga *et al.* (1997) reported that the kinetics of the degradation processes could be enhanced as a result of the presence of an immobilised balanced bacterial consortium, provided that they do not grow too dense and hamper mass transfer diffusion in the reactor. Trnovec & Britz (1998) also found that continual inducement of shock conditions, such as increases in OLR or changes in substrate pH, resulted in an elevated granular bed. If this happens, part of the granule bed must be removed. In this study, removal of 20% of the granular bed was done to prevent biomass washout, occurring because of a very high granular bed. This too large granule bed was ascribed to growth in the microbial community and to the accumulation of fine solids from the substrate in the bed. Congestion due to non-biodegradable particles in the substrate, which were then retained in the granule bed, was probably the main cause of the increased granular bed. In time, the bioreactor once more reached COD removal values above 90% (Steps 12 - 14) (Fig

2A). The stepwise decrease in substrate pH (6.3 to 4.7) and increase in OLR (19.92 to 30 $kgCOD.m^{-3}.d^{-1}$) was continued from Steps 14 to 18. Efficiency parameters, such as COD removal and biogas production, with the exception of fluctuations in system alkalinity, did not show any indication of possible instability in the bioreactor (Fig 2A and 2C). From day 260 to day 285 (Fig 2A) a drop in the substrate COD was experienced. This was due to the use of a specific low level COD containing batch of distillery effluent, which at that time was the only wastewater available. The COD concentration was re-adjusted once a higher COD containing effluent was obtained and the OLR increased until 30.0 $kgCOD.m^{-3}.d^{-1}$ was reached. This did, however, not influence the removal efficiency of this system as was expected with such a large fluctuation in substrate COD concentration.

In conventional anaerobic treatment systems, neutralisation of reactor substrates are needed to obtain optimum operational pH values between 6.6 and 7.4 (Moosbrugger *et al.,* 1993; Wentzel *et al.,* 1994). Distillery effluents are known to have a pH ranging from 3.5 to 4.5 (Romero *et al.*, 1988; Carcía-Bernet *et al.*, 1998) and in this study the distillery effluent used had an average pH of 4.3 (ranging from 3.9 to 4.7). The conditioning of the anaerobic granular community to function effectively at such a low pH as 4.7, as has been done in this study, can reduce operational costs considerably. However, further reduction in substrate pH was not attempted because the bioreactor required an extended recovery period (several days) in terms of pH stabilisation, after substrate pH reductions. A certain amount of neutralisation is therefore still needed. This was taken as an indication that the bioreactor was reaching the minimum substrate pH in terms of operational efficiency. However, at a substrate pH of 4.7, the bioreactor could still maintain an acceptable alkalinity, effluent pH and COD removal of 6 000 $mg.l^{-1}$, 7.5 and 93%, respectively.

When looking at the influence of substrate pH on operational efficiency, it has been reported that the internal biorector pH affects the microbial enzymatic activity as well as the average growth rate of the bacterial community involved in the anaerobic process. A near neutral pH, the desired operational pH range for digester bacterial growth being between 6.6 and 7.4, is vital for the successful operation of any anaerobic bioreactor system (Moosbrugger *et al.*, 1993). The bioreactor pH is maintained by the buffering capacity of the system, which is dictated by the alkalinity levels (Borja & Banks, 1995). The pH of the system will therefore directly influence the treatment efficiency.

The pH in an anaerobic bioreactor is dictated by the resultant of the alkalinity produced by the conversion of organic acids to CO_2 and the conversion of proteins to NH_3.

For each pH change to the bioreactor, there will be an equilibrium alkalinity and CO_2 partial pressure. Fluctuations in alkalinity values can subsequently be ascribed to the fluctuations in CO_2 partial pressure due to changing organic acid conversion tempos as well as changing tempos at which protein compounds are converted to NH_3. In general, a ratio of total VFA's (as acetic acid) to total alkalinity (as $CaCO_3$) of less than 0.1 is desirable (Nel & Britz, 1986). An alkalinity level ranging between 2 500 and 5 000 $mg.l^{-1}$ is desirable for complex wastewaters such as distillery effluent, because it provides a buffering capacity for a much larger increase in volatile fatty acids (Nel & Britz, 1986). When VFA's are treated directly (an effluent containing a mixture of pure VFA's) a large buffer capacity is not required.

The whole driving force behind the conversion process is based on the rate of methanogenesis from the acids. Thus, when the acetate to methane reaction is not rapid enough to prevent the accumulation in VFA (pH drop), the system must be able to counteract this drop in pH to maintain a favourable pH environment for the functioning of the bacteria. This is thus known as the system's buffering capacity (Nel & Britz, 1986). The alkalinity, buffering capacity and bioreactor effluent pH are thus all inter-linked and play an important role in minimising overloading effects. Alkalinity is also a good indicator of instability (Lane, 1984). During the first phase (Steps 1 to 8) it was seen that the reactor effluent pH was, on average, 7.4 (± 0.3) and the alkalinity increased from 1 700 to 3 625 $mg.l^{-1}$. Thereafter, the substrate pH was decreased from 7.5 to 6.8 because of the stability shown by the reactor in the previous steps (Steps 1 to 8) in terms of COD removal and the system's increased buffering capacity shown by means of the alkalinity (Table 2).

In this study, a drop in the alkalinity was found after most decreases (Fig. 2A) in substrate pH, which probably induced a measure of stress on the microbial environment. After a 4 d period of adjustment it was found that the alkalinity started to increase and stabilise once more. It is thus evident that a sudden change in the alkalinity level can be used as an indication of bioreactor stability when stress conditions are induced on the bacterial community. The increase in reactor substrate concentration also aided the buffering capacity of the reactor which in turn, generally, with the exception of Steps 11 to 14, led to an increase in the alkalinity (up to 6 000 $mg.l^{-1}$). This can probably be because of the high potassium bi-tartrate concentration reported to be present in distillery effluents (Ross, 1991). With an increase in the concentration of potassium bi-tartrate in the reactor substrate resulting from the increased OLR, the concentration of free potassium ions will

increase (Wentzel *et al.*, 1994). This will probably contribute to an increased buffering capacity in the system which can accommodate larger fluctuations in system pH more effectively (Ross, 1991). The bioreactor's efficiency in this particular study was aided by the increase in alkalinity leading to an adequate buffering capacity which helped control fluctuations in system acidity. The reactor's bacterial community could thus, because of a favourable pH environment created by the effective buffering capacity of the system, degrade the accumulated VFA's without any pH inhibition.

Conclusions

The anaerobic process has in the past only been partially successfully implemented in the treatment of high strength distillery effluents. In certain cases high OLR's and good COD removals were obtained, but often long HRT times of up to 12 d were needed before acceptable efficiency levels were obtained (Maiorella *et al.*, 1983; Borja *et al.*, 1993). During this study, it was found that the UASB technology could be used to successfully treat the high strength distillery effluent at OLR's of up to 30 $kgCOD.m^{-3}.d^{-1}$ and a HRT of 24 h. Excellent COD removal efficiency, with an average COD removal value of 90%, was achieved. Continual increases in alkalinity and R-rate showed good UASB bioreactor stability and efficiency in terms of buffering capacity and organic removal, respectively. This was true even when a reduction in bioreactor substrate pH of 7.5 to 4.7 was implemented and the OLR increased from 2 to 30 $kgCOD.m^{-3}.d^{-1}$.

The bioreactor used in this study could therefore be operated at a substrate pH of 4.7 with little or no neutralisation of the full strength raw distillery effluent. These results will enable distilleries to treat their effluent directly without the accumulation of discharged effluent in holding tanks. It will also minimise the need for excessive dilution and neutralisation of the raw distillery effluent prior to the treatment process.

The accumulation of suspended solids present in the distillery effluent, in the bioreactor was at times found to be problematic and caused the bioreactor efficiency to decrease. Removal efficiencies as well as alkalinity levels within the reactor decreased and the granular bed increased to such a degree that biomass washout occurred. This necessitated the removal of part of the granular biomass so as to increase system dynamics, enhancing operation and further granulation. Filtering the reactor substrate before feeding it to the reactor is advisable in the future so as to minimise the accumulation

of fine solids within the bioreactor. Further studies must also still be conducted on the influence of a further shorting of the HRT so as to investigate the effect of bio-solid accumulation on the overall efficiency of the microbial community.

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Chapter 4

DEVELOPMENT OF AN INEXPENSIVE AND RELIABLE METHOD FOR SCREENING ANAEROBIC GRANULES FOR ACTIVITY

Summary

The activity method of Owen *et al.* (1979), as adapted by Lamb (1995) was evaluated in terms of efficiency and applicability in determining the activity of granular samples. The method was found to be inaccurate as well as time consuming and it was thus modified accordingly so as to shorten the incubation time from 72 to 25 h. The assay volume was also reduced from 250 to 20 ml, as well as securing the vial from possible gas leaks, by means of rubber septa secured with aluminium crimp tops. Results obtained with the modified assay method were found to be more accurate, and the impact of the different test substrates (glucose, lactate, acetate and formate) on activity was more evident. The activity of seven different anaerobic granules was subsequently evaluated. In this study activity was not measured in volume of gas produced per unit COD converted or volatile suspended solids (VSS), which requires more time (determining the COD or VSS), but as tempo of gas production ($ml.h^{-1}$) in a standardised basic growth medium. The activity data obtained were also displayed as bar charts giving valuable information about population dynamics as well as possible substrate inhibition. The S-type granules, for example, showed a shift in population from 5 to 10 h of incubation from dominant, typical acidogenic catobolisers to a population consisting of both methanogenic (acetate and formate-utilising) and acidogenic species. This was evident from the fact that from the 5 to 10 h evaluation period, the biogas activity remained constant, but the methanogenic activity increased from 0 to 0.04 $ml.h^{-1}$ for acetate as substrate and 0 to 0.03 $ml.h^{-1}$ for formate. Calibration scales constructed from the data also improved the ease with which the activity of different granule types could be compared with each other. However, future work could be done on expanding the range of test substrates used as well as incorporating a larger variety of anaerobic granules treating different effluents.

Introduction

The success of the upflow anaerobic sludge bed (UASB) process is mainly due to the capability of retaining the active biomass in the reactor. Over the years, several methods have been developed to characterise and quantify sludge activity but each has advantages and disadvantages. There is thus an increasing need for a rapid method to evaluate the activity of the granular biomass.

Typical activity investigations have included the determination of sludge biomass activity, assessment of inhibition levels and the measuring of kinetic constants. Most of these were performed in large closed vessels incubated under varying conditions and the methane production used to indicate the activity. Measuring the amount of biogas produced has been the most popular method to characterise methanogenic activity (James *et al.*, 1990; Angelidaki *et al.*, 1998; Verstraete & Vandevivere, 1999). Early methods relied on the liquid displacement of external gas collectors (Chernicharo & Campos, 1991; Angelidaki *et al.*, 1998; Verstraete & Vandevivere, 1999) or by displacement of a piston in gas lock syringes (Owen *et al.*, 1979; Dolfing & Bloemen, 1985; James *et al.*, 1990). More recent modifications included the introduction of a pressure transducer method (Angelidaki *et al.*, 1998). This system permitted continuous or discontinuous measurement of the pressure increase in a series of linked vials by means of multiplexer chips and a BBC microcomputer. The method opened the field of sequential automated methanometry (SAM), with the most recent method being the computerised automatic biogas activity monitoring (BAM) system (Angelidaki *et al.*, 1998).

Many researchers have observed that the data from the different activity tests varied depending on the methodology used. The most important variables that influenced microbial community growth were identified as initial activity, inoculum size, age and decline in metabolic activity and death of members of the consortium resulting from unsuitable storage conditions. Although recent developments in methanogenic activity testing have lessened the difficulties previously associated with method execution, there is still a lack of a simple method to show the activity of the different trophic groups in their specific environment within the anaerobic granule.

In most methods, sludge or mashed granules are used, which is not a true reflection of the granule activity. In many cases, the tests are performed over several days and it was concluded by Angelidaki *et al.* (1998) that true activity was not being measured but rather metabolic activity resulting from the growth of members of the microbial consortium.

The aim of this study was to investigate the applicability of a known activity test and to develop a method that will give a fast indication of direct granular activity. As part of the study, the activity levels of different types of granules will be calibrated so as to develop a scale of reference for future use.

Materials and methods

The test procedures and different test substrates (carbon sources) that were used in the four different experimental studies are summarised in Table 1. In each study the specific carbon sources used, the presence or absence of glucose in the control, and the addition of distillery effluent to the Nutrient-solution (Table 2), are indicated.

Study I. *Evaluating the applicability of a known activity test using anaerobic granules.*

In the first study an established method, as described by Lamb (1995), was used to determine the applicability in accessing the activity of anaerobic granules from a laboratory-scale UASB bioreactor treating distillery effluent (Chapter 3 of this thesis). The Lamb (1995) method is a modification and combination of the anaerobic toxicity assay (ATA) and the biological methane potential assay (BMP) (Owen *et al.*, 1979).

A Basic-medium consisting of a Nutrient-solution (Valke & Verstraete, 1993), glucose (2.0 $g.l^{-1}$) and distillery effluent with either one of three different COD concentrations: 250, 500 or 1 000 $mg.l^{-1}$. In each case a granular inoculum of 3.0 g of water drained anaerobic granules, was used. The inoculum and 200 ml Basic-medium were dispensed into 250 ml glass containers with a final headspace of 50 ml. The headspace was limited to improve the accuracy of the gas production determinations (Owen *et al.*, 1979).

The addition of different carbon sources and carbon concentrations to the Basic-medium were also evaluated and included ($g.l^{-1}$): 2.0 glucose; 0.1, 0.5 and 1.0 acetate; and a combination of 2.0 glucose with either 0.1 or 0.5 acetate. These test substrates were added to the test containers and the pH adjusted to 7.0 with 1M NaOH. The final pH of each test was also determined at the end of the assay. The assays were prepared in triplicate, sealed with rubber stoppers and incubated at 35°C. The rubber stoppers were equipped with glass tubes combined with rubber tubes that were sealed off to make gas measurements possible.

Table 1. A summary of the test procedures and substrates used in the four experimental studies.

Control with 2 g.l^{-1} glucose	Control without 2 g.l^{-1} glucose	Nutrient medium plus distillery effluent	Carbon sources (g.l^{-1})										
			2 glucose and 0.1 or 0.5 acetate	1 glucose	2 glucose	2 lactate	4 lactate	0.1 acetate	0.5 acetate	1.0 acetate	0.1 formate	0.5 formate	1.0 formate
Study I: Evaluation of activity test													
✓		✓	✓		✓			✓	✓	✓			
Study II: Design criteria evaluation of Study I													
✓				✓	✓	✓	✓	✓	✓		✓	✓	
Study III: Evaluation of glucose in nutrient medium.													
	✓			✓	✓	✓	✓	✓	✓		✓	✓	
✓				✓	✓	✓	✓	✓	✓		✓	✓	
Study IV: Screening different types of granules for activity													
✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 2. The composition of the Nutrient-solution used as part of the Basic-medium in the activity tests (Valke & Verstraete, 1993).

Compound	Concentration ($g.l^{-1}$)
KH_2PO_4	2.5
K_2HPO_4	1.0
NH_4Cl	1.0
$MgCl_2$	0.1
$Na_2S.7H_2O$	0.1
Yeast extract	0.2
Urea	1.0
pH	7.0

For each test combination, a control was prepared in the same way as for the samples (Basic-medium with granule inoculum) but no additional carbon source was added. The control was used as a point of reference to compare the relative influence of the specific carbon source on the granule microbial community relative to those with no additionally added carbon source.

The biogas samples were taken after 5 h and then each 24 h period for three days and analysed gas chromatographically. This was sufficient time for all the carbon sources to be depleted. The biogas-production readings were taken by using a gas-tight and free moving 10 ml syringe equipped with a 12 gauge needle (Owen *et al.*, 1979). Biogas measurement was done by injecting the needle into the rubber sampling tube and holding the syringe horizontally, allowing the plunger to move freely until equilibrium was reached. Readings were verified by withdrawing the plunger past the equilibrium point and then allowing it to return to its original position. The readings obtained were corrected for standard temperature and pressure.

The methane composition was determined by injecting a gas sample (1.0 ml) into a Fisons GC equipped with a thermal conductivity detector and 2.0 m x 3.0 mm i.d. column packed with Porapak Q (Waters Ass. Inc, Milford, MA), 80/100 mesh. The oven temperature was set at 55°C and helium was used as carrier gas at a flow rate of 30 $ml.min^{-1}$ (Lamb, 1995; Ronquest, 1999). The biogas readings were expressed as cumulative volumes.

Study II. *Evaluation of the effect of different design criteria (substrate changes, inoculum size and assay volume variations) on the activity method used in Study I.*

In the second study, the method used in Study I was modified for increased ease and reliability in testing the methanogenic activity of a granular sample. Smaller containers in the form of test vials (20 ml) were used that could be sealed with aluminium crimp tops and rubber septa. The rubber septa and aluminium tops minimised the possibility of gas leaks and decreased the effort associated with the pervious experimental design i.e. securing the rubber stoppers to the glass containers by means of a wire restraint (see Fig. 1). The duration of the assay was also shortened to 25 h to limit gas production due to cell growth. It is believed that the initial rate of biogas accumulation is the optimal estimate of the performance of the biomass as bacterial growth and adaptation of biomass change the biomass metabolite characteristics. Adaptional changes included changes in concentrations of substrate

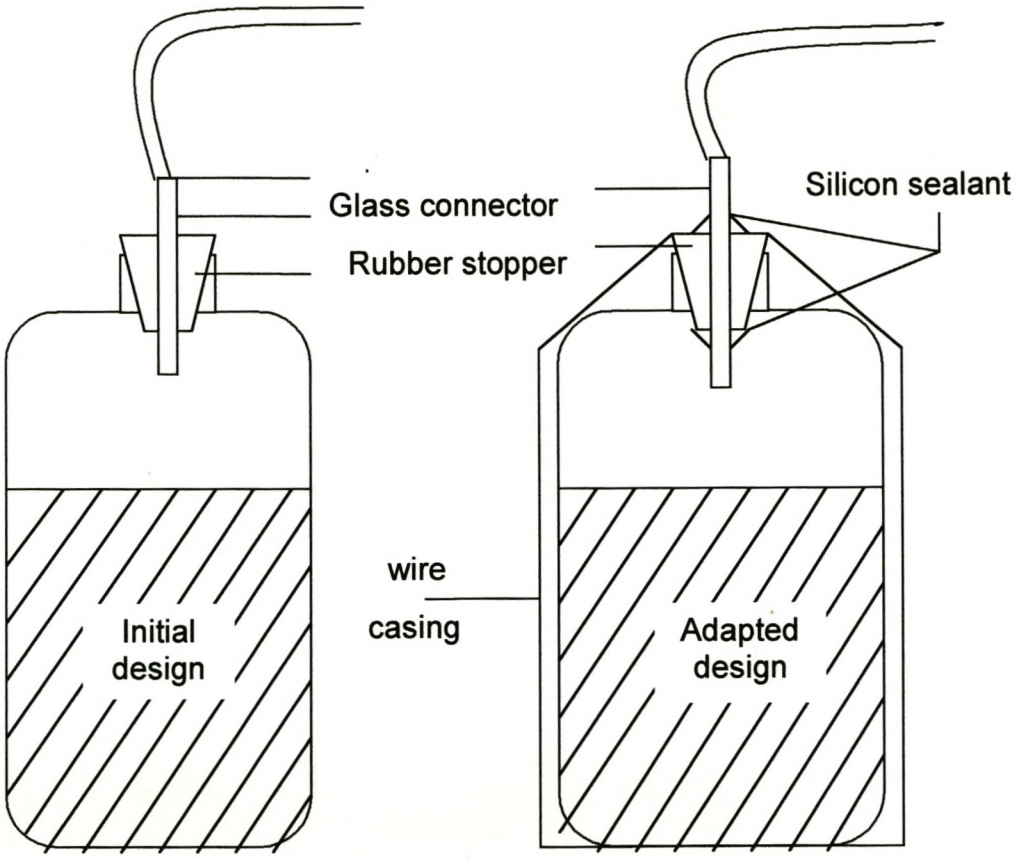

Figure 1. Initial and adapted experimental assay design used in Study I.

and nutrients in the assay bottles as the degradation process took place (Sørensen & Ahring, 1993). An incubation period of 25 h was thus considered to be adequate as negligible additional biogas was produced thereafter. The time period for the most reliable activity assays performed in the literature are in the order of a few hours to a day (James *et al.*, 1990; Ince *et al.*, 1995; Angelidaki *et al.*, 1998). Further changes in design criteria included the omission of distillery effluent to the Basic-medium. The inoculum size was also varied (1, 2 and 3 g water drained anaerobic granules) to establish which inoculum size would give the best results. The test substrates used as carbon sources were also altered and included 1 and 2 $g.l^{-1}$ glucose, 2 and 4 $g.l^{-1}$ lactate, 0.1 and 0.5 $g.l^{-1}$ acetate and 0.1 and 0.5 $g.l^{-1}$ formate.

The different inoculum sizes, Basic-medium (without distillery effluent, but with 2 g glucose) together with the different test substrates were placed in the 20 ml glass vials leaving a headspace of 7 ml and the vials sealed with crimp tops and incubated at 35°C. The headspace was kept at 7 ml to ensure sufficient pressure build-up necessary for gas measurements to be taken. A smaller headspace was found to decrease the accuracy in determining biogas production as some of the biogas produced could be dissolved in the assay media by a reverse osmosis mechanism (Angelidaki *et al.*, 1998). Biogas readings were taken at time 5, 10 and 25 h, to ensure that sufficient pressure had developed necessary to measure it by means of a gas-tight syringe. The pH of all the test vials were set at 7.0 with 1M NaOH. The final pH of each assay vial was also determined at the end of the study. As with the previous study, a control was prepared for each test substrate in the same way as for the samples (Basic-medium with granule inoculum but no additional carbon source). The contents of the assay bottles were gently mixed at regular intervals throughout the incubation time to ensure that no substrate limitation occurred due to mass transfer restrictions. All tests were prepared in triplicate. Biogas, carbon dioxide and methane determinations were done gas chromatographically on a Fisions GC.

Study III. *Evaluating the influence of the addition of glucose to the Basic-medium on the effectivity of the activity test.*

In this study, the necessity of including a suitable carbon source (glucose) as part of the Basic-medium, was verified. This was done by using the assay set-up described in Study II and using the Basic-medium with either no added glucose or 2.0 $g.l^{-1}$ added glucose (as in Studies I and II) in the assay set. Both these set-ups were also

evaluated using the same additional carbon sources (lactate, acetate and formate) as in Study II (Table 1).

The rest of the procedures were the same as those used in Study II (headspace volume, incubation time, as well as temperature and pH adjustment of assay vials). The CH_4 and CO_2 content of all biogas samples, as well as the final pH of each assay vial, were determined.

Study IV. Methanogenic activity of different types of anaerobic granules.

In this study, the method described in Study II was applied to determine the activity of seven different types of anaerobic granules. This was done to establish an activity calibration-set to be used as a reference for future granule activity estimates. Each granule type was ascribed a letter (as shown in Table 3) to aid in classifying them in terms of their original source.

The assay method used was as follows: 20 ml glass vials were inoculated with 3 g anaerobic granules and 12 ml of the Basic-medium (2 $g.l^{-1}$ glucose and nutrient medium) added to the vials. The different test substrates (1 and 2 $g.l^{-1}$ of glucose, 2 and 4 $g.l^{-1}$ lactate, 0.1, 0.5 and 1.0 $g.l^{-1}$ acetate and 0.1, 0.5 and 1.0 $g.l^{-1}$ formate) were then added to each specific assay set. Each vial was sealed, the pH set at 7.0 and the vials incubated at 35° C for 25 h. The assay vials were prepared in triplicate and biogas samples were taken at time 5, 10 and 25 h. The biogas samples were analysed for CH_4 and CO_2 content gas chromatographically, using a Fisons GC equipped with a thermal conductivity detector and 2.0 m x 3.0 mm i.d. column packed with Porapak Q (Waters Ass. Inc, Milford, MA), 80/100 mesh. The oven temperature was set at 55°C and helium was used as carrier gas at a flow rate of 30 $ml.min^{-1}$ (Lamb, 1995; Ronquest, 1999). The final pH of each vial was determined at the completion of the study.

Results and discussion

Study I. Evaluating the applicability of a known activity test using anaerobic granules.

In the first study, the applicability of an established method adapted by Lamb (1995) from Owen *et al.* (1979), for testing the activity of anaerobic granules, was evaluated. In this study anaerobic granules from a lab-scale UASB bioreactor treating distillery effluent, were used as inoculum.

Table 3. Different anaerobic biogranules evaluated in Study IV for Granule activity.

Label number	Origin	Effluent type
S-type	industrial UASB	Distillery effluent[1]
O-type	lab-scale UASB	Distillery effluent[2]
C-type	industrial UASB	Fruit juice effluent[3]
M- type	lab-scale UASB	Winery effluent[4]
G-type	lab-scale UASB	Cannery effluent[5]
W-type	industrial UASB	Brewery effluent[6]
V-type	industrial conventional bioreactor	Gelatine effluent[7]

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5. Mr. G.O. Sigge, University of Stellenbosch, SA
6. Dr. A. Wood, SA Breweries, Amanzimtoti, SA
7. Dr. M. Van der Merwe, Pietersburg, SA

In a preliminary set of experiments (data not shown), it was found that the proposed method had some shortcomings in terms of reproducibility relating to problems experienced with the design of the assay. Biogas readings from triplicate samples showed large differences, which was attributed to faulty design criteria. The problem was that the experimental set-up had to be made completely gas-tight. The rubber stoppers were thus secured to the assay containers by means of a wire restraint, so as to prevent the stoppers from being dislodged due to pressure building up within the test container. The opening for the glass connector in the rubber stopper was also made gas-tight by using silicone sealant to seal off the openings between the glass connector and the rubber stopper. The preliminary and adapted assay design can be seen in Fig. 1.

The assay was then repeated with the new design and the data obtained illustrated in Fig 2A, B and C. The graphs represent the cumulative production of biogas with 250 (A), 500 (B) and 1000 (C) $mg.l^{-1}$ COD distillery effluent added to the Basic-medium. Each control data point represented the cumulative biogas production of triplicate assay samples that did not contain any additional test substrate and thereby represented the activity of the microbial community in the absence of any stimulatory or inhibitory compounds. It was, therefore, not necessary to indicate the net difference in production between the test assays and the control. As can be seen in Fig. 2A, B and C, the tests were performed over a period of 120 h but the data clearly shows that the major biogas production took place within the first 30 h for all three the COD concentrations. Distillery effluent was added to the Basic-medium to simulate the environment in which the granules had been functioning previously. According to Ronquest (1999), the bacterial communities within anaerobic granules are able to adjust in such a manner that bacteria, especially adapt in breaking down a specific compound in the substrate, are included in the granule structure. Those specific granules are, therefore, more effective at treating a specific substrate. Glucose, acetate and a combination of the two were also added as carbon sources to investigated the activity of the different trophic groups within the specific anaerobic granules used in this study.

From the results (Fig. 2) it can be seen that the best cumulative gas production was obtained when using 250 and 500 $mg.l^{-1}$ COD distillery effluent in the Basic-medium (Fig. 2A and B). When 250 $mg.l^{-1}$ COD distillery effluent was used in the Basic-medium plus a combination of 0.1 $g.l^{-1}$ acetate and 4 $g.l^{-1}$ glucose as test substrates, the most biogas was produced by the end of the incubation period. However, the 0.1 $g.l^{-1}$ acetate assay produced more biogas than all the other assays for

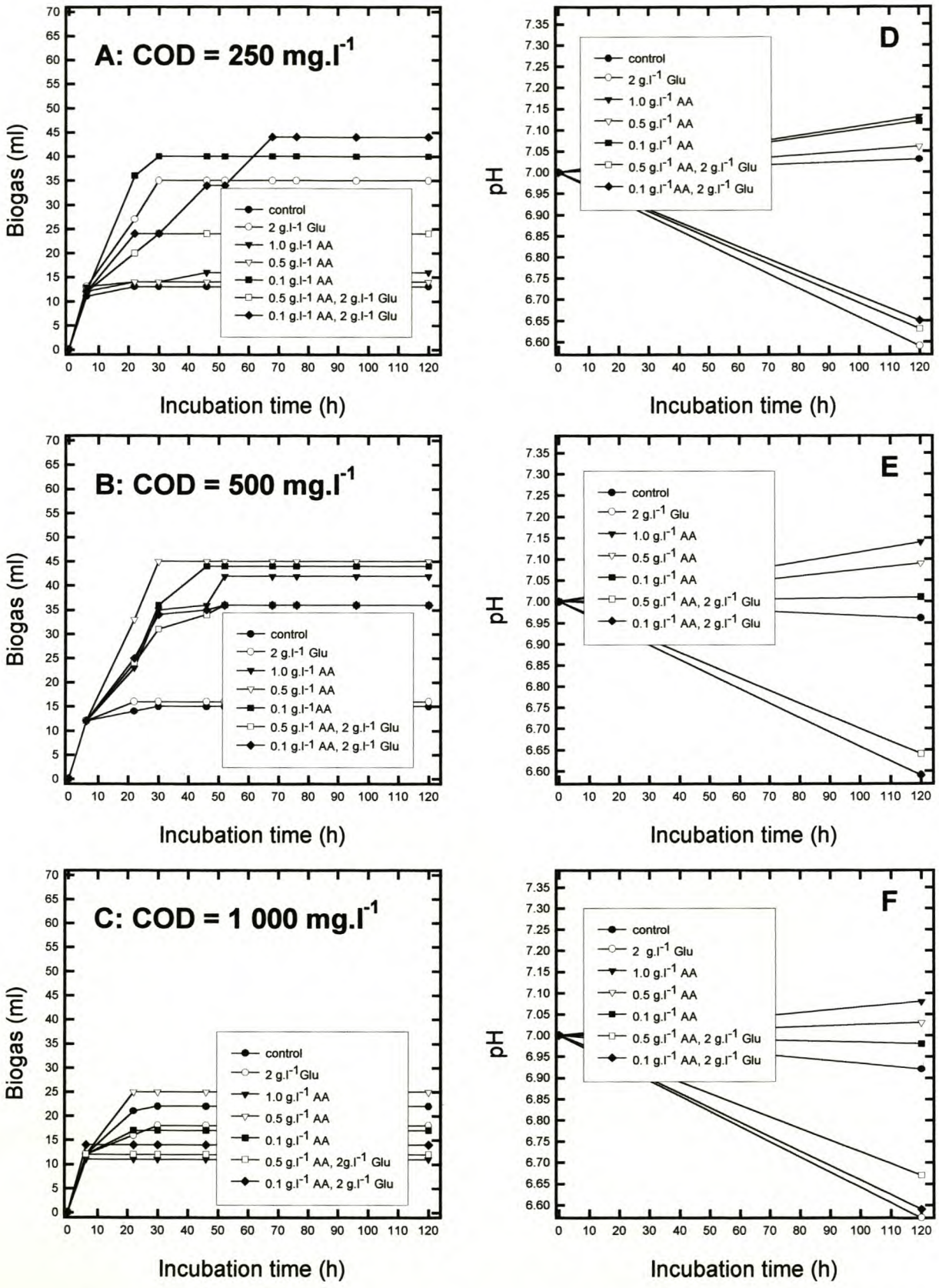

Figure 2. Cumulative biogas production (ml) of granules at different COD concentrations (mg.l^{-1}), (250(A), 500(B) and 1000(C)), and with different carbon sources. Final pH values are shown for each COD concentration (250(D), 500(E) and 1000(F)). (Glu = glucose, AA = acetate).

the same COD concentration after 20 h. Thus, when evaluating the effect of different test substrates on biogas production, the specific biogas profile of each test substrate should be considered. When 500 mg.l^{-1} COD distillery effluent was added to the Basic-medium, the most biogas produced at the completion of the assay was with 0.5 g.l^{-1} acetate (Fig. 2B). The 0.1 g.l^{-1} acetate assay produced very similar volumes of biogas. Similar profile trends, but much lower volumes were obtained when a 1 000 mg.l^{-1} COD distillery effluent was used in the Basic-medium (Fig. 2C). The use of 0.5 g.l^{-1} acetate as test substrate also gave the highest biogas production after 20 h for this COD concentration (Fig. 2C). Although no general activity profile for the different carbon sources used can be seen with the different concentrations of distillery effluent, it would appear that the samples containing acetate generally produced more biogas (Fig. 2A - C). The higher biogas production activity associated with the acetate containing assay sets can possibly be ascribed to the activity of the methanogens, which can directly convert acetate to methane.

It was also observed that in most cases no additional gas production took place after 30 h of incubation with the 250 and 500 mg.l^{-1} COD distillery effluents in the Basic-medium (Fig. 2A and 2B) and after 20 h for the 1 000 mg.l^{-1} COD distillery effluent (Fig. 2C). It was thus concluded that an incubation time of 120 h was unnecessary because most of the usable carbon sources were probably metabolised by that time. For the subsequent studies the incubation time was thus shortened to 25 h.

The data indicating the impact of the different test substrates on final system pH are illustrated in Fig. 2D, E and F. For all three the COD concentrations of distillery effluent used in the Basic-medium, the largest pH drop was where the 2 g.l^{-1} glucose was added as test substrate, and a pH drop from 7.00 to 6.59 was found. The accumulation of volatile fatty acids (VFA) was probably the cause of this, but precisely, which was not determined. It can thus not be concluded which group of acidogenic bacteria was most active in terms of substrate utilisation and VFA production.

The highest pH increase for all three cases was obtained with the addition of 1.0 g.l^{-1} acetate as test substrate, with an increase from 7.0 to 7.1. The increase in the pH that occurred when acetate was used as test substrate can be attributed to the release of sodium ions as acetate was metabolised (Morgan *et al.*, 1990). In the pH range of this assay, methanogens and other anaerobic bacteria involved in the process can still function with ease (Nel & Britz, 1986) and it was concluded that system pH did, therefore, not significantly influence the gas production.

There was, however, no similarity between the test substrate's biogas production profiles at the different distillery effluent concentrations. The accuracy of this particular assay method in terms of specific substrate utilisation is, therefore, unreliable. Similar studies done on the activity of anaerobic bacteria according to this method also showed large variability (Lamb, 1995). Lamb (1995) ascribed the variability in microbial activity to differences in biomass age. Older biomass samples were found to produce less biogas than biomass (sludge) more recently sampled. The biomass in the form of granules, as used in this study, was of the same age and the variability in biogas production and pH changes was thus rather ascribed to the inadequacy of the method and not because of differences in biomass age.

Study II. *Evaluation of the effect of different design criteria (substrate changes, inoculum size and assay volume variations) on the activity method used in Study I.*

In *Study II,* the set-up was modified to make use of smaller test vials (20 ml) that could be properly sealed using septa and crimp tops which resulted in greater biogas measuring accuracy. The optimum inoculum size was also determined (Fig. 3) in triplicate by using 1.0, 2.0 and 3.0 g of water drained anaerobic granules and combining with either ($g.l^{-1}$): 1.0 and 2.0 glucose; 2.0 and 4.0 lactate; 0.1 and 0.5 acetate; or 0.1 and 0.5 formate, as different carbon sources. Formate was added as an extra test substrate to include the investigation of the activity of formate-utilising methanogens within the methodology. In this study, the assay duration was shortened to 25 h and the biogas, methane and carbon dioxide determined after 5, 10 and 25 h. The duration of the assay was shortened to 25 h to limit gas production due to cell growth.

The data are presented in Fig. 3 and 4. The dotted line in Fig 3A, B and C represents the cumulative volume of biogas produced at time 25 h for the control containing a 3 g inoculum. It was thus possible to estimate the net difference in production between the added carbon sources and the control, which contained no additional carbon sources.

In all the studies, the use of the 3 g granular inoculum resulted in the production of the largest biogas volume (Fig 3A - E) and the 1g inoculum test vials the lowest amount of biogas. It was, therefore, decided that 3 g granular inoculums would be the most suitable to ensure the production of biogas and to enhance the visual evaluation of the data. The use of a 25 h incubation period was adequate for

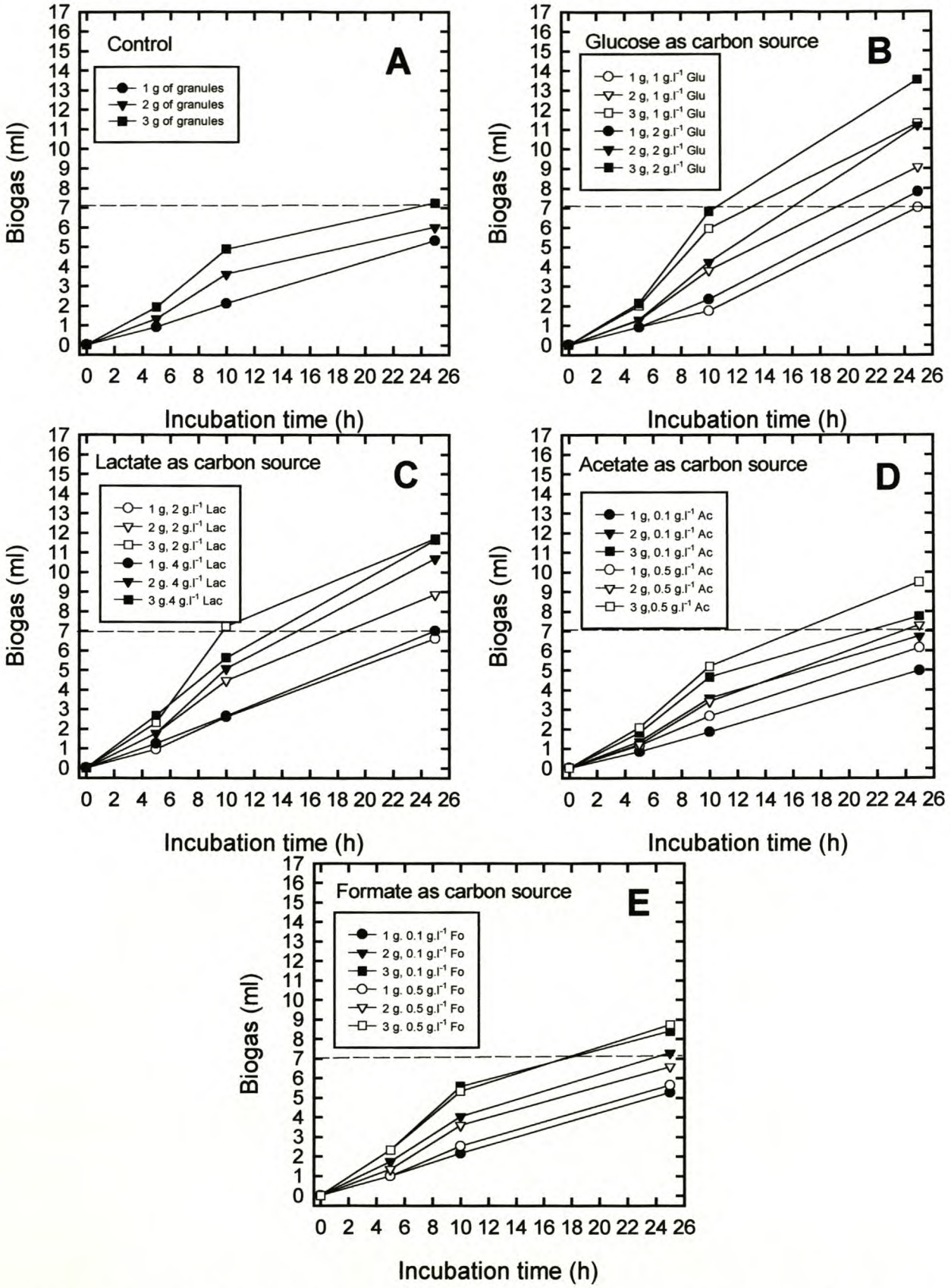

Figure 3. The cumulative biogas production of anaerobic granules with different carbon sources as well as different inoculum sizes (1, 2 and 3 g).(Glu = glucose, Lac = lactate, Ac = acetate, Fo = formate) (---- = cumulative biogas production for the 3 g inoculum control after 25 h)

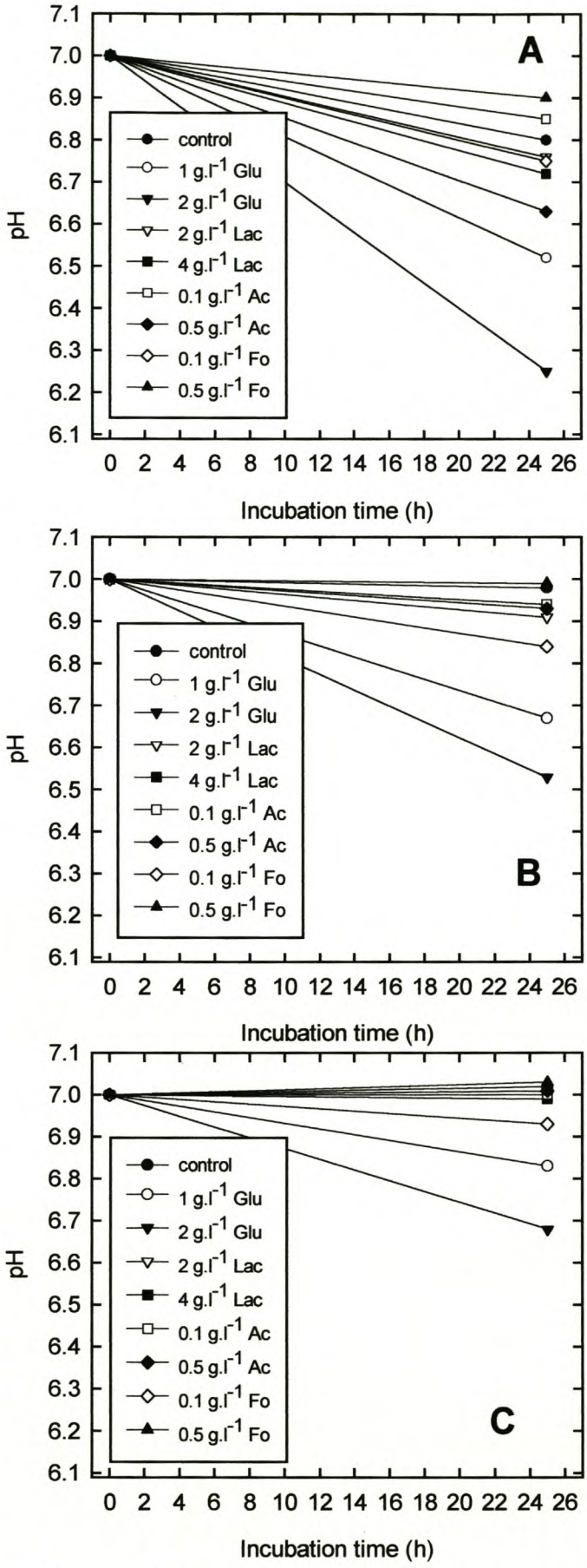

Figure 4. Final pH of vials with different carbon sources for 1 (A), 2 (B) and 3 g (C) granular inoculum.

substrate depletion to take place as no substantial volume of biogas was produced thereafter. In Fig. 3 (A - E) it can be seen that all the test substrates used enhanced the biogas production. The highest value amount of biogas was produced with glucose as test substrate (Fig. 3B), which was an indication that the acidogenic community of the granules used in this study were well adapted and able to effectively degrade the carbon source. The 2 $g.l^{-1}$ glucose assay produced more biogas than when only 1 $g.l^{-1}$ of glucose was used and this was ascribed to the availability of more degradable carbon. The final pH of the 2 $g.l^{-1}$ glucose assay (Fig. 4A, B and C) was also the lowest for all three inoculum sizes, which could be due to the accumulation of the volatile fatty acids that could not be utilised by the methanogenic bacteria during the test period.

Lactate was used as a test substrate to investigate the possible presence of lactate-utilising bacteria. The reason for this was that Britz *et al.* (1999) postulated that when an anaerobic microbial consortium is put under 'stress' conditions (with an additional rich carbon source such as glucose), lactate-utilising bacteria would be able to function best and lactate would be utilised with a subsequent increase in biogas production. The 4 $g.l^{-1}$ lactate vials produced more biogas than the 2 $g.l^{-1}$ lactate vials (Fig. 3C). It was also found that the lactate containing assays produced less biogas than the glucose samples, but more than those containing acetate and formate as carbon sources (Fig. 3D and E).

Although, based on the data from Study II, it was expected that the biogas production of the acetate assays would be relatively high due to the high CH_4 (> 80%) content in the biogas samples after 25 h, the acetate samples did not produce more biogas than the glucose assays. The 0.5 $g.l^{-1}$ acetate assays produced more biogas than the 0.1 $g.l^{-1}$ acetate assays for all the inoculum sizes and the reason for poor biogas production can, therefore, not be attributed to substrate inhibition (Fig. 3D). The final pH values of the acetate vials were between 7.0 and 7.1 (Fig. 4) with the formate vials having the most alkaline pH of between 7.1 and 7.2. The elevated pH's obtained with the acetate vials are probably due to the release of sodium ions as the acetate was metabolised (Morgan *et al.*, 1990).

The lower volume of biogas obtained for acetate in comparison with lactate and glucose can possibly be attributed to the fact that the absorption of carbon dioxide is increased at an alkaline pH (Morgan *et al.,* 1990). Thus, although the total gas yield was smaller due to greater carbon dioxide absorption in the liquid phase, its methane content was significantly higher: 82% in comparison with 68% in the biogas produced with glucose and lactate.

The data for this study showed that the modified set-up led to a faster and more accurate indication of granule activity. This study, using three different granule inoculum sizes and each test in triplicate, was repeated at three separate times and in total gave a biogas variation of less than 10%. The shorter assay period was also more convenient in terms of laboratory handling.

One aspect that was found to help stabilise and make the modified method more accurate was the addition of glucose to the basic assay medium. This was verified by comparing the adapted method with added glucose to the Basic-medium and one set where it was omitted from the Basic-medium.

Study III. *Evaluating the influence of the addition of glucose to the Basic-medium on the effectivity of the activity test.*

In *Study III,* the necessity of including a suitable carbon source (glucose) as part of the basic test medium, was determined. This was done by repeating the method used in Study II and using the Basic-medium with either 2.0 $g.l^{-1}$ added glucose or the Basic-medium with no added glucose.

The results of tests obtained without and with the added glucose to the Basic-medium are illustrated in Fig. 5 and 6, respectively. It was clear from the data in Fig. 5B and 6B that the addition of 1.0 and 2.0 $g.l^{-1}$ glucose to the basic medium enhanced biogas production, and especially the 2.0 $g.l^{-1}$ addition made the assay easier to evaluate. This was especially so when determining the activity of the lactate catabolising anaerobes, and of the acetate and formate-utilising methanogens (Fig. 5C - E and Fig. 6C - E). Although the basic trend in biogas production was very similar for both the media where glucose was added and where the glucose was omitted, the glucose containing Basic-medium assay results were more clear in terms of visual evaluation. Similar tests as reported in the literature performed without the addition of glucose to a basic medium, usually required a different means of gas sampling (e.g. pressure transducers) than just a gas-tight syringe (Angelidaki *et al.*, 1998). This type of measuring instrument (pressure transducer) is more accurate for determining small amounts of biogas, but it is much more expensive than the method used in this study. Based on the results obtained, it was decided that it was advantageous to add glucose as part of the Basic-medium.

The final pH values obtained with glucose in the Basic-medium were generally

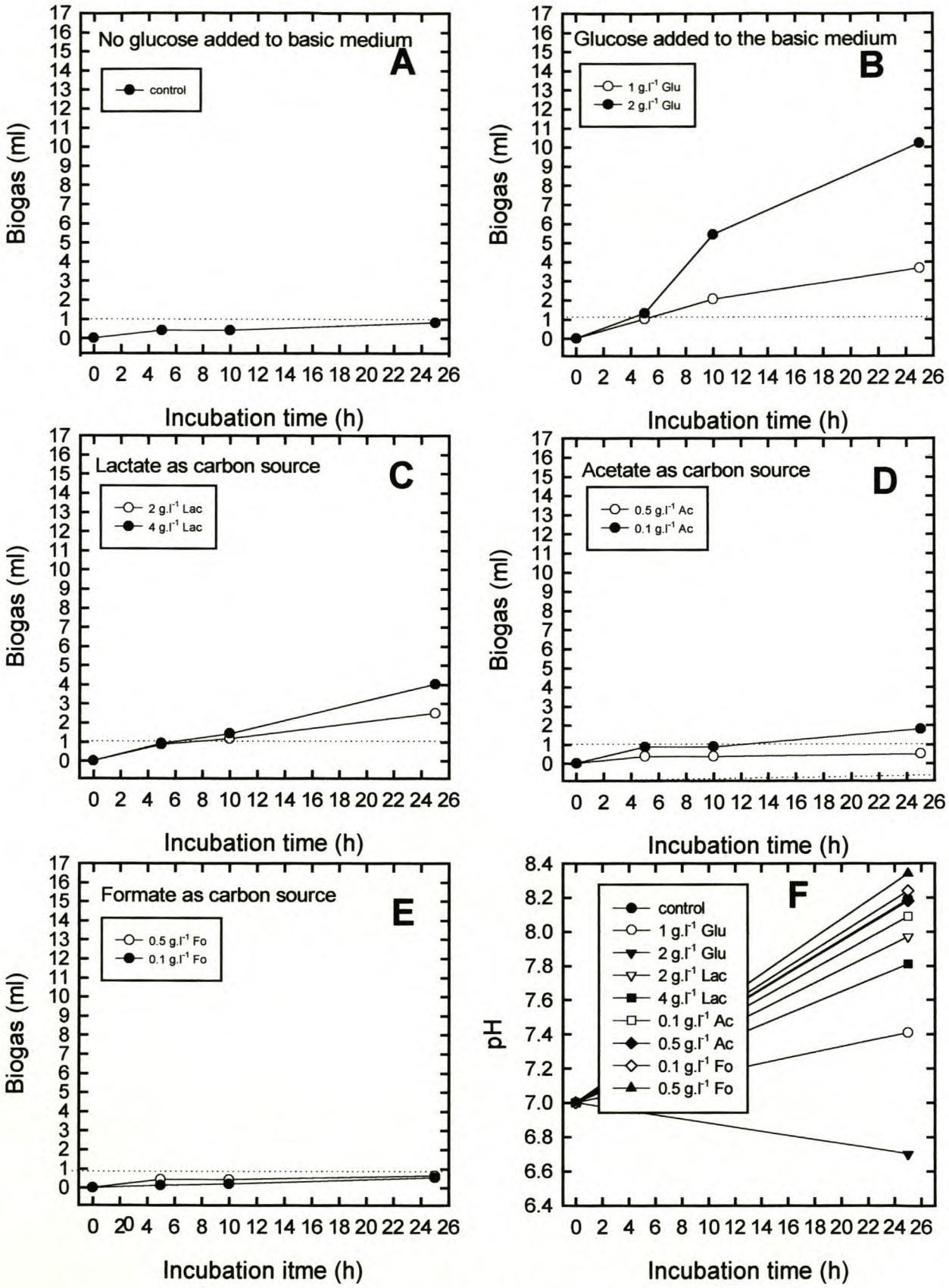

Figure 5. Cumulative biogas production by anaerobic granules where no glucose was added to the basic medium (A) and with the addition of different other carbon sources (B - E) as well as the pH changes (F) determined at the end of the assay period. (Glu = glucose, Lac = lactate, AA = acetate, Fo = formate) (----- = the cumulative value of biogas production for the control after 25 h)

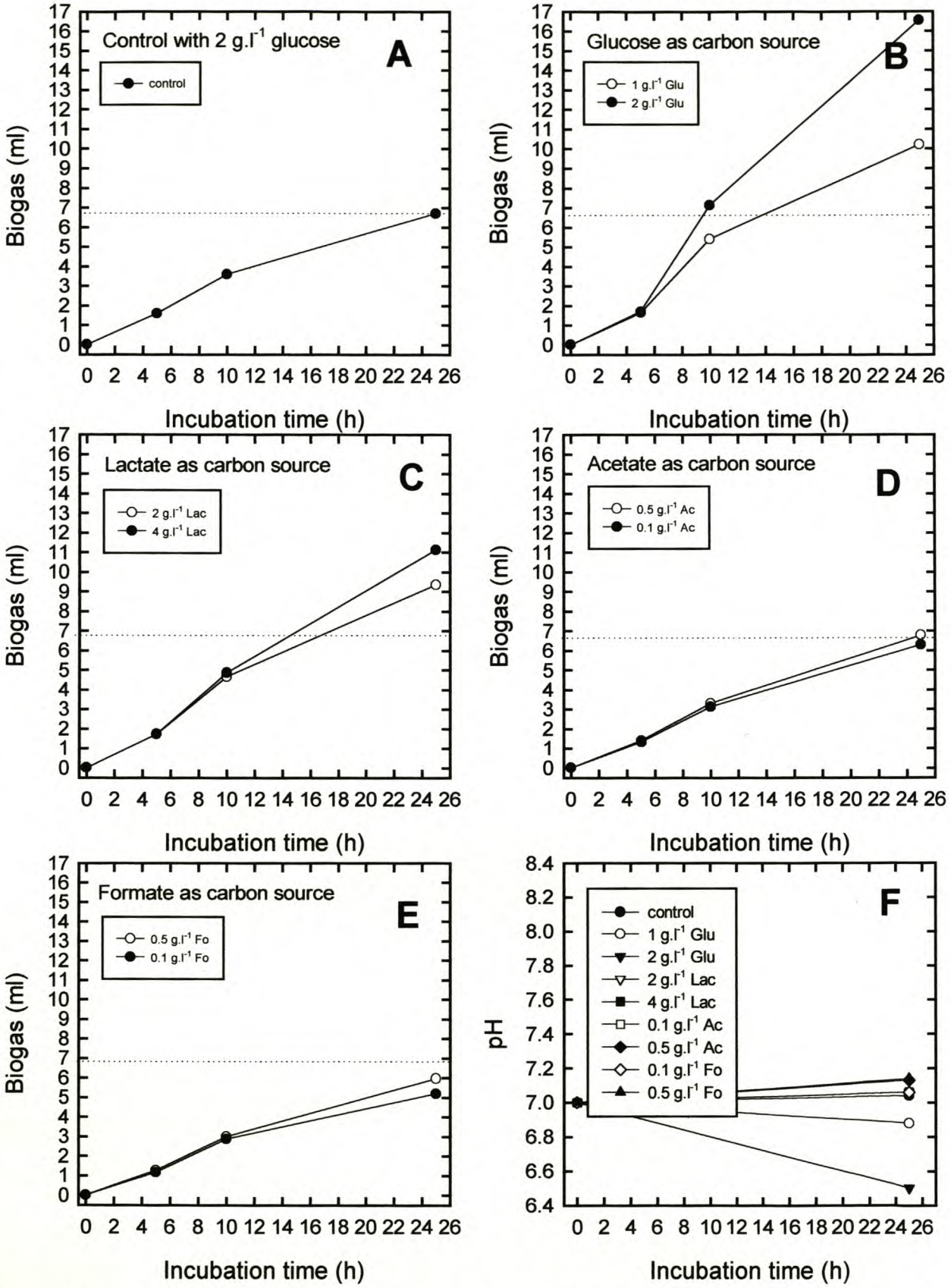

Figure 6. Cumulative biogas production of anaerobic granules where glucose (2 $g.l^{-1}$) was added to the basic medium (A) and with different other carbon sources (B - E), as well as the pH changes (F) determined at the end of the assay period. (Glu = glucose, Lac = lactate, AA = acetate, Fo = formate) (---- = the cumulative value of biogas production for the control after 25 h)

lower than the values where glucose was not present in the Basic-medium (Fig. 5F and 6F). This was ascribed to the possible accumulation of more volatile fatty acids, which the methanogenic populations were unable or partially unable to metabolise during the incubation period employed in this study. Although the final pH values for the test assays where glucose was added (Fig. 5F and 6F) were within the operational range for methanogen activity (Fig. 6F), the formate assay vials resulted in a final pH of above 8.0 (Fig. 5F) when glucose was omitted. This high pH could possibly be inhibitory to the methanogens or may change the structure of the test substrate in such a manner that the methanogens are unable to utilise their specific metabolites.

Study IV. Methanogenic activity of different types of anaerobic granules.

In Study IV the modified assay, as described in Study II, and with the addition of 2.0 $g.l^{-1}$ glucose to the Basic-medium, was used as a standardised method to determine and compare the activity of seven different granule types. These granules were obtained from industrial and lab-scale UASB bioreactors treating the following effluents: winery, distillery, brewery, cannery and fruit juice effluent (Table 3).

After the cumulative biogas and CH_4 production of each type of granules were determined, the data was converted to represent the activity of each type of granule relative to the others. In this study the activity of each type of granule is thus described in terms of the tempo of biogas and CH_4 production. The tempo of gas production was determined using the slope or gradient (the cumulative gas production (CGP) over time for each of the time intervals (T = 5, 10 and 25 h)). The gradient was determined as follows: e.g. for 10 h: $(CGP_{10h} - CGP_{5h})/(T_{10h} - T_{5h})$. The tempo of biogas production (S_B for biogas and S_M for methane) was thus given in $ml.h^{-1}$ and was taken as representative of the activity of the granules. These values were then plotted against time for all the granules and for the other carbon sources evaluated to give a visual representation of the activity of the different granule types.

The biogas and methanogenic activities of the seven granule types are shown in Fig. 7 and 8, respectively. In Fig. 7 (A - K) it is clear that the different granule types could be divided into two main groupings in terms of the tempo of biogas production (S_B) after 5 h (activity). The groups were the 0-, M-, G- and C- type granules (the first group) which exhibited more activity (± 0.3 $ml.h^{-1}$) than the S-, V- and W-type granules (the second group) for the different carbon sources. After the 10 h incubation period, a larger variation could be seen between the activities of the different granules for the test substrates.

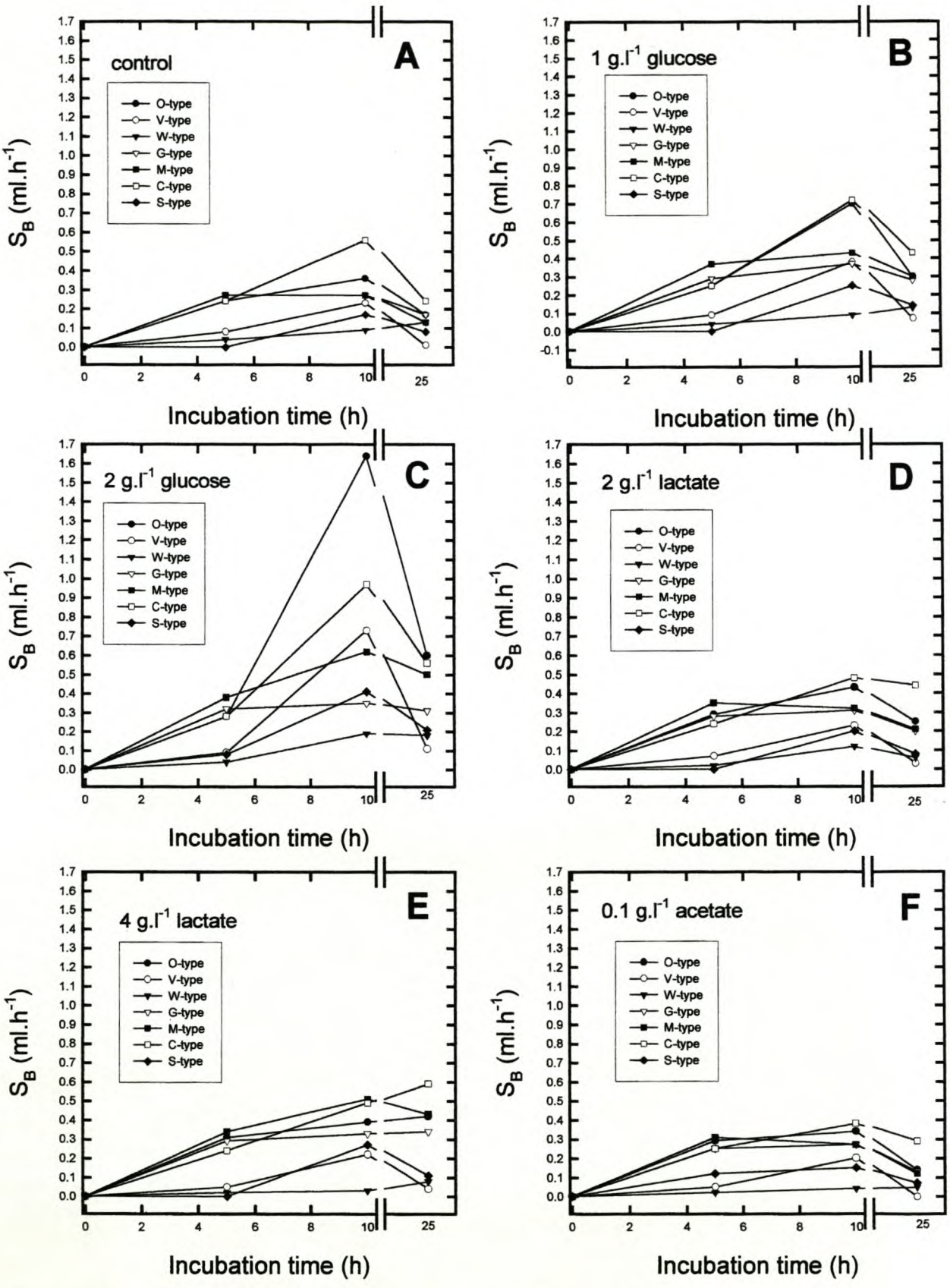

Figure 7. Comparison of the activity of different types of anaerobic granules with different carbon sources added to the Basic-medium. The data are averages of triplicates. (S_B = tempo of biogas production ($ml.h^{-1}$)

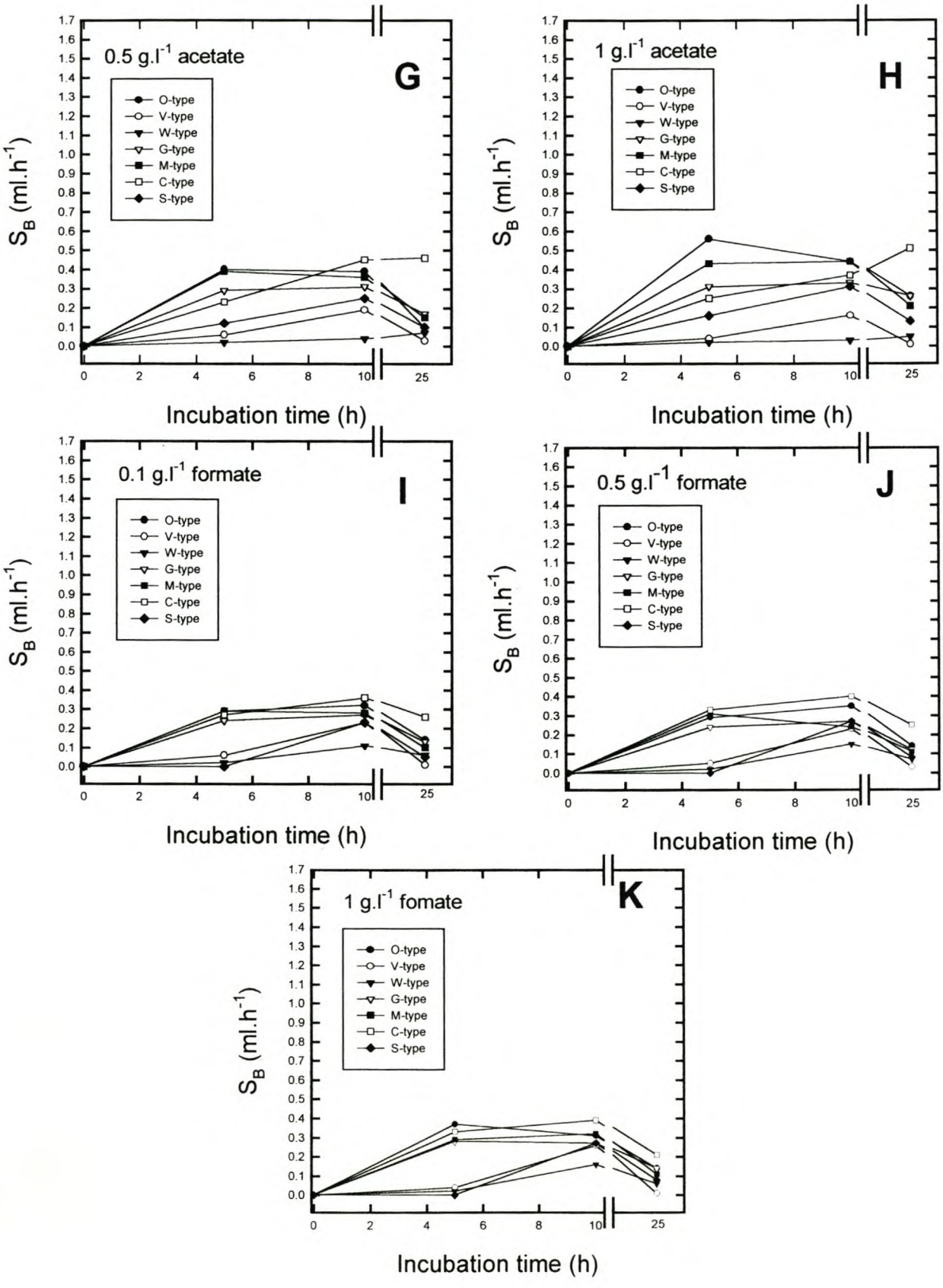

Figure 7. continued

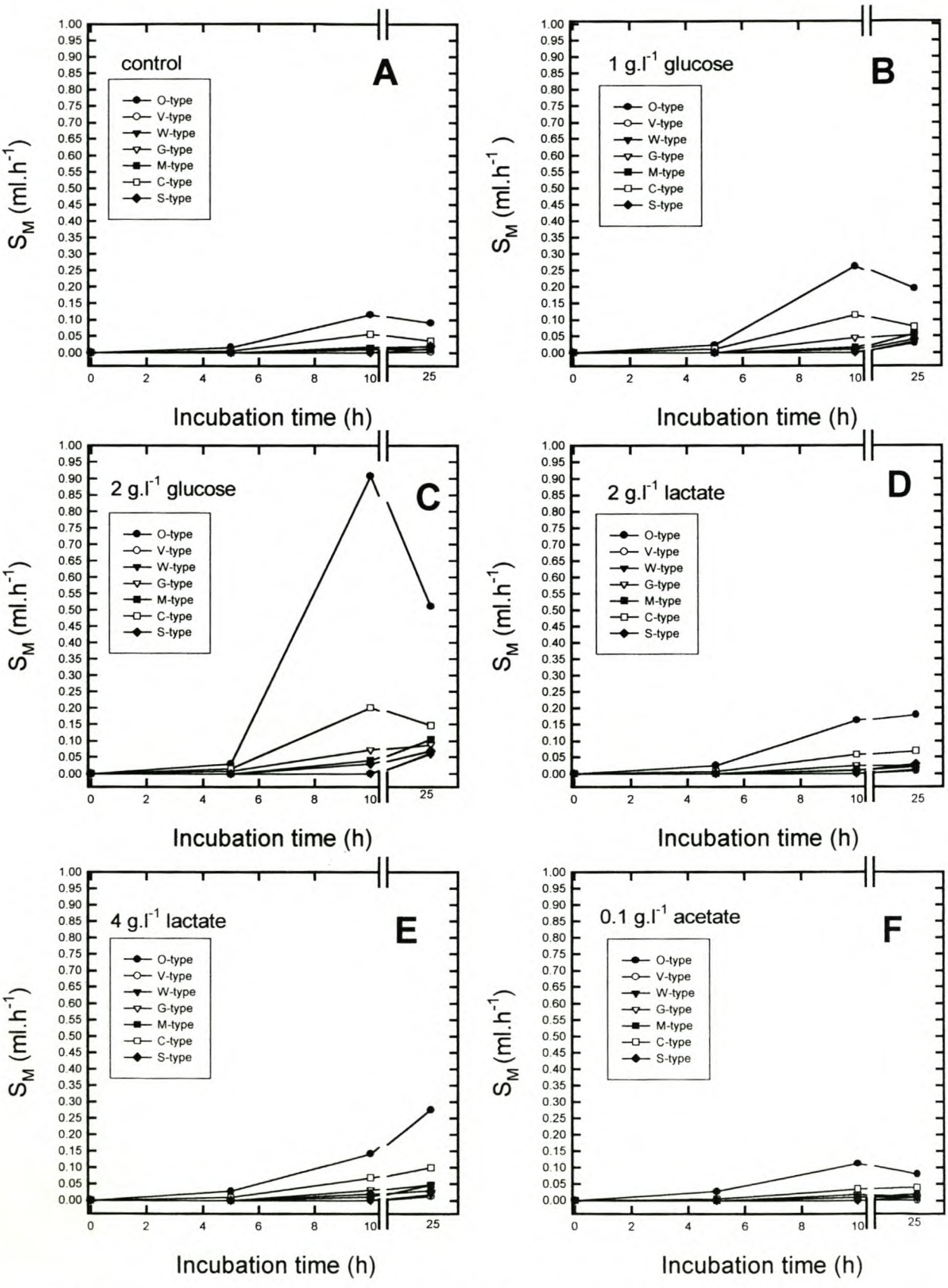

Figure 8. Comparison of methane activity of different anaerobic granules with different carbon sources added to the Basic-medium. The data are averages of triplicates. (S_M = tempo of methane production ($ml.h^{-1}$)

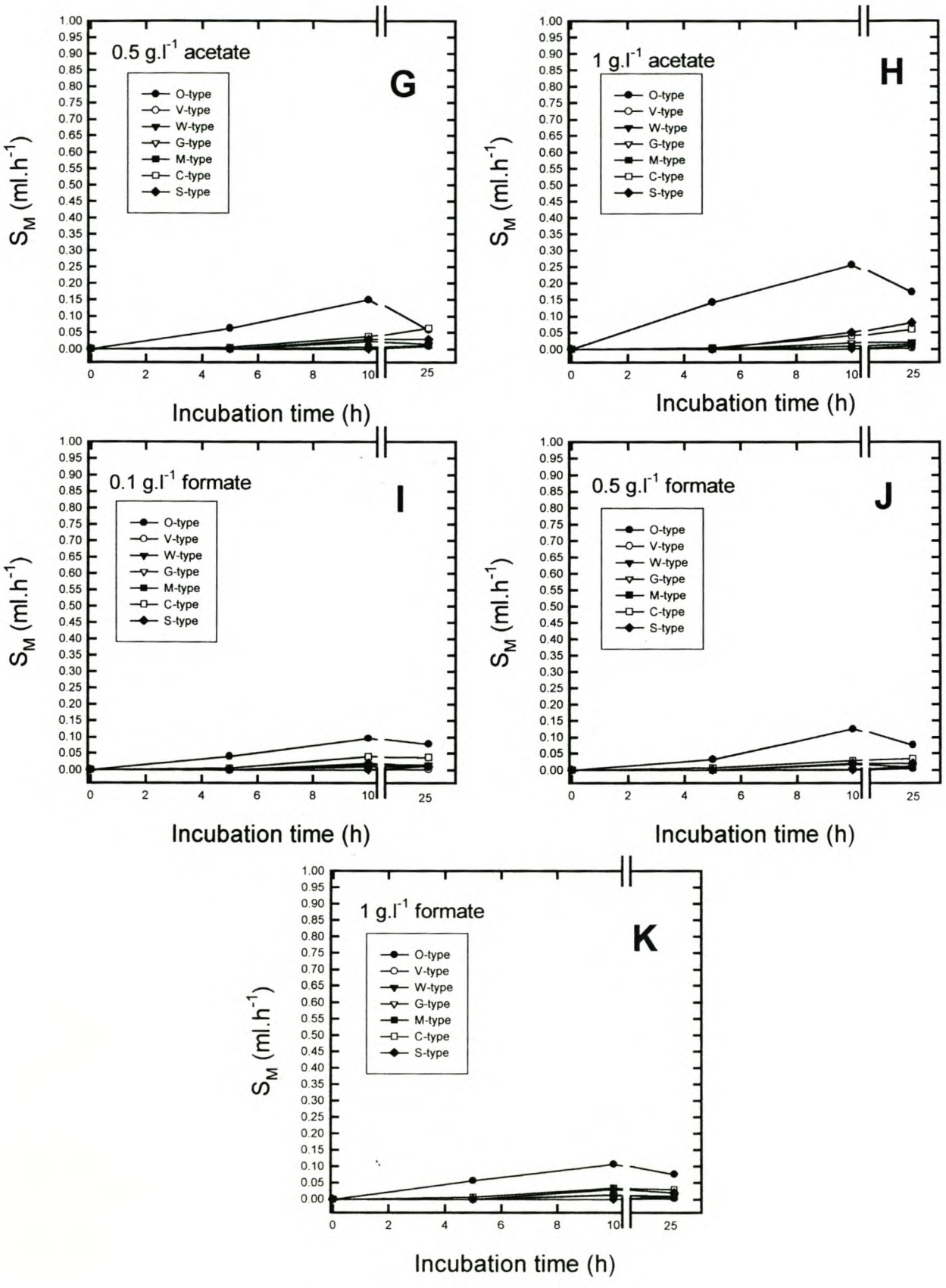

Figure 8. continued

In Fig. 8 (A - K) the methanogenic activity (S_M) (tempo of CH_4 production) of the granules relative to one another and with the different carbon sources, is illustrated. The methanogenic activity of all the granule types for all the carbon sources used was very low or even absent after 5 h of incubation (< 0.05 $ml.h^{-1}$). The activity of the O-type granules, however, showed a higher activity (0.08 and 0.15 $ml.h^{-1}$) with the added 0.5 and 1.0 $g.l^{-1}$ acetate as carbon source (Fig. 8G and 8H). From this, it was concluded that the methanogens in these granules were able to effectively utilise the substrate, with the acetate probably being directly metabolised to methane. At an acetate concentration of 0.1 $g.l^{-1}$ (Fig. 8F), the activity of the O-type granules did not vary significantly from the others, possibly indicating that the availability of acetate was not adequate for optimal CH_4 production activity. At 10 h of incubation, a larger variability in methanogenic activity (Fig. 8) was found and subsequently the influence of the different carbon sources was more apparent. No specific groups were evident as was found for the biogas and each test substrate was evaluated separately by means of bar charts. The rate at which biogas and CH_4 were produced decreased after the 10 to the 25 h incubation time (Fig. 7 and 8) for most of the granules, indicating that this further incubation period of up to 25 h was found not to be of value in indicating activity.

Activity evaluation of different types of granules using bar charts (histograms)

To more clearly illustrate the impact of the different carbon sources ($g.l^{-1}$: 2 glucose, 4 lactate, 1.0 acetate and 1.0 formate) on the activity of the different types of granules, the activity data for both the biogas (S_B) and CH_4 (S_M) were converted into bar charts (Fig. 9 - 16). The activity of the control assay of each granule type was plotted together with the value obtained for the assay of that particular carbon source at time 5 and 10 h of incubation. The net effect of the specific carbon source on the activity of the granules can therefore be estimated by visually comparing the control with the sample bar on the chart. It is **important to note** that the activity values discussed in the following paragraphs are those where the control value had been subtracted from the value of the tested samples. In certain cases, it was also found that a measure of inhibition (the control value greater than the sample value) was present but the activities of those specific granule types were still recordable in the activity range.

Glucose as carbon source - From the data summarised (in the form of bar diagrams) in Fig. 9, it can be seen that after 5 h of incubation, the M-type granules showed the highest biogas activity (0.15 $ml.h^{-1}$) with glucose as carbon source. The O-, G- and C-type granules were, however, the only granules that produced methane by the

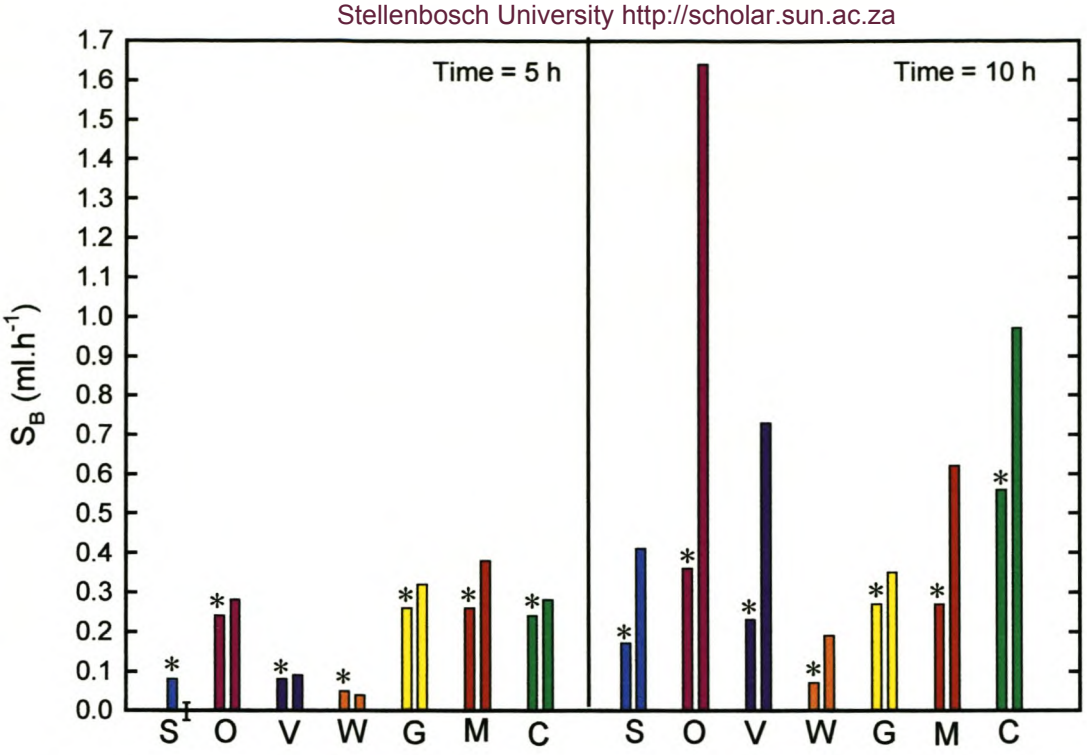

Figure 9. Activity of different anaerobic granules evaluated in the Basic-medium (as control) and with an additional 2.0 g.l^{-1} of glucose as carbon source. (S_B = tempo of biogas production (ml.h^{-1}), (* = control, I = no gas production)

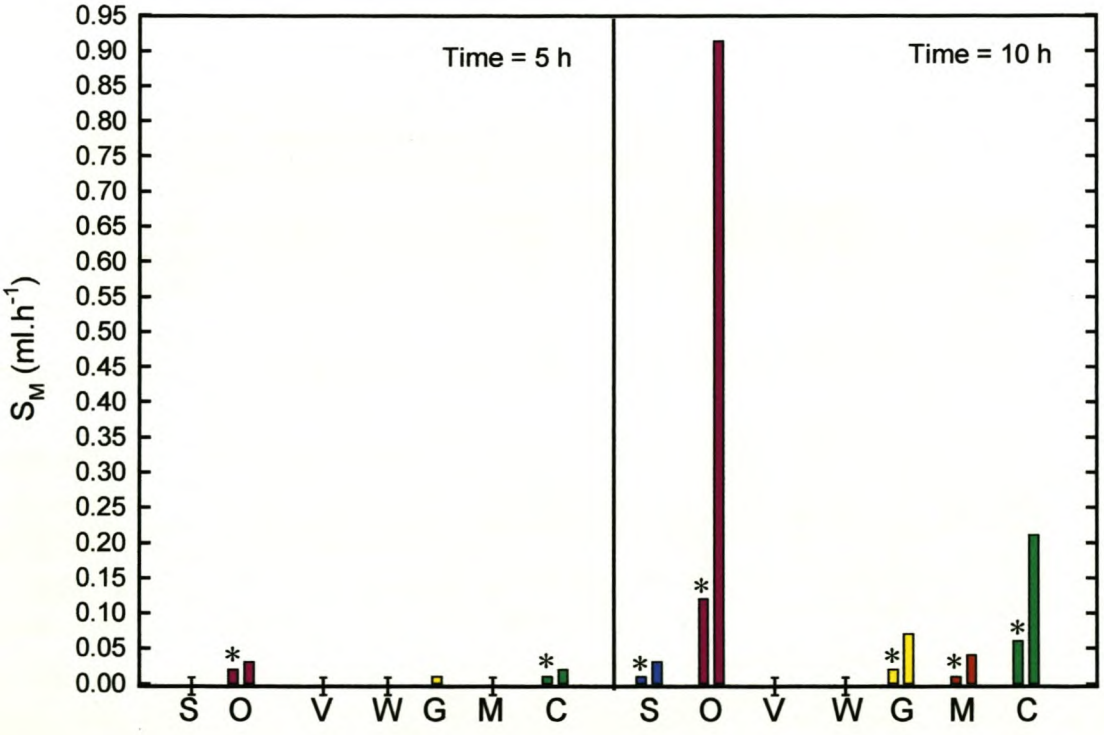

Figure 10. Methanogenic activity of different anaerobic granules evaluated in the Basic-medium (as control) and with an additional 2.0 g.l^{-1} glucose as carbon source. (S_M = tempo of CH_4 production (ml.h^{-1}), (* = control, I = no gas production)

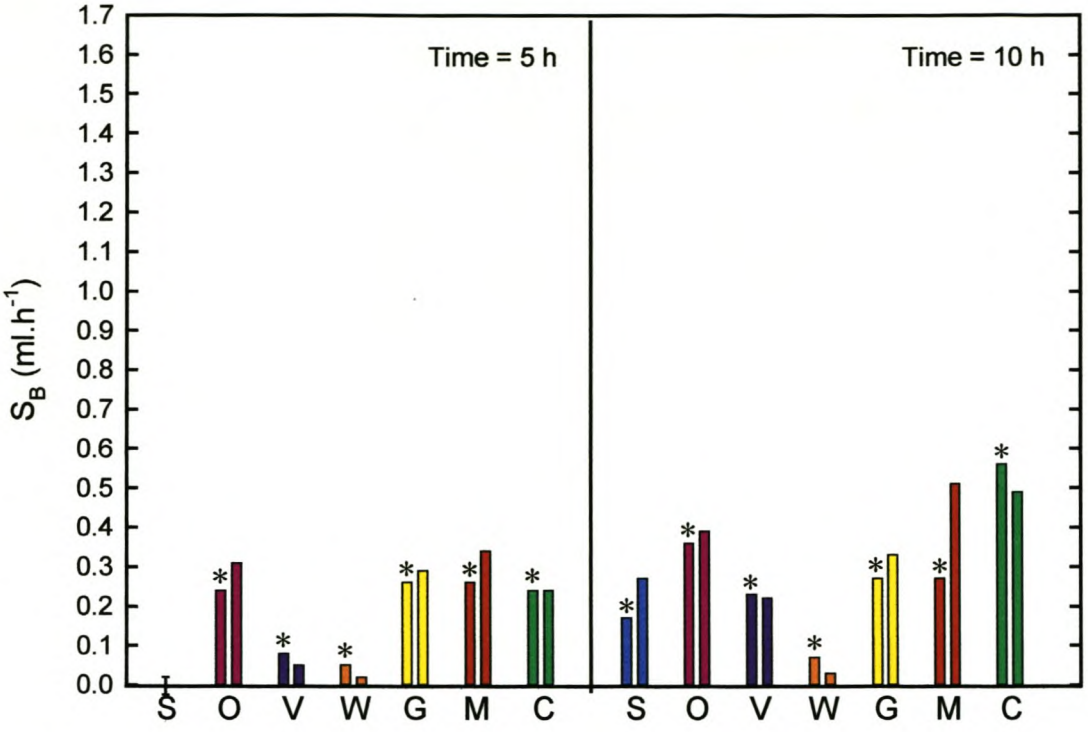

Figure 11. Activity of different anaerobic granules evaluated in the Basic-medium (as control) and with an additional 4.0 g.l^{-1} lactate as carbon source. (S_B = tempo of biogas production (ml.h^{-1})), ($*$ = control, I = no gas production)

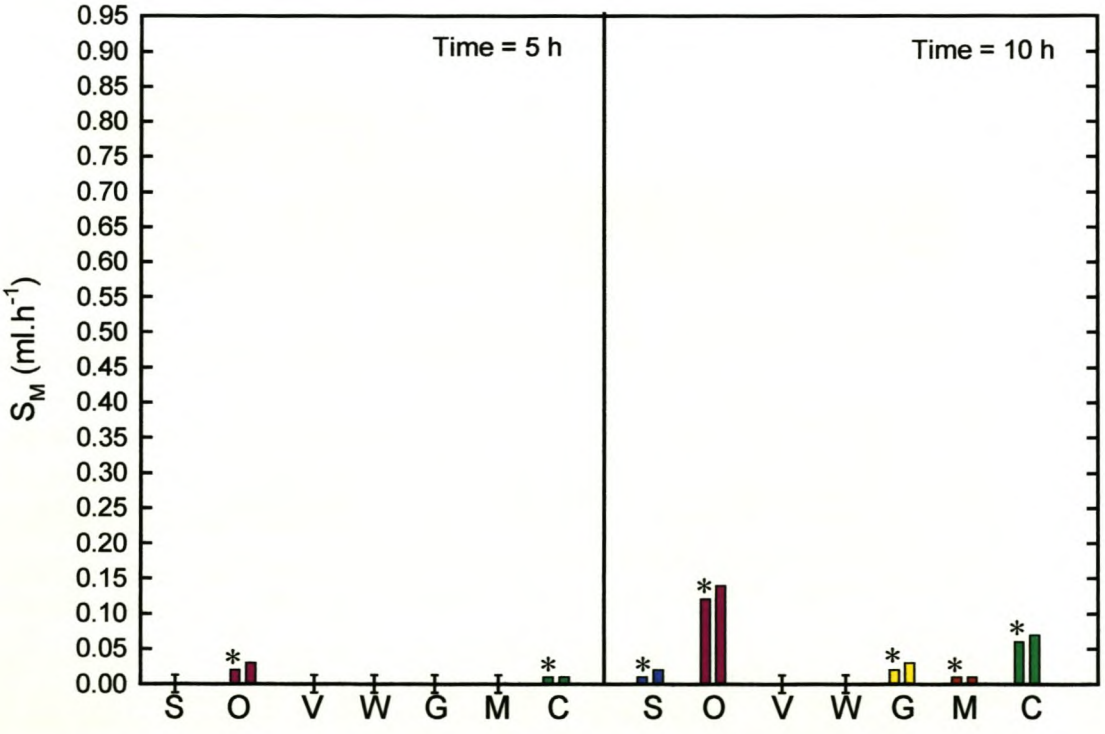

Figure 12. Methanogenic activity of different anaerobic granules evaluated in the Basic-medium (as control) and with an additional 4.0 g.l^{-1} lactate as carbon source. (S_M = tempo of CH_4 production (ml.h^{-1})), ($*$ = control, I = no gas production)

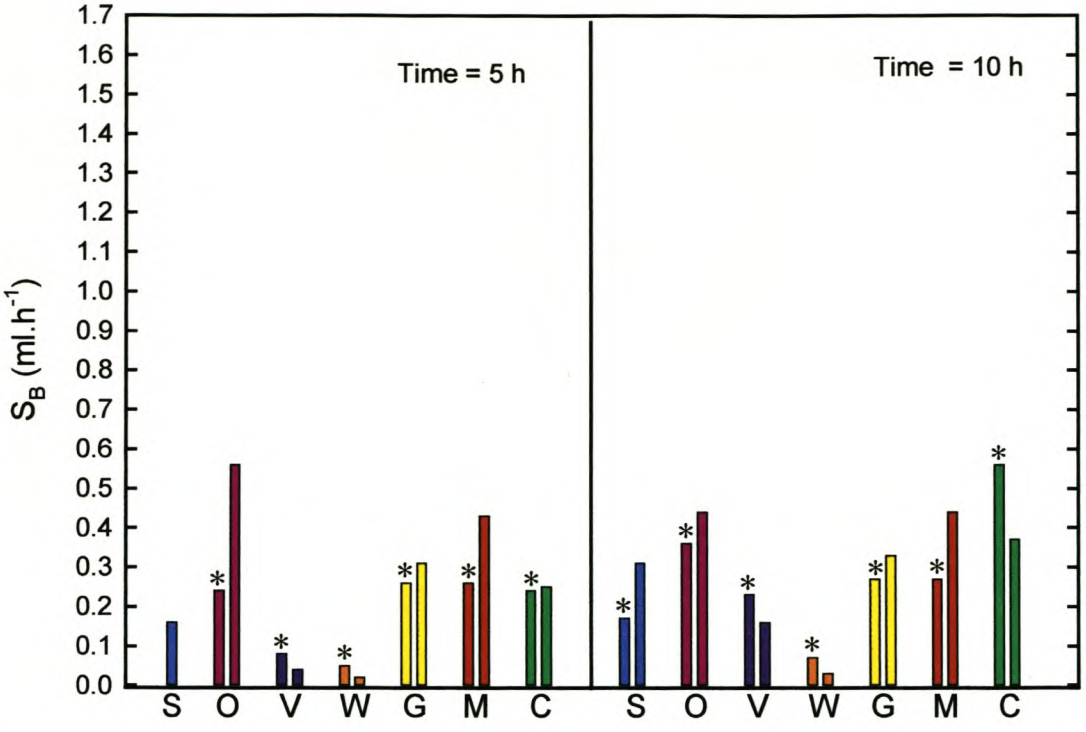

Figure 13. Activity of different anaerobic granules evaluated in the Basic-medium (as control) and with an additional 1.0 g.l^{-1}acetate as carbon source. (S_B = tempo of biogas production (ml.h^{-1})), (∗ = control, I = no gas production)

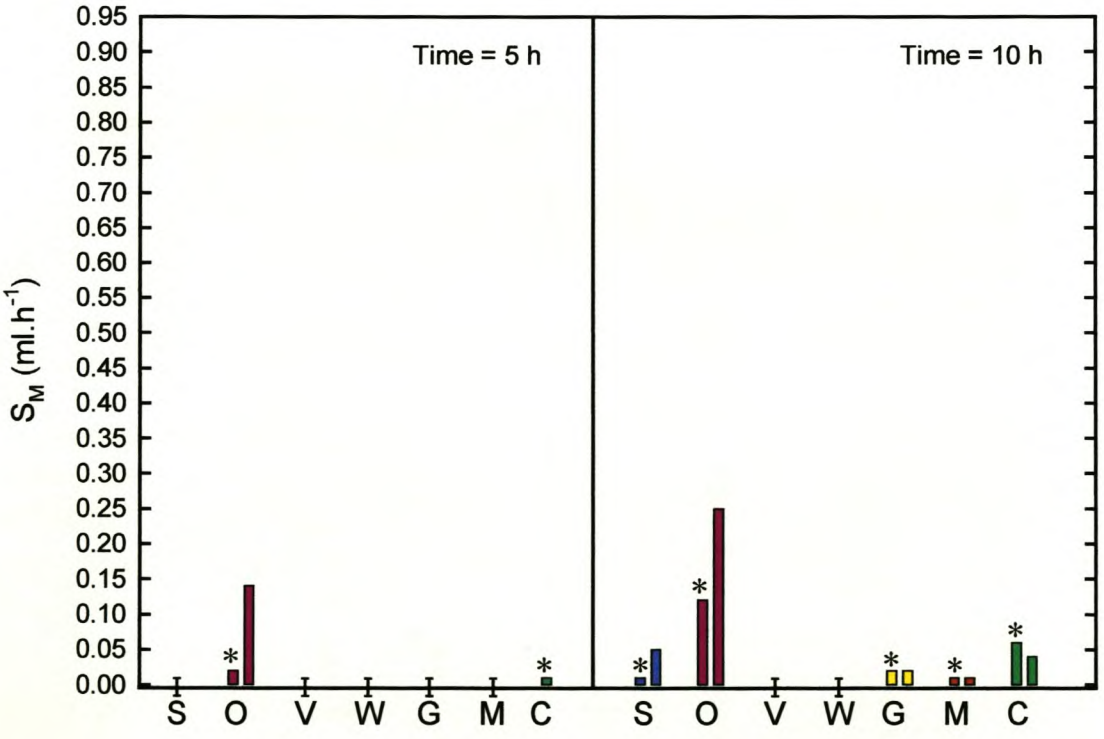

Figure 14. Methanogenic activity of different anaerobic granules evaluated in the Basic-medium (as control) and with an additional 1.0 g.l^{-1} acetate as carbon source. (S_M = tempo of CH_4 production (ml.h^{-1})), (∗ = control, I = no gas production)

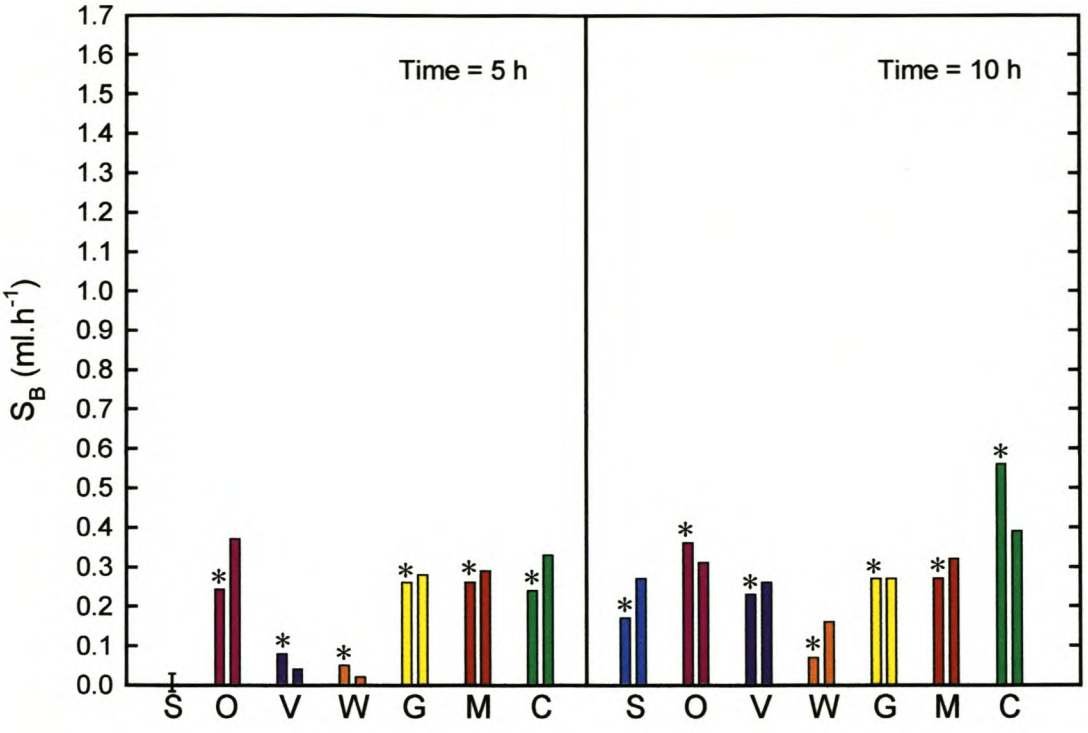

Figure 15. Activity of different anaerobic gganules evaluated in the Basic-medium (as control) and with an additional 1.0 g.l^{-1} formate as carbon source. (S_B = tempo of biogas production (ml.h^{-1})), (∗ = control, I = no gas production)

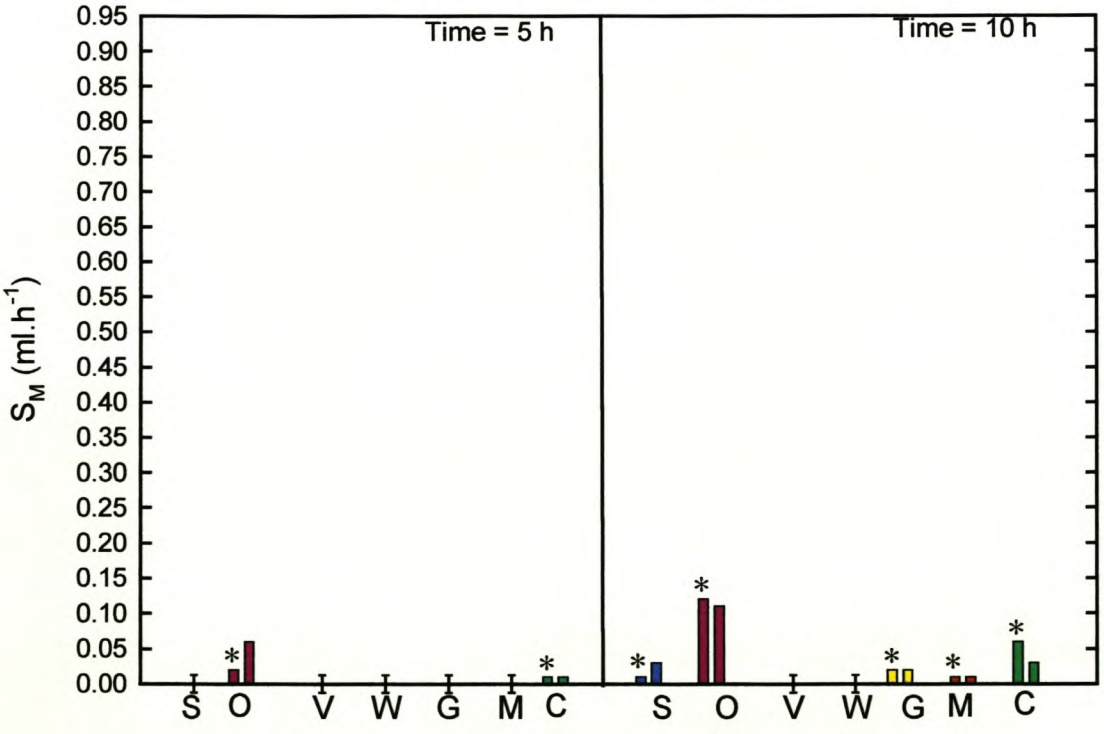

Figure 16. Methanogenic activity of different anaerobic granules evluated in the Basic-medium (as control) and with an additional 1.0 g.l^{-1} formate as carbon source. (S_M = tempo of CH_4 production (ml.h^{-1})), (∗ = control, I = no gas production)

5 h period (Fig. 10) and thus the presence of active methanogens was confirmed within these granules. The M-type was found to produce no CH_4 after 5 h. From this absence of methane in the biogas and largest biogas activity, it was subsequently concluded that the acidogenic population in the M-type granules probably was the most active of the granule consortium at this specific time of incubation (5 h).

The biogas activity profiles for the granules at an incubation time of 10 h (Fig. 9) showed higher values and more variability for the different types of granules tested. The highest biogas activity (1.28 $ml.h^{-1}$) was achieved by the O-type granules with 2.0 $g.l^{-1}$ glucose (Fig. 9). This was followed by the V-type granules with an activity of 0.5 $ml.h^{-1}$. The C- and M-type granules were next with 0.43 and 0.35 $ml.h^{-1}$, respectively. The order of biogas activity (S_B) after 10 h for the different types were: O > V > C > M > S > W > G. The O-type granules were also found to show the highest methanogenic activity (S_M) (Fig. 10), followed by the C- and G-types (0.81 $ml.h^{-1}$ compared to 0.15 and 0.05 $ml.h^{-1}$, respectively). No CH_4 was produced by the V- and W-type granules. Thus, it was concluded that the acidogenic populations of the V- and W-type granules were active (V = second on the biogas activity scale (S_B)), but not the methanogenic populations, as seen by their inability to produce CH_4. The same conclusion was reached for the S-, G- and M-type granules, which showed relatively high biogas activities, but only low methanogenic activity (S_M) at the 10 h period (Fig. 9 and 10). The order of the methanogenic activity (S_M) of the granule types after 10 h were: O > C > G > S > M.

Lactate as carbon source - Similar activity profiles were found when lactate was used as carbon source (Fig. 11 and 12) but the activity values for the granules were generally lower than those obtained with glucose as test substrate. In this study with lactate, it was also found that the S-type granules had not produced biogas by the 5 h period and the S-, V-, W-, G- and M-type granules, furthermore, did not show any methane activity after 5 h (Fig. 10). The biogas and methane activity scales after 5 h of incubation were M > O > G > C > V > W and O > C, respectively. After 10 h of incubation the biogas and methanogenic activity scales changed to M > S > G >O > V > W > C and O > S = G = C > M, respectively. The V-, W- and C-type granules did produce biogas after 5 and 10 h, but always lower than for the control, which was an indication of inhibition, possibly caused by the use of lactate as carbon source or possibly because of the absence of a lactate-utilising population. However, the C-type granules did produce a small amount of methane with the granules showing a low methanogeinc activity (S_M) value of 0.02 $ml.h^{-1}$. The V- and W-type granules still did not

produce any methane after 10 h with lactate, as was also found with glucose as carbon sources. It could therefore safely be concluded that the methanogens are either totally inactive, present in too low numbers or not present at all in these granules.

Acetate as carbon source - When acetate was used as carbon source, all granule types showed activity, with the O-type granules, after 5 h of incubation, giving the highest biogas activity (S_B) (Fig. 13). No biogas was produced in the control vials for the S-type granules after 5 h of incubation. The V- and W-type granules, after 5 h, once again showed a measure of inhibition, which was similar to when lactate was used as carbon source (Fig. 11). The biogas activity scale (S_B) after 5 h was found to be: O > S > M > G > C > W > V. After the 10 h period, the S-type control did produce biogas and these granules were ranked second on the biogas activity scale. The biogas activity (S_B) of the O-type granules decreased from 0.33 to 0.09 $ml.h^{-1}$ (Fig. 13), probably due to the rapid depletion of acetate within the first 5 h of incubation. The V-, W- and C-type granules showed similar patterns of inhibition as when lactate was used as carbon source (Fig. 11). The biogas activity scale (S_B) after 10 h was M >S > O > G > W > V > C (Fig. 13). The methanogenic activity (S_M) (Fig.14), again, as with glucose and lactate as carbon sources (Fig. 10 and 12), showed that the methanogenic population of the O-type granules were far more active that those of the other types. Only the O-type granules and the control of the C-type granules producing CH_4 after 5 h. The granules in the C-type control vials, therefore, contained a small amount of active methanogenic bacteria although no methane was produced in the sample vial. After 10 h, the methanogenic activity for the O-type granules of incubation was again the highest (0.14 $ml.h^{-1}$) followed by the S-type granule with 0.04 $ml.h^{-1}$. Although the G- and M-type granules did produce CH_4, their activity values were not more than those of their corresponding control values, suggesting that a measure of inhibition might have occurred in the presence of acetate or that the acetate could not be utilised by any consortium members. The C-type granules showed a direct inhibition in the presence of acetate. The V- and W-type granules, again, were found to produce no CH_4 which was the same as for all the carbon sources tested (Fig. 10, 12 and 14). The methanogenic activity scale after 10 h was O > S > G = M > C (Fig. 14).

Formate as carbon source - The biogas (S_B) and methanogenic (S_M) activities obtained at 5 and 10 h of incubation with formate as test substrate are shown in Fig. 15 and 16, respectively. The biogas and methanogenic activity scales after 5 h were O > C > G = M >W > V and O > C, respectively. In this study, the S-type granules, again, showed no biogas activity after 5 h and the S-, V-, W-, G- and M-type granules also did

not produce any methane after 5 h of incubation. However, the O-type granules did show an active formate-utilising methanogenic population after 5 h as seen in Fig. 16. The V- and W-type granules at 5 h incubation clearly showed an inhibition effect.

At 10 h of incubation, the S-, W- and M-type granules showed a notable tempo of biogas activity (0.1, 0.1 and 0.05 $ml.h^{-1}$, respectively) with formate as carbon source (Fig. 15). The S-type granules were the only granules exhibiting a positive methanogenic activity of 0.03 $ml.h^{-1}$ after 10 h (Fig. 16), with the O-, G-, M- and C-types possibly being inhibited by the presence of formate. The biogas (S_B) and methanogenic (S_M) activity scales for formate at 10 h were S > W > M > V > G > O > C and S > G = M > O > C, respectively.

Overall from the data it appeared as if the O- and S-type granules were thus better equipped to metabolised acetate and formate, which was taken as an indication of an active methanogenic (acetate and formate-utilising) population.

Calibration scales - Both the activity scales for biogas and methane are presented as calibration scales in Fig. 17 and 18. The activity values for biogas (S_B) and methane (S_M) of all the granule types were plotted on a scale ranging from the poorest activities (at the bottom) to the most active (at the top). This was done for all four the carbon source ($(g.l^{-1})$: 2 glucose, 4 lactate, 1.0 acetate and 1.0 formate) and at 5 h and 10 h of incubation since the activity profiles change for some substrates as the period of incubation increases. These calibration scales can now be used to classify the general biogas (Fig. 17) and methanogenic activities (Fig. 18) of any biogranule relative to active (O-type) and inactive (W-type) anaerobic granules, provided that the same method of activity testing is used. For example, in Fig. 17, it is clear that for most of the granules tested the biogas activity (S_B) was between 0.25 and 0.50 $ml.h^{-1}$ for all the carbon sources. It is also clear that the O-type granules have an activity value above those of the other granules after 5 h of incubation and when acetate was used as carbon source.

The segregation of granules into different activity ranges widens from 5 to 10 h incubation (three activity ranges at 5 h and five activity ranges at 10 h). There are also less activity ranges present in the calibration scales for methanogenic activity, with most granules types having a methanogenic activity (S_M) value below 0.15 $ml.h^{-1}$ and only a few in the second range from 0.15 to 0.30 $ml.h^{-1}$ (Fig. 18). It should be noted that these activity ranges are only for the quick assessment of granule activity and statistical evaluation is not necessary. An estimated activity value can thus be obtained to

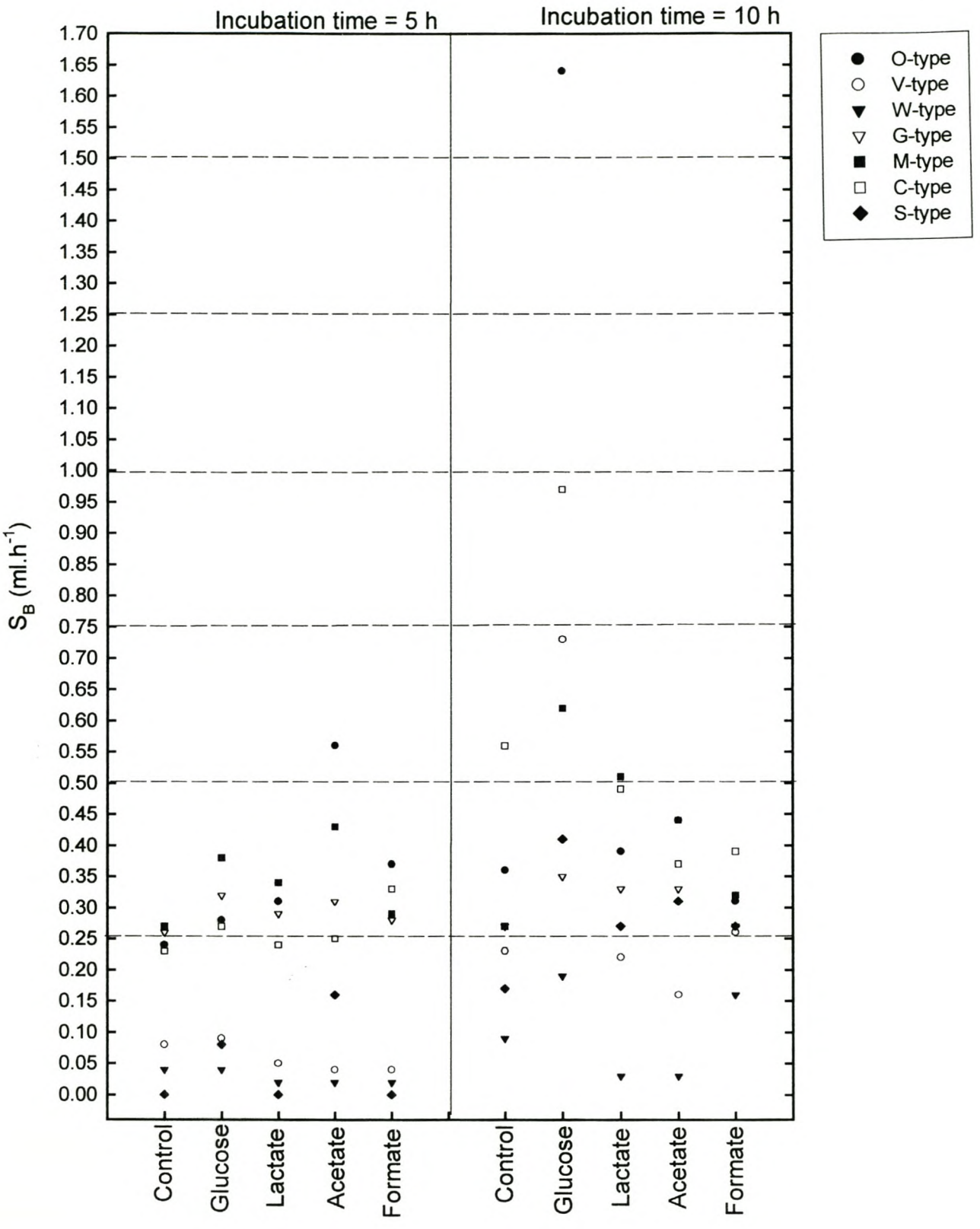

Figure 17. Calibration sheet for different anaerobic granules and different carbon sources in terms of general biogranule activity.
S_B = tempo of biogas production (ml.h^{-1})

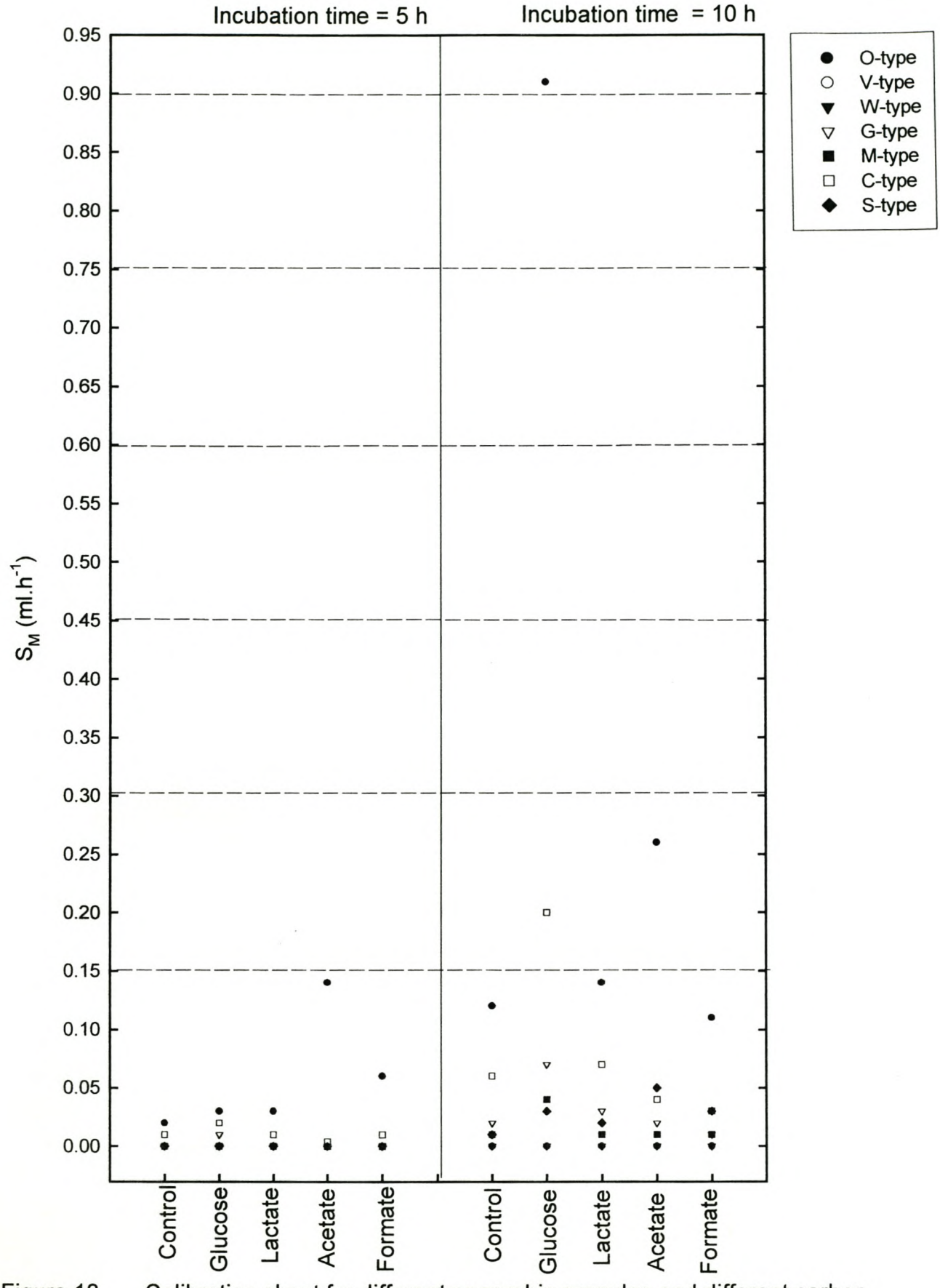

Figure 18. Calibration sheet for different anaerobic granules and different carbon sources in terms of methanogenic activity.
S_M = tempo of methane production (ml.h^{-1})

determine if an inoculum is suitable for bioreactor start-up or if the maximum space loading rate of an operational system must be estimated.

Conclusions

In the past, much research was done on the development of different efficient tests for determining the activities of the bacteria involved in anaerobic digestion (Colleran *et al.*, 1992; Coates *et al.*, 1996; Garcia-Morales *et al.,* 1996; Codina *et al.,* 1998). Many of these methods are either time consuming and unreliable or require expensive monitoring equipment, which could put further strain on an already tight budget (Angelidaki *et al.,* 1998) and this type of research does not really provide an easy, reliable and fast method of indicating direct granular activity. Once the activity level of the sample has been determined using these methods, it is still not entirely clear how the level of activity compares to a good or a poor level of activity.

In this study, a known activity method was drastically modified to give a rapid indication of granular activity. This was necessary because very poor and unreliable results were obtained which were ascribed to the impractical physical set-up of the method. The experimental set-up was thus modified by using smaller vials, aluminium crimp tops to seal the vials, shorter incubation times, as well as experimenting with different inoculum sizes. From the three different inoculum sizes (1, 2 and 3 g) evaluated, the 3 g granular inoculum gave the best results in terms of volume of biogas produced and, therefore, visual evaluation of the data. These adaptations gave more accurate and reliable results and some deductions could be made concerning the bacterial activity of the different trophic groups in the granules, when different test substrates were used.

The influence of the addition of glucose to the Basic-medium on the effectivity of the activity test was also evaluated. It was found that the addition of glucose increased the volume of biogas produced, again aiding in the visual evaluation of the data. However, lower pH ranges were obtained for samples where glucose was added to the Basic-medium. This was ascribed to the accumulation of more volatile fatty acids, which the methanogens were not fully able to degrade within the specific incubation time of the assay. However, these pH values were still within the operational pH range for methanogenic activity and the addition of glucose to the Basic-medium was thus incorporated into the new adapted methodology.

In Study IV, this new adapted method was applied to granules treating different effluents, and the subsequent general and methanogenic activities determined. The different activities were expressed in terms of the tempo of biogas (S_B) and methane production (S_M) ($ml.h^{-1}$), and also displayed in the form of bar charts and calibration scales. The use of bar charts can considerably improve the ease with which the activity of different types of granules can be compared to each other. According to the acquired data, the O-type granules were found to be the most active in terms of both general biogas (S_B) and methanogenic (S_M) activity in contrast to the V-type granules which displayed the lowest S_B and S_M.

These illustrative forms of histograms (bar charts) and calibration scales of S_B and S_M values to evaluate biogas and methanogenic activity do, therefore, not only provide useful activity data, but it can also give valuable information regarding the status of the different tropic groups (acidogens and methanogens) within the different types of granules. The bar charts can also be used to indicate a possible shift in population dynamics or inhibition due a specific carbon source used. For example in Fig. 13 and 14, the S-type granules showed a shift in population from 5 to 10 h of incubation from a dominant acidogenic to a population consisting of both methanogenic (acetate and formate-utilising (Fig. 16)) and acidogenic species. This was evident from the fact that from 5 to 10 h the biogas activity remained constant (Fig. 13), but the methanogenic activity increased from 0 to 0.04 $ml.h^{-1}$ for acetate and from 0 to 0.03 $ml.h^{-1}$ for formate. Substrate inhibition can also be detected as seen in Fig. 15 and 16, where both the biogas and methanogenic activities of the O-type granules decreased to below the values for the control, from 5 to 10 h of incubation, when formate was used as carbon source. Future work must include expanding the type of granules evaluated with this specific activity method by which the calibration scales could be adjusted to accommodate a wider variety of anaerobic granules.

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Chapter 5

GENERAL DISCUSSION AND CONCLUSIONS

Background

Water conservation and recycling is becoming increasingly important for the South African fermentation industry. Distilleries, as part of the fermentation industry, generate large volumes of effluent that urgently need to be treated. High biodegradable dissolved solids (up to 50% present as reducing sugars), high ash content, high temperature and a low pH, makes this effluent difficult to treat. Several treatment options are in use presently, but they all have disadvantages of some nature, such as long retention times, bad odours or the need for large areas of land. Considerable interest has been shown in the application of anaerobic digestion, especially the UASB design (upflow anaerobic sludge blanket), to treat this high strength waste water. Anaerobic digestion can achieve a greater reduction in COD, much lower sludge yields, biogas production and requires less nutrient supplementation which leads to lower running costs than when using aerobic systems (Lettinga *et al.*, 1997). The nature and strength of the distillery effluent provides more favourable conditions for anaerobic digestion than aerobic systems. Although the anaerobic digestion process may have advantages, the formation, amount and specific activity of the granules dictate the operational efficiency and performance of the UASB system. In addition, distillery effluent also contains phenolic compounds that can lead to a high inhibitory and anti-bacterial activity on the anaerobic digestion consortium such as the methanogenic populations (Nel & Britz, 1986).

UASB bioreactor optimisation

Research on establishing the efficiency parameters of anaerobic systems, and specifically the UASB bioreactor, whilst treating distillery effluent at various organic loading rates, needs to be done. The use of a mesophilic UASB system was, therefore, evaluated as an option in the treatment of high strength distillery effluent. The main aim was to optimise the efficiency of the bioreactor in terms of the organic loading rate (OLR) and feeding at a lower substrate pH, so as to minimise neutralisation costs. The OLR was thus gradually increased from 2.01 to 30.00 $kgCOD.m^{-3}.d^{-1}$ (Chapter 3). The results obtained with the lab-scale design clearly showed that the UASB bioreactor could successfully treat the full-strength distillery effluent.

The 2.3 l lab-scale UASB used obtained excellent COD removal values (> than 80%) with OLR's as high as 30 $kgCOD.m^{-3}.d^{-1}$ with a positive alkalinity value, showing excellent bioreactor stability and efficiency. This was true even when a reduction in bioreactor substrate pH from 7.5 to 4.7 was implemented with simultaneous increases in OLR from 2 to 30 $kgCOD.m^{-3}.d^{-1}$. The bioreactor operational efficiency level was further supported by a continual increase in the R-rate with each increase in OLR. The data also showed that the bioreactor could successfully treat effluents with a pH of as low as 4.7, and still deliver a final bioreactor effluent with a pH of above 7. The economical implications of the reduction in neutralisation costs are considerable. However, a post-treatment will have to be implemented to reduce the final bioreactor COD level (3 000 $mg.l^{-1}$) so as to conform to local standards of 75 $mg.l^{-1}$. A further shortening of the HRT was not attempted as literature generally dictates an HRT of 2 - 6 d for such a high OLR and high solids containing effluent (Braun & Huss, 1986; Ehlinger *et al.*, 1992). This still makes the treatment time of 24 h, as used in this study, highly acceptable. The UASB bioreactor in terms of OLR's, substrate pH and biogas yields, was more efficient when compared to results reported by Souza *et al.* (1992). These authors did a similar study using a thermophilic UASB bioreactor to treat distillery effluent (COD = 25 – 30 $kgCOD.m^{-3}.d^{-1}$) and only achieved a maximum of 72% COD removal at an HRT of 24 h.

The accumulation of suspended solids, which were present in the distillery effluent, in the bioreactor was at times found to be problematic and caused the bioreactor efficiency to decrease. These accumulated suspended solids caused excessive expansion of the granular bed, which lead to the washout of the granular biomass. The loss of active biomass was found to be detrimental to the efficiency of the bioreactor. Pre-treatment options, such as filtering the reactor substrate before feeding to the digester are advisable in the future, as this will minimise the accumulation of fine solids within the granular bed of the bioreactor.

Further studies must also be conducted on the influence of a further shortening of the HRT so as to investigate the effect of bio-solid accumulation and the overall efficiency of the microbial community as well as the possible inhibitory effect of phenolic compounds that are present in the effluent.

Activity tests

The acidogenic and methanogenic activity of any type of anaerobic granules is of great importance and can aid reactor operators in predicting maximal space loading

rates, especially during the critical start-up period. Activity levels can also provide useful information for the selection of anaerobic samples suitable for inoculation processes and can help in the detection of an unbalanced situation between the microbial populations normally present in an anaerobic bioreactor.

A range of different activity methods have been described in the literature (Colleran *et al.*, 1992) but most of them are either laborious, non-specific to the different trophic groups found within the granules, or require expensive monitoring equipment such as pressure transducers (Angelidaki *et al.*, 1998). An easy, reliable and economic method must be developed to determine the activity level of anaerobic granules. Such a method could also be applied during the selection of granules to treat different waste waters and give information on the activity relative to other types of granules and can give an indication of the general lack or presence of methanogenic activity.

In this study, a known activity method (Lamb, 1995) was evaluated and found to be inadequate. It was thus drastically modified (Chapter 4: Study I and II) to give a rapid indication of granular activity. The experimental set-up was modified by using smaller vials, aluminium crimp tops to seal the vials, shorter incubation times, as well as optimising the different inoculum size. It was found that a 3 g granular inoculum gave the best results in terms of volume of biogas produced and helped to facilitate the visual evaluation of the data. The modified assay technique gave more accurate and reliable results and information could be gathered concerning the bacterial activity of the different trophic groups in the granules, especially when different test substrates were used. The addition of glucose to the basic medium was also evaluated (Chapter 4: Study III) since traditional activity methods do not include extra glucose in the basic medium. It was found that the addition of glucose increased the volume of biogas produced, and this aided in the visual evaluation of the data, although a lower pH range was obtained. This lower pH was attributed to the accumulation of more volatile fatty acids, which the methanogens were probably not able to metabolise within the specific incubation time. The final pH values for the test assays where glucose was added were, however, still within the operational range for acidogenic and methanogenic activity.

Histograms and "Calibration scales"

The modified method developed and evaluated in this study was applied to granules that had been used to treat different effluents (Chapter 4: Study IV), and the subsequent general (biogas) (S_B) and methanogenic (S_M) activities determined. Activity

levels were not given as volume of gas produced per unit COD converted or volatile suspended solids (VSS) or as the tempo at which the headspace of assay vials increased, as traditionally is the case, but as tempo of biogas or methane production ($ml.h^{-1}$). This is easier to determine and does not require any expensive equipment.

The data obtained was used to construct bar charts (Histograms) and "calibration scales", which could in the future be used for the comparison of different granule activities in terms of their relative efficiency. The bar charts can also be used to indicate possible substrate inhibition on granular activity as well as a possible shift in population dynamics within the granules. For example, the S-type granules showed a shift in population as the incubation period progressed from a dominant acidogenic to a population consisting of both methanogenic (acetate and formate-utilising) and acidogenic species when acetate and formate were used as carbon source. This was evident from the fact that the biogas activity remained constant, but the methanogenic activity increased when acetate or formate was used as carbon sources. With this method, substrate inhibition can also be detected as was seen with the C-type granules, which produced both biogas and methane with glucose and lactate as substrate, but produced less biogas and methane than the corresponding control values when acetate and formate were used. The use of bar charts also considerably improved the ease with which the activity of different types of granules could be compared. The "calibration scales" can be used to group the general biogas (S_B) and methanogenic activities (S_M) of any new biogranule relative to active (O-type) and inactive (W-type) anaerobic granules, providing that the same method of activity testing is used. The "calibration scales" can thus be used to give a fast indication of how the activity value of one sample relates to the activity values of other granules, even when using different test substrates.

Recommendations for future research

During this study, it was found that the UASB technology could be used to successfully treat the high strength distillery effluent at OLR's of up to 30 $kgCOD.m^{-3}.d^{-1}$ and an HRT of 24 h. The accumulation of suspended solids, present in the distillery effluent, in the bioreactor was at times found to be problematic and caused the bioreactor efficiency to decrease. Filtering the reactor substrate before feeding it to the reactor is advisable to minimise the accumulation of fine solids within the bioreactor.

A post-treatment step should also be investigated, as the final COD which was reduced from 30 000 to 3 000 $mg.l^{-1}$, must be reduced to 75 $mg.l^{-1}$, before discharge

can take place. Further studies must also still be conducted on the influence of shorter HRT's so as to investigate the effect of bio-solid accumulation on the overall efficiency of the microbial community.

Future work regarding activity testing should also include expanding the variety of granules and including different test substrates. The granules evaluated in this study only included granules treating food related effluents and the inclusion of granules treating, for example, petrochemical and dairy wastes should also be considered since activity data is also crucial for such anaerobic digestion processes. By applying this method on a wider scale, the "calibration scales" can be adjusted to accommodate a wider variety of anaerobic granules and thus find a much broader application.

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