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Anaerobic digestion of lactose wastewater

Final report by Elina Lapshina

Introduction

This experimental work was performed as part of the CROPGEN project, which addresses bio-energy generation from crops and agricultural wastes. At the time the project began Organic Power (OPL) were investigating the treatment of a cheese-farm wastewater by anaerobic digestion using the Maltin® System demonstration plant at Horsington, Somerset. This system has a multiple-tank design consisting of 8 digesters in series, each with a retention time of approximately two days. The system is effectively a plug-flow model, which, due to its multi-tank design can provide a natural separation between different microbial communities involved in various stages of the anaerobic digestion. This in turn could provide a digester system with high performance potential in terms of energy production and waste treatment. This design is also ideal for studying different stages in anaerobic digestion processes. The aims of current work were to undertake a detailed laboratory-scale study into anaerobic digestion of a cheese-farm wastewater to provide initial understanding of the processes, which enable successful and efficient treatment of this waste and generate biogas as a potential energy source. Current work uses lab-scale continuously stirred tank reactors (CSTRs) to investigate potential degradability of this waste, as well as to gain understanding of the processes that are likely to take place in a plug-flow reactor. The results of this work provide a basis for full-scale trials with the Maltin® System digesters.

Materials and methods

Substrate

Substrate used in this experimental work was a waste-water from a cheese farm (Barbers, Somerset). The farm produces two types of waste, one of which was used for this study. The source of the substrate is a cheese permeate - a watery mixture of left-over whey protein and lactose. This is centrifuged to recover whey protein, which is then converted to whey powder and used as a commercial product. The left-over solution contains about 6% lactose, which is further filtered to recover some of the water. The water is free from chlorine and is used to wash the cheese-making equipment at the factory. The remaining solution of lactose is now 12-18% concentrated. The main characteristics of this substrate are represented in Table 1.

When fresh, this solution is about at 38^{O}C, bright yellow colour and more viscous than water. It has high COD contents (between 120-170 g l^{-1}), up to 75-95% of which is dissolved COD, and high total solids contents (170 g l^{-1}), up to 95% of which is volatile dissolved solids. Elemental analysis of this substrate was performed during the Experiment 3. It was found that nitrogen contents were less than 1%. Carbon content is ~40%, hydrogen content is ~6%. This suggests that the main component of the waste is dissolved lactose ($C_{12}H_{22}O_{11}$, M_w = 342.3 g/mol, theoretical carbon content 42%, hydrogen content 6.4%). The calorific value of this substrate was found to be 15.65 kJ/g.

Table 1. General characteristics of the lactose substrate

Characteristic, units	Values
Total COD, g l^{-1}	120-170
Dissolved COD, g l^{-1}	154-162
Total solids, g l^{-1}	~170
Volatile solids, g l^{-1}	~160
Total carbon, %	40
Total hydrogen, %	6
Total nitrogen, %	<1
pH (when fresh at 38^{O}C)	5.5
Calorific value, kJ g^{-1}	15.65

The pH of the fresh waste is around 5.5, and the solution is quickly acidified to lower pH on standing. In cool environment and on drying in the air, lactose precipitates, but quickly re-dissolves on stirring or shaking the solution. At the farm, this waste presents an extra cost. About 145 m^3 of this waste is produced daily. Some of the solution is given out to farmers free of charge to use as a pig feed additive. This is, however, a very small proportion of the total volume produced. The rest is stored in the containers, and then taken away by a contractor waste collection company. The cost of disposal is £120 per tank. Together with other wastes produced at the farm, the total cost is about £120-£240 a day. Utilisation of this waste through anaerobic digestion is considered as one of the possibilities to reduce the on-going costs and utilise this waste as an energy source. In terms of the CROPGEN, this waste is regarded as highly degradable agricultural waste, and is used for investigation of its potential utilisation in the Maltin® System reactors. For the current studies, the lactose wastewater was collected, transferred into 250 or 500 ml plastic bottles and frozen on the same day. It was then thawed at room temperature for 20-24 hours prior to utilising it as a substrate for the digesters.

Digester seed

The inoculums used in this study were either anaerobic digester sludge (Experiments 1-3) from a sewage digester (Millbrook, Southampton) or cattle slurry (Experiment 1) obtained from a farm (Somerset). Both inoculums were used for Experiment 1. This was done to compare sewage sludge and cattle slurry as two potential inoculums for the full-scale digesters. It was found that there was no significant difference in reactor performance. Cattle slurry can therefore be used for the full-scale trials as it is readily available at the site. Sewage sludge was used for all subsequent laboratory-scale trials as it was a more convenient source at this site. Both inoculums were sieved through a 2 mm mesh. The time gap between collection, sieving and inoculation was minimised as much as was possible, and was no more than 1-2 days. The basic parameters are shown in the Table 2 below:

Table 2: Basic parameters of the inoculums

Characteristic, units	Cattle slurry	Sewage sludge
TS, g l^{-1}	71.7	29.2
VS, g l^{-1}	53.8	17.8
TCOD, g l^{-1}	82.4	27.9

Experimental set-up

During this study, three types of the experimental set-up were used.

Experiment 1:

This experiment was set-up as a preliminary investigation into possible inoculums and potential substrate loading rates. The digesters were 250 ml glass conical flasks. The flasks were arranged in a heated shaking water bath. Temperature was set at 36.5-37.0^{O}C. Ten flasks were inoculated with 50 ml of either sewage sludge or cattle slurry (five flasks each) and briefly sparged with nitrogen gas to exclude air. 1.25 ml of substrate diluted with distilled water to 2.5 ml was added into each flask. The gas production was recorded over next several days. The lactose was added again at the same concentration only when

gas production levelled out. This feeding rate gave COD loading of approximately 2.6 g l^{-1} per every 3-4 days. This regime was maintained for 22 days, during which the digesters were fed 6 times (including the initial addition of feed). From day 22 intermittent daily feeding regime was introduced: a sample of 1.25 ml was taken from each flask and the substrate of the same volume was added. This regime represented a loading of 2 g COD l^{-1} day^{-1}. From day 46 the loading was increased to 3 g COD l^{-1} day^{-1}. The gas was collected into glass collectors filled with acidified water (pH<4) to avoid CO_2 dissolution. No nutrient supplements were added during this experiment. The overall hydraulic retention time (HRT) was 50 days.

Experiment 2a:

Four 5-litre mechanically stirred plastic CSTRs were used in this experiment. The digesters were inoculated with 3.75 litres of sewage sludge and briefly sparged with nitrogen gas. Initially the substrate was added to the digesters at organic loading rate (OLR) of 1 g COD l^{-1} day^{-1}. The HRT was chosen to be 16 days (to approximate the HRT of the full-scale demonstration plant at OPL). To achieve these conditions 30 ml of substrate were diluted to 250 ml with distilled water and basal nutrient medium. The nutrient medium was added to provide necessary macronutrients: ammonia (as NH_4Cl at 0.53 g l^{-1}), calcium (as $CaCl_2.2H_2O$ at 0.08 g l^{-1}), magnesium (as $MgCl_2.6H_2O$ at 0.1 g l^{-1}); and micronutrients as 1ml of trace element solution per litre of substrate (trace element solution contains: 5.1 ml HCl 36%, 1.5 g $FeCl_2.4H_2O$, 60mg H_3BO_3, 100 mg $CoCl_2.6H_2O$, 70 mg $ZnCl_2$, 25 mg $NiCl_2.6H_2O$, 15 mg $CuCl_2.2H_2O$, 25 mg $NaMoO_4.2H_2O$ (Pfenning *et al.*, 1981)). The digesters were operated at these conditions for 30 days. Alkalinity was added to the digesters on day 39 in the form of $NaHCO_3$ (2 g l^{-1}).

Experiment 2b:

After this period, the digesters were re-seeded with 2 litre of fresh sewage sludge and were left to stabilise for 6 days. The OLR and the feed preparation were as in Experiment 2a, 1 g COD l^{-1} day^{-1}. Digester 1 was switched to 50 day HRT. Digester 2 was run at 16 day HRT (as before). Digester 3 was run at 16 day HRT with solid-recirculation regime. The solids were recovered from a 250 ml sample daily by centrifugation. Supernatant was discarded, and the pellet was mixed with the feed and added to the reactor. Reactor 4 was switched to batch operation to imitate plug-flow conditions (the set-up is described in the Experiment 3). The gas was collected by displacement of acidified water (pH<4). Alkalinity was added to the digesters at various stages in the form of $NaHCO_3$ (2 g l^{-1}).

Experiment 3a:

Four 5-litre CSTRs were used as in Experiment 2. The reactors were seeded with sewage sludge. The feeding regime was once every 14-16 days. The substrate was added to the digesters at different volumes from 260 - 780 ml, equivalent to 7.2-23.3 g COD l^{-1}. Nutrient medium was used as in the Experiment 2. The gas was collected by acidified water displacement into a gas collector.

Experiment 3b:

Digesters were completely re-seeded with fresh sewage sludge. A set volume of the substrate (500 ml, 17-19 g COD l^{-1}), containing necessary nutrients as in previous experiments, was added to all four digesters. The gas collection was switched to gas bags via an on-line gas count device. Ports for a pH probe and a sampling tube were installed in digesters 1 and 2. An on-line pH monitoring was performed on digester 1 for two runs (1 and 2) and on digester 2 for the remaining runs (3-5). pH control via addition of NaOH solution (1 or 2 *N*) was used during the last two runs.

Sampling and analytical methods

Total and volatile solids content of the inoculums and the substrate were measured prior each feeding (Standard methods). COD of the substrate was measured prior every feed (Standard methods: acid digestion and FAS titration). Elemental analysis of the substrate

was also performed to confirm its composition (Flash Elemental Analyser 1112 series).Total volume of biogas produced was corrected to the atmospheric pressure and temperature each day. Gas samples were taken from the head-space (Experiment 1), from the gas collector (Experiment 2 and 3a) and from the gas bags (Experiment 3b). Gas composition was analysed using gas chromatograph (GC Virian 3800). Samples were analysed on COD, alkalinity (three-point titration method as described by Ripley *et al*.[18]), VFA (acidified by formic acid and analysed using Shimadzu GC 2010), and pH (standard pH electrode probe) at least once a week (Experiment 2 and 3). On-line gas counters and pH measurements were set-up for the Experiment 3b. Gas counters were made in-house. Data Taker software was used for on-line data collection. Ammonia contents were determined in some of the samples (alkali digestion, distillation, and acid titration method and spectrophotometric method). The calorific value of the substrate was measured using Gallenkamp Ballistic bomb calorimeter.

Literature review

A literature search was carried out on anaerobic digestion of cheese whey and lactose. It was found that most of the experimental work done so far in this field favours 2 stage processes.[1-4] Various digester designs have been used for acidogenic and methanogenic stages.[1-7] The optimum HRTs of 24h for acidogenic stage[1] and 2-4 days for methanogenic stage[1-3], and the COD removal of up to 95-97% have been achieved at high-loading (20-25 g l^{-1} day^{-1}) with high-performance digesters (e.g. UASB reactors).[2] It was also found that all of the work done so far was carried out using either raw cheese whey or deproteinated whey powder that has been diluted to various concentrations. In all experiments, the alkalinity of the process was controlled by addition either NaOH, $NaHCO_3$, or $Ca(OH)_2$ [3, 4, 7], and the digestion of cheese whey without alkalinity supplementation was found 'not feasible'.[3]

A literature review on hydrogen bio-fermentation was carried out after it was found that the biogas produced during the initial stages of the Experiments 3a and 3b had high contents of hydrogen, to gain knowledge about possible processes that take place during this stage and so that to adjust the experimental procedures accordingly.

Biohydrogen production is considered as an advantageous step in anaerobic digestion as production of this valuable gas is accompanied by effective pre-treatment of the waste providing soluble products such as acetate, butyrate, propionate, and ethanol, utilised by methanogens.[8,9] H_2 is considered as ideal fuel with less pollution than fossil fuel based economy.[10] Advantages include: water vapour as a sole combustion product, 50% higher efficiency and 2.75 times higher energy content than any hydrocarbons.[10] Fuel cells can also be used to double the conversion efficiency of H_2 to electricity.[10] The gas can be easily stored as metal hydride, and transmission of H_2 through natural gas pipelines is estimated to be more efficient than the transmission of electricity down the power lines.[10] A wide range of suitable and low-cost crop-based substrates for biohydrogen fermentation from sugar-containing crops (such as sugar beet, sweet sorghum, starch-based crops corn and wheat, and ligno-cellulosics like fodder grass and miscanthus) are suggested by various sources and include sucrose, starch, glucose, molasses, rice winery wastewater, sugar beet and sugar beet extracts.[10-12] These substrates (particularly sugar beat extract and rice winery wastewater) are similar to the lactose wastewater used for our experiments, although the COD contents were still much lower than for lactose (20-40 g l^{-1} for rice winery, 150 g l^{-1} for lactose). Mixed culture inoculums with or without heat pre-treatment are reported to be as effective as pure obligate anaerobe hydrogen-producing cultures of *Clostridia*, *Escherichia*, *Citrobacter*, *Bacillus*.[8-17] These include anaerobic digester sludge, compost, different types of soil, landfill sediments, silow etc. [8-17]. All sources agreed that butyric acid and ethanol-type fermentations were preferred for hydrogen production, providing stability to the methanogenic stage and therefore to the overall process of waste treatment. Successful hydrogen fermentation does require nutrients (phosphate, and iron, as well as nitrogen source) and the hydrogen-producers are extremely sensitive to oxygen and pH changes, so constant (automatic) source of feeding, pH control and exclusion of air are required.[12] Also, removal of H_2 from the system is necessary as its accumulation (increase in partial pressure) is reported to inhibit the process.[11]

Ethanol-type fermentation was originally described by Ren et *al*. (1997)[13] as a new type fermentation in addition to propionic and butyric acid-type fermentations. Butyric acid and ethanol type fermentations were reported by the authors as having higher acetogenic rates than propionic acid-type fermentation and therefore provide the necessary stability for the methanogenic stage. The authors reported that in their experiments of treatment of molasses in a CSTR seeded with activated sludge and fed at loadings of 5-10 kg COD m^{-3} d^{-1} for 40 days ethanol-type fermentation was observed during the steady-state at pH lower than 4.5 and was accompanied by hydrogen evolution. At this pH ethanol is neutral and therefore helps to maintain a stable NADH/NAD^+ balance. The authors also advised that pH 5.5 should be avoided because of the risk of propionic acid accumulation and that the hydrogen partial pressure higher than 50 kPa also may result in inhibition of further hydrogen fermentation.

Lay (2000)[14] performed experiments on starch substrate for a chemostat reactor at a loading of 6 kg COD m^{-3} day^{-1}. He found that hydrogen production was maximum at pH 5.2 (61% of total biogas or 1600 l m^{-3} day^{-1}), but the main soluble products were acetate and propionate. At pH 4.5, however, butyric acid-type fermentation was predominant with negligible levels of propionate and higher alcohol levels. The H_2 production was lower (56% or 1156 l m^{-3} day^{-1}). This study confirmed that pH has to be maintained at a level that allows sensible and effective balance between optimal H_2 production and butyrate and/or ethanol type fermentations if the products are used for further utilisation at methanogenic stage.

Fang and Yu (2001)[15] report that an up-flow reactor seeded with granular sludge, from which methanogens were washed out, and operated at a loading of 1.97 COD l^{-1}, pH 5.5 and temperature of 55^{O}C provides the optimum conditions for lactose acidification with hydrogen fermentation. At these conditions authors report that the main products were acetate, propionate and ethanol, and the main gaseous products were found to be carbon dioxide and hydrogen. HRTs of 2-24 hours were tried, but these did not influence the process significantly. These experiments were run for up to 41 days. The results of this paper showed that the acidification of lactose was achieved very quickly (98% within 24 hours).

Ginken and Sung (2001)[10] reported that pH value around 5.6 is a 'dividing line' between acid and alcohol production. In their hydrogen fermentation experiments they used 250 ml stirred batch bottles seeded with various heat-treated inoculums and fed on sucrose substrate at a loading of 7.5 g COD l^{-1} at 37^{O}C. At these conditions they achieved the highest hydrogen production (74.7 ml H2 l^{-1} h^{-1}) and substrate conversion efficiency. However, the authors do not report which acids predominated at these conditions. The high levels of hydrogen production were also facilitated by sparging with nitrogen to avoid inhibitory levels of H_2.

Fang and Liu (2002)[16] did a detailed study on effect of pH on hydrogen fermentation. In their experiments they used a stirred tank reactor fed on glucose at 7 g COD l^{-1} with addition of nutrients. They explored the effect of pH in a range of 4-7 at 0.5 increment increases with steady states achieved at every step. They found that the optimal H_2 production was at pH 5.5 (64%) and with 99% glucose conversion rate. The specific H_2 yield was 2.1 mol H_2 mol^{-1} hexose. This indicated butyrate type fermentation and was confirmed by VFA analyses which showed that 35% was butyrate, followed by acetate (29.2%) and ethanol (10.1%), with less than 3% propionate.

Yu et *al*. (2002)[12] used UASB reactor seeded with sludge acclimated with 10 g COD l^{-1} glucose for 21 days in a 5 litre CSTRs, for the rice winery wastewater treatment. In this study, the authors report a maximum effective H_2 production of 9.33 l g^{-1} of VSS per day (1.37-2.13 mol H_2 mol^{-1} hexose) at HRT 2 hours, and OLR 34 g COD l^{-1}, at controlled pH of 5.5 (controlled automatically with 6*N* NaOH or 6*N* HCl), and at 55^{O}C. The authors report that specific hydrogen production in their case is higher than the values reported for CSTRs. However, at all tested conditions propionic acid levels were similar to or higher than the levels of other products such as acetate, butyrate and ethanol and the distribution of these products was found to be highly dependent on pH, loading rates and temperature but not on HRT. These findings confirm the results from previous studies.

Horiuchi et *al.* (2002)[17] also report that pH is an important factor that influences selective acid production during the fermentation. In their study a CSTR was inoculated with sewage sludge and fed on glucose, and pH was controlled between 5 and 8. The results showed that butyric and acetic acids production predominated in the pH range 5-7, but the presence of propionic acid producers was evident at higher pH (7-8). Reversing the conditions to the lower pH levels showed that the acid production switched back to predominantly butyrate, accompanied by higher hydrogen fermentation. These studies confirmed the importance of pH control for sustainable hydrogen production.

In their review, **Hawkes et *al***. (2002)[11] summarised the principle conditions that had to be met for a sustainable fermentative hydrogen production. These included a carbohydrate-rich feedstock, high-solids inoculum, inorganic nutrients (particularly nitrogen and phosphorus), short HRT, pH around 5.5, temperature at 30^{O}C, low ORP, removal of H_2 by sparging, and constant feed supply to avoid shock loadings and gaps in a feeding regime. They stated that the fermentation should be directed away from ethanol production and towards VFA.

Theoretically a mole of hexose can give 2-4 moles of hydrogen in the process of fermentation with acetic and butyric acids being the main products:

$C_6H_{12}O_6 + 2H_2O = 2CH_3COOH + 2CO_2 + 4H_2$
$C_6H_{12}O_6 + 2H_2O = 2CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$

Production of propionate and/or lactate is not generally accompanied by hydrogen evolution. In refined sucrose and sugar beet extract studies by Hussy et *al*.[9], CSTRs were inoculated with anaerobic digester sewage sludge sieved through 1.18 mm mesh, and initially sparged with N_2. The pH in their experiments was controlled at 5.2-5.3. The shortest lag phase before the H_2 production observed was 16 hours. Stable H_2 production was achieved by the authors in a 45-day experiment. Sparging with N_2 proved to improve H_2 production, maximum achieved was 1.7-1.9 mol mol^{-1} hexose. Butyrate/acetate metabolism was shown to be preferred over the ethanol metabolism for H_2 production, and the redox increase to over -130 mV was shown to be a threshold value associated with the micro-organism washout and propionic acid accumulation.

A very detailed and comprehensive account on hydrogen fermentation processes was given by **Wang et *al***.[8]. They have undertaken investigations into relationship between hydrogen production and propionic acid accumulation using CSTR fed on sugar beet wastewater at 8 kg COD m^{-3} day^{-1} for 60-70 day experiments at 30^{O}C, in which they altered either pH or ORP levels during different periods of the experiment. The authors based their discussion around the following acidogenic intracellular reaction of hydrogen production:

$2NADH + 2H^+ = H_2 + 2NAD^+$

This reaction is catalysed by hydrogenase - an iron-containing enzyme, activity of which is inhibited by low pH.[14] Therefore appropriate ratio of NADH/NAD$^+$ and a pH level have to be maintained in the cells for stable hydrogen fermentation. Propionic acid type fermentation is also known to reduce the accumulation of NADH in the cells. But this acid is reported as an inferior substrate for methanogens. **Wang *et al*.** reported that shock loadings, ethanol type fermentation and low ORP levels favour hydrogen fermentation. However in these conditions high acidogenic activity results in high levels of NADH and both propionic acid and hydrogen fermentations are employed for returning to the appropriate balance between NADH and NAD$^+$. The work done by the authors suggests a solution that promotes hydrogen fermentation without propionic acid accumulation by controlling pH at low levels of 4.2 under anaerobic conditions (-150mV). At this pH level ethanol-type fermentation was observed that did not depend on ORP values. pH 5.5 was found to favour propionic acid fermentation the levels of which varied with different ORP. At pH 6 butyric acid-type fermentation was observed, which again depended on ORP fluctuations.

Overall, the information found on hydrogen production was useful as it signified the importance of pH in the process of hydrogen production. It also showed that the pH for hydrogen production has to be maintained at relatively low values. The exact value is somewhat undefined and seems to depend on the substrate used as some sources prove

5.5 to be the optimum, whereas others suggest lower values (e.g. 4.2) and report 5.5 as a pH of propionic acid accumulation.

Results and discussions

Experiment 1.

Experiment 1 showed that both sewage sludge (SS) and cattle slurry (CS) were suitable for digester seeding. Cattle slurry showed a better performance in terms of gas production during the start-up (Fig. 1). This is likely to be due to higher alkalinity and nutrients available. By the end of the experiment the difference in gas production diminished and both types of the digesters produced the same total amount of gas (Fig. 2).

First 20 days (start-up period) allowed to establish that a loading rate of 0.65-0.85 g COD l^{-1} day^{-1} (or ~2.6 g COD l^{-1} every 3-4 days) was a suitable way to start the process, and that this period was enough for acclimatisation. The estimated average daily gas production rate was 31 ml and 33 ml for SS and CS inoculated digesters respectively. The methane contents were 34% for both types of seed.

The intermittent daily feeding from day 22 showed that the processes within the digesters were stable. The digesters were able to cope with a loading rate of 2 g COD l^{-1} day^{-1} between days 22 to 46, and then with a higher loading of 3 g COD L^{-1} day^{-1} from day 46 until the end of the experiment on day 76. The daily gas production between days 22-46 was 62 and 59 ml for SS and CS inoculated reactors respectively with 52-53% methane. During days 46-76 daily gas production was 125 and 98 ml for SS and CS, with 49% and 36% methane respectively. No nutrients or alkalinity were added during this experiment.

These results suggested that treatment of lactose by anaerobic digestion can be achieved in a single stage digester without nutrient or alkalinity supplements. The results were contradictory to the published evidence on the digestion of cheese-farm waste, which report that single-stage digestion is difficult to achieve and that the two-stage processes are preferred.[1-7] The alkalinity adjustment is reported to be necessary after the acidification and before the methanogenic stage.[3] As noted above, the digesters were fed intermittently, i.e. there were frequently 2-3 day gaps between the addition of feed, which could explain the observed apparently stable results.

Fig. 1. Experiment 1. Average cumulative gas production
Start up, days 1 - 22

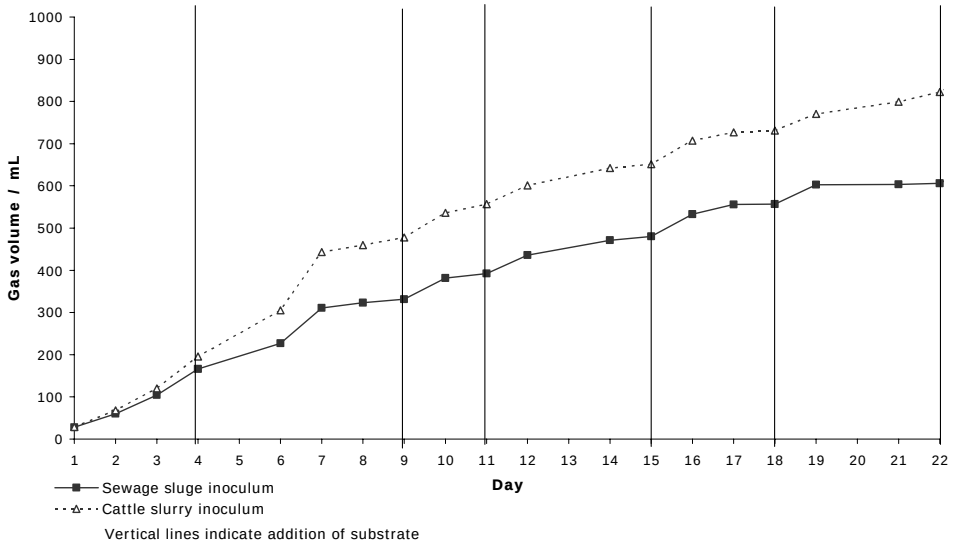
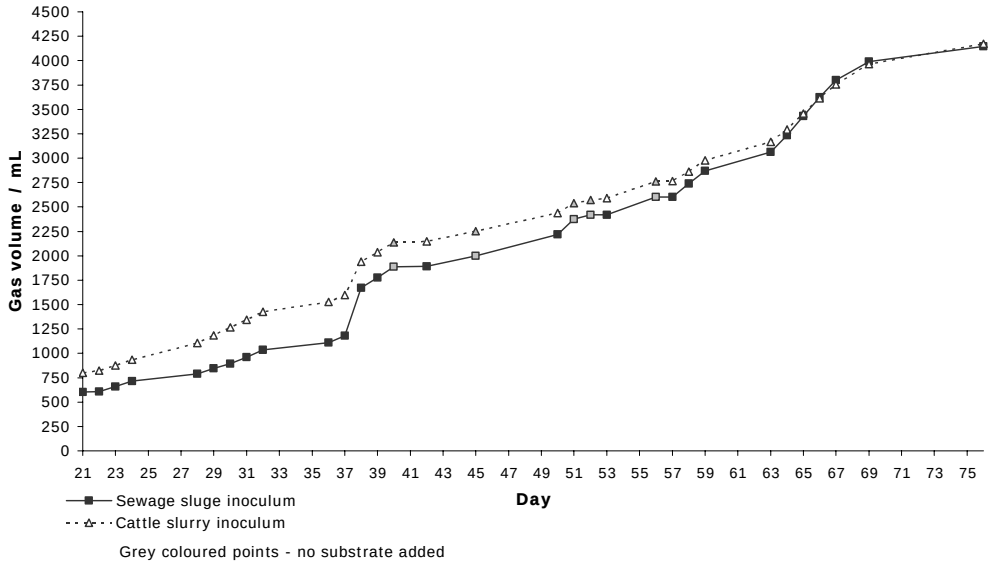


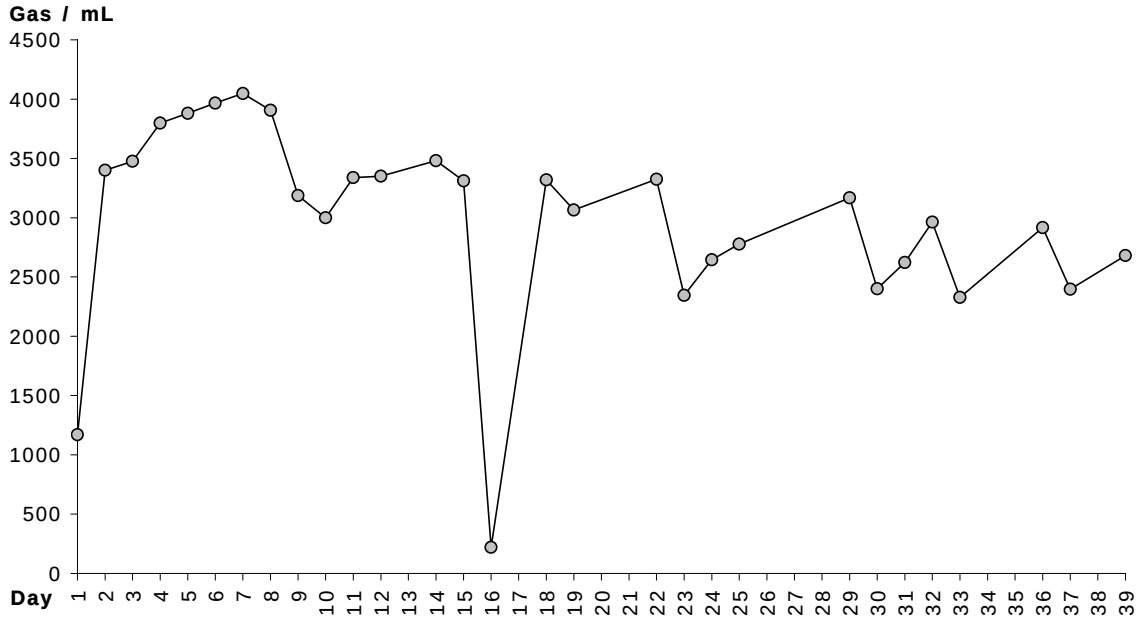
Fig. 2. Experiment 1. Average cumulative gas production
Days 21 - 76



Experiment 2a.

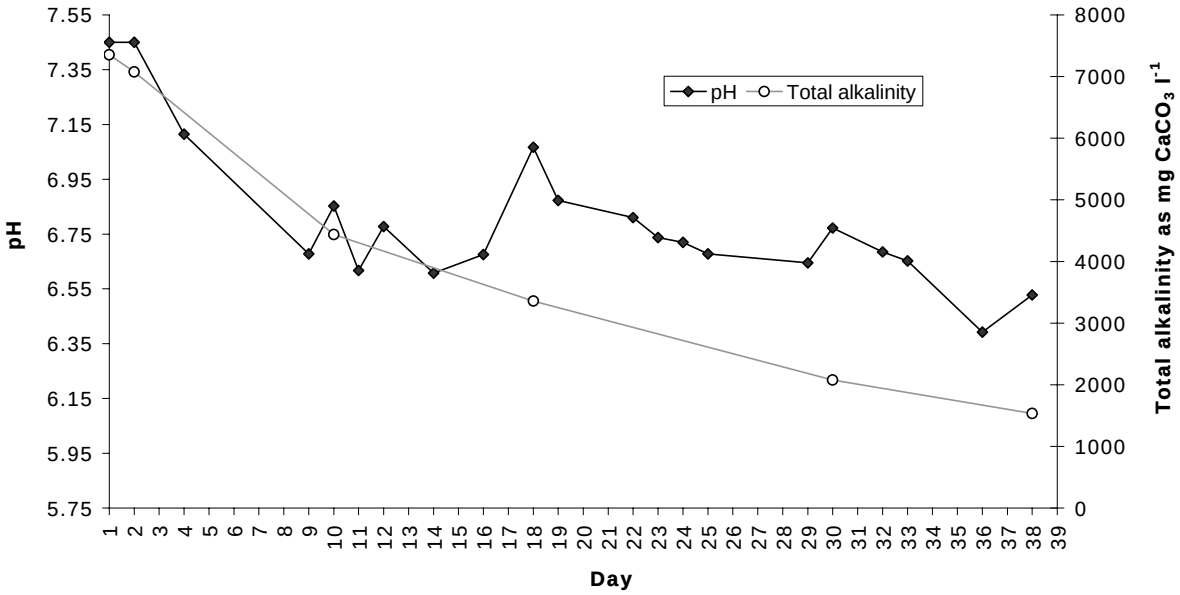
This experiment was set-up to scale-up the Experiment 1 for better and more parameter control. During the first 39 days of the experiment digesters were fed at a loading close to 1 g COD l^{-1} day^{-1}. The average estimated specific methane production over this period was 0.37 l g^{-1} of COD added. However, the daily total biogas production (Fig. 3), pH and alkalinity (Fig. 4) were falling. By day 39, pH fell from 7.43 to 6.53, and the total alkalinity dropped from 8149 to 1534 mg as $CaCO_3$ l^{-1}. The intermediate to partial alkalinity ratio (IA:PA), which represents VFA to alkalinity ratio and acts as an indicator of digester stability, increased from 0.5 to 0.8. These results indicated VFA overload and compromised methanogenic activity.

Fig. 3 Experiment 2a - Average daily gas production



The daily gas production is average for four digesters, each received the same loading of 1 g COD l^{-1} day^{-1}.
The low value on day 16 is due to the digesters not being fed on the previous day (15).

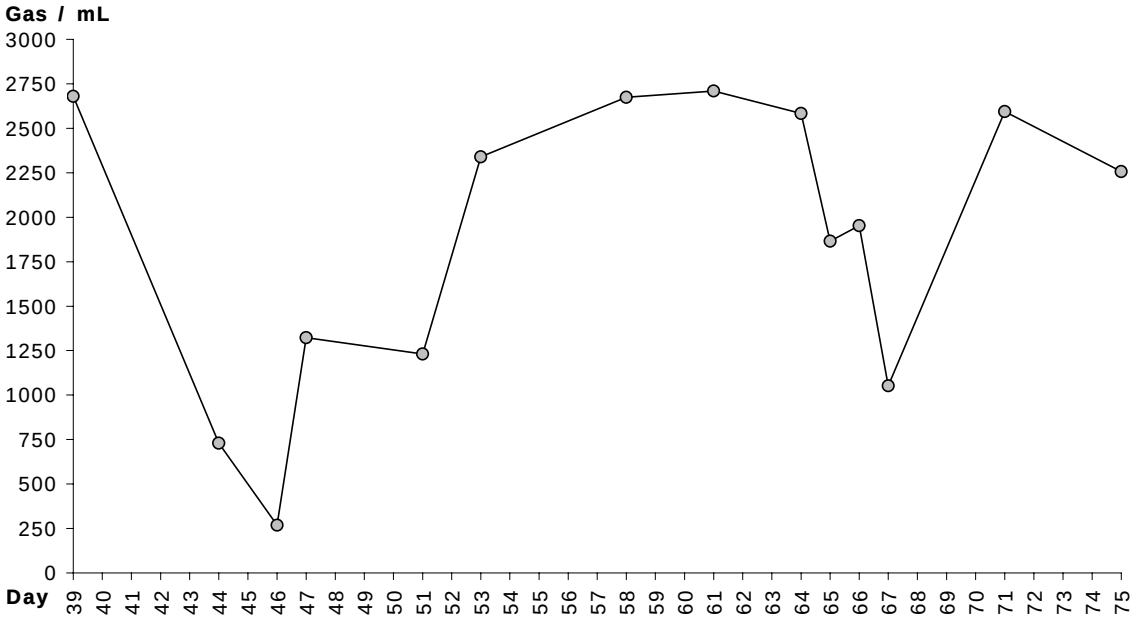
Fig. 4 Experiment 2a - Average pH and total alkalinity change



The pH and alkalinity change shown is related to the same period as in Fig. 3.
Points indicate the pH and/or total alkalinity measured on that day

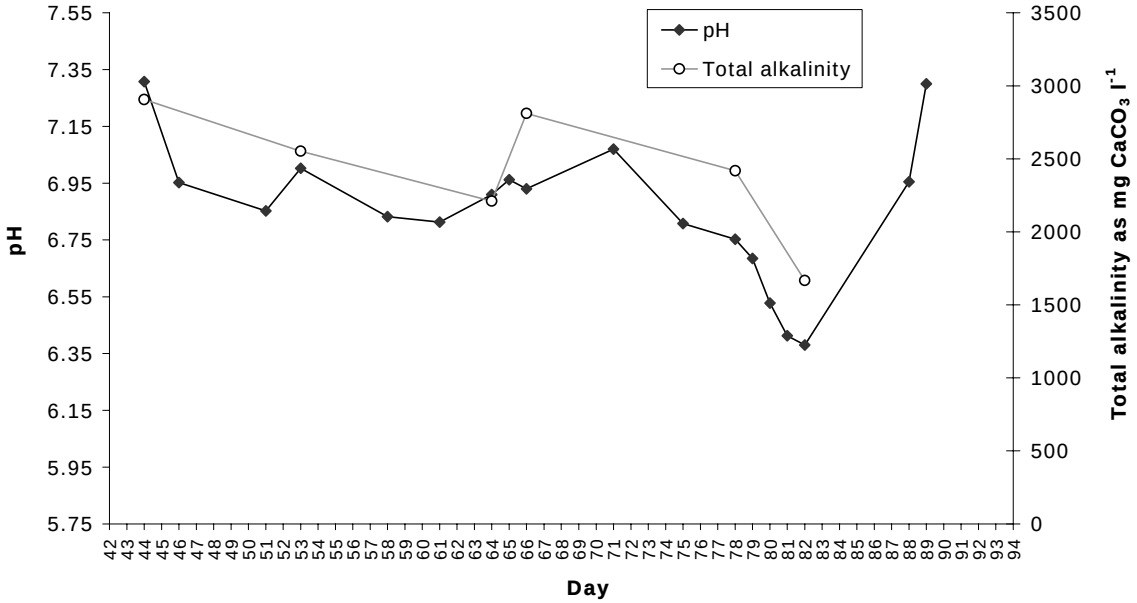
Feeding was stopped on day 39, and $NaHCO_3$ (~2g/L of reactor) was added to improve the alkalinity. Feeding was then restarted on day 44 with the same loading of 1 g COD l^{-1} depending on the digesters' pH and alkalinity, which were measured prior to feeding. A slight improvement in gas production, pH stability and an increase in alkalinity were observed (Fig. 5 and 6). The IA:PA dropped to 0.39. It was proposed that the instability observed could be due to micro-organisms acclimatisation, and the loading perhaps was too high during the start-up period. The improvement after the addition of alkalinity observed suggested that digesters were acclimatised.

Fig. 5 Experiment 2a - Average daily gas production after alkalinity supplement



The daily gas production is average for four digesters. Each digester received a loading of 1 g COD l^{-1} on the days represented by grey coloured circles. Black circle indicate addition of $NaHCO_3$ at 2 g l^{-1}. Clear circles indicate days when digesters were not fed.

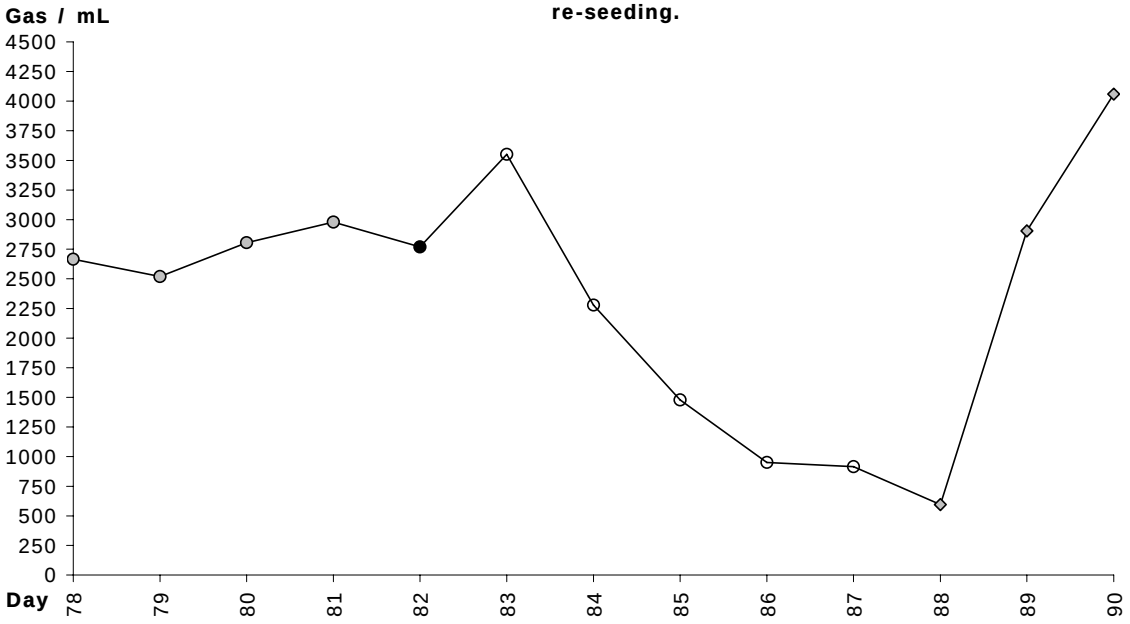
Fig. 6 Experiment 2a - Average pH and total alkalinity change after alkalinity supplement



The pH and alkalinity change shown is related to the same period as in Fig. 5. Points indicate the pH and/or total alkalinity measured on that day. From day 78 the loading was increased to 1.5 g COD l^{-1} day^{-1}

To test this proposal, the loading was increased to 1.5 g COD l^{-1} day^{-1} from day 78 for 4 days. During this period although total biogas production was stable at around 0.46 m^3 kg^{-1} COD added (Fig. 7), pH and alkalinity were falling (Fig. 6), and the IA:PA increased to over 1.

Fig. 7 Experiment 2a - Average daily gas production during higher loading and after re-seeding.



The daily gas production is average for four digesters. Grey circles indicate feeding at the loading of 1.5 g COD l^{-1}. Black circle indicates re-seeding the digesters with sewage sludge. Grey squares indicate re-start of feeding at the loading of 1 g COD l-1.

The sludge in the digesters was very thin by this point. The total solids in the beginning of the experiment were 28.5 g l^{-1} (VS 56.5%), and fell down to 8.79 g l^{-1} (VS 51%) by day 55. Each digester was re-seeded with 2 litres of sewage sludge on day 88 and was left to stabilise for 6 days, after which feeding re-started at a loading rate of 1 g COD l^{-1} day^{-1}.

The results from this experiment suggested that conditions in the digesters were generally unfavourable for methanogenic micro-organisms growth. The retention time of 16 days although was close to the retention times used for industrial-scale digesters, was much shorter than the one used in the smaller-scale Experiment 1 (50 days). This lead to the

micro-organisms being washed-out, and as methanogens grow much slower than acidogens, accumulation of the hydrolysis products resulted, which further deteriorated the conditions by lowering the pH. This was apparent even at such a low loading rate as 1g COD l^{-1} day^{-1} and with nutrient supplements. The results were in accordance with published sources reporting that a single-stage digestion of cheese-farm and lactose-based wastewater without alkalinity control is not possible.[3] Regular control of alkalinity was not investigated due to operational difficulties and extra costs that it would incur on a larger scale.

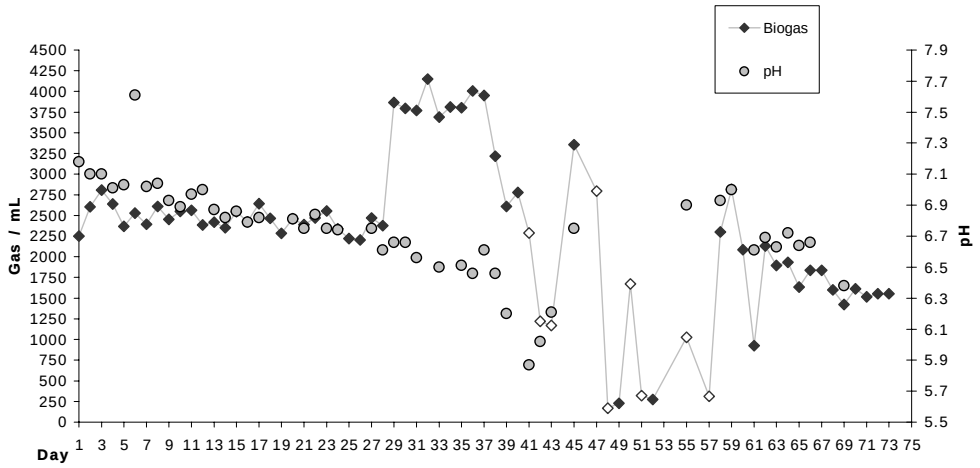
Experiment 2b

This experiment was performed to investigate which of the factors - retention time, solids wash-out or continuous hydrolysis due to regular feeding - were the reasons for digester failure in the Experiment 2a.

Effect of the retention time:

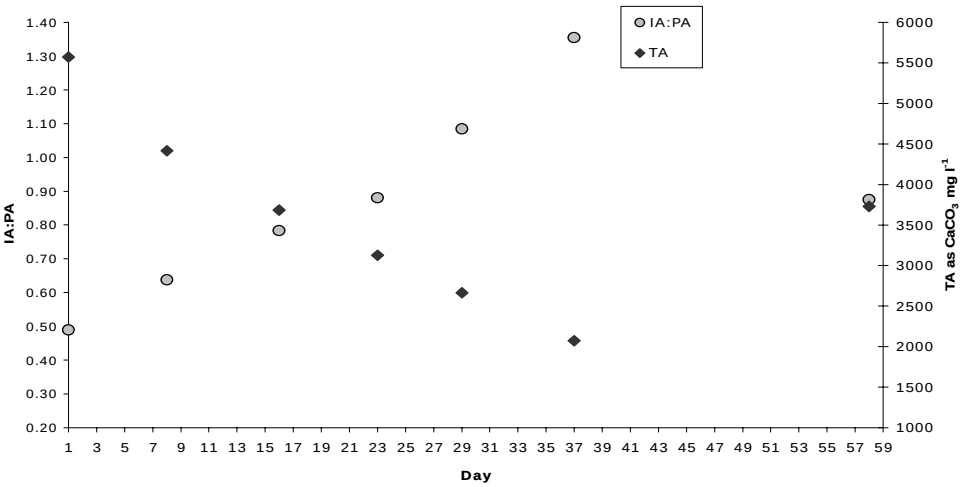
One of the re-seeded digesters from Experiment 2a was switched to a 50 day hydraulic retention time. During the first 30 days, stable daily gas production was established (Fig. 8). Total biogas production was 0.69 m^3 kg^{-1} COD added with methane content of 45%, corresponding to an average specific methane production of 0.27 m^3 kg^{-1} COD added. However, gradual decrease in pH from 7.18 (day 1) to 6.61 (day 28) correlated with a steep decrease in total alkalinity (TA) capacity (from 5571 to 2665) and an increase in IA:PA ratio was indicating the VFA accumulation and weak methanogenic activity.

Fig. 8 Experiment 2b - Digester 1 Daily gas production and pH change



Clear squares indicate no substrate addition on these days.
Days 1-28: loading of 1 g COD l^{-1} day^{-1} used.
Day 28-40: increased loading of 1.5 g COD l^{-1} day^{-1}.
Days 45, 49, and 52: loading of 0.75 g COD l^{-1} with 87.5 mg $NaHCO_3$ l^{-1} day^{-1}.
Day 58-73: 1 g COD l^{-1} day^{-1} with 12.5 mg l^{-1} yeast extract addition.

Fig. 9 Experiment 2b - Digester 1 TA and IA:PA



When the COD loading was increased to 1.5 g l^{-1} between days 28-40, although gas production seemed stable between days 28-38 (0.63 m^3 kg^{-1} COD added, with 46% CH4), a further decrease in pH from 6.61 to 6.46 was observed, the alkalinity dropped from 2665 mg as $CaCO_3$ l^{-1} (day 29) to 2073 mg as $CaCO_3$ l^{-1} (day 37), and the IA:PA ratio over 1 indicated a VFA accumulation. Within the next two days gas production fell, the digester was left to stabilise and was fed irregularly at a loading of 0.75 g COD l^{-1} with addition of $NaHCO_3$ at 87.5 mg l^{-1} to supplement the alkalinity. On day 58, when the pH, the alkalinity and the IA:PA were within the acceptable values for a stable digester (6.93, 3728 mg as $CaCO_3$ l^{-1} and 0.87) feeding re-started at a loading of 1 g COD l^{-1} day^{-1} with yeast extract supplement at 12.5 mg l^{-1} day^{-1} to provide extra nutrients and vitamins. However, the gas production remained low and was falling, as did the pH. A slight increase of TA was observed on day 69 (4075 mg as $CaCO_3$ l^{-1}), but this was accompanied by a high IA:PA ratio (1.38), which indicated instability of the methanogenic microbial community within the digester.

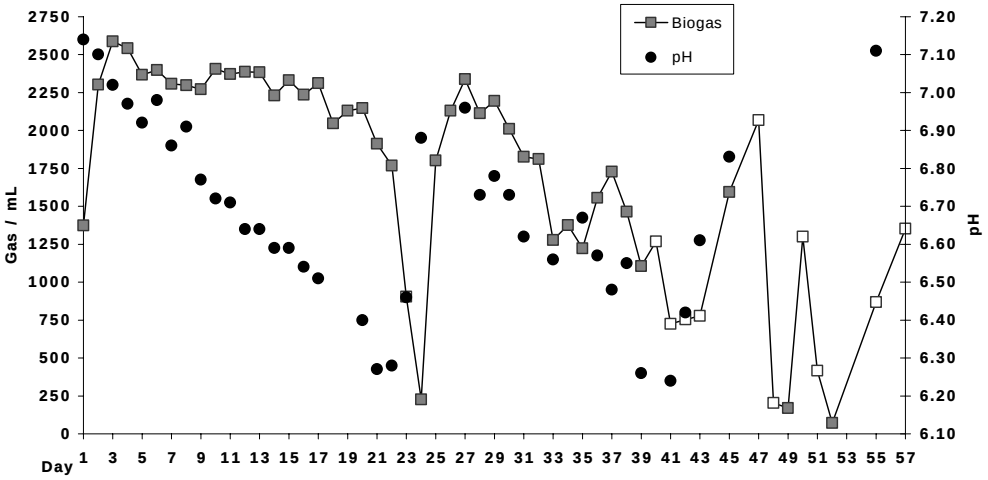
The results of this experiment showed that a wash-out of micro-organisms was taking place even at the longer retention time of 50 days, suggesting that the conditions were unfavourable for methanogenic bacteria growth to sustain a stable healthy population. As a result, intermediate alkalinity, which directly corresponds to VFA concentration, was rising, and although the pH was within the range of optimal pH for methanogens (>6.3), the buffering capacity of the digester was low and any further acid production was compromising the digester processes.

Comparison between the retention times; effect of late alkalinity supplement.

Digester 2 was run under the conditions of the Experiment 2a. The biogas production observed was lower than in the Experiment 2a with 0.23 m^3 methane kg^{-1} COD added over the period of the first retention time of 16 days (Fig. 10). A steady decrease in gas production over the first 22 days was accompanied by a decrease in pH from 7.14 to 6.28, a drop in alkalinity from 4965 to 1327 mg as $CaCO_3$ l^{-1}, and an increase in IA:PA from 0.50 to 0.93 between days 1 and 23 respectively (Fig. 10 and 11). On day 23 the alkalinity supplement was added in the form of $NaHCO_3$. The amount added was calculated on the basis of the alkalinity measured on that day to bring the buffering capacity to an acceptable level of 4000 mg as $CaCO_3$ l^{-1}. This amount, however, raised the alkalinity only to 2200 mg as $CaCO_3$ l^{-1}, but improved the IA:PA to 0.48, and pH to 6.88. The feeding was then re-started at the initial loading of 1 g COD l^{-1} with daily alkalinity supplement of 87.5 mg $NaHCO_3$ l^{-1}. This was continued between days 24-29. The initial improve in gas production within the next three days was quickly followed by steep decline and a fall in pH and alkalinity.

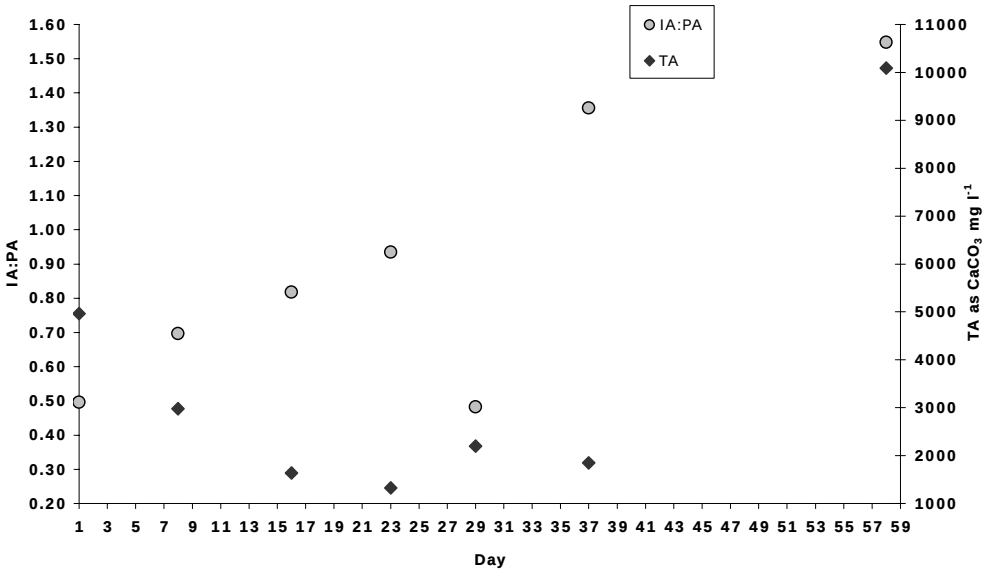
The results of this experiment confirmed the observations of the Experiment 2a and showed that the micro-organisms within the digester were highly sensitive to the pH and buffering capacity. The attempts to recover the process by 'late' addition of alkalinity were not successful. The observations suggested that the substrate is very easily acidified. The acidogenic bacteria utilises the substrate quickly, which promotes its growth and establishment. However, more slowly reproducing methanogens, are not able to utilise all the acids produced. Either the acid concentration is too high for them, or (more likely) the type of the acid is not preferred for methanogenic acitivity. It is reported that at pH ~7 propionic acid-type fermentation predominates.[17] Propionic acid is less preferred by methanogens than acetic or butyric (the production of which takes place at lower pH values).[8,17] This may slow down the growth of methanogens initially, followed by a steady daily wash-out at a rate higher than their reproduction. This leads to further accumulation of acids and deteriorates the process. The addition of alkalinity at this stage seems to improve the overall conditions, but as the acid accumulated is likely to be propionic, it is not taken up effectively by the methanogens, so when the feeding is restarted, a misbalance is observed even quicker.

Fig. 10 Experiment 2b - Digester 2 Daily gas production and pH change



Clear squares indicate no substrate addition on these days.
Days 1-39: loading of 1 g COD l-1 day-1 used.
Day 23: 2.25 g l-1 NaHCO3 was added.
Days 24-39: 87.5 mg l-1 day -1 NaHCO3 was added with substrate between days 24-40.
Days 45, 49, and 52: loading of 0.75 g COD l-1 with 87.5 mg NaHCO3 l-1 day-1.

Fig. 11 Experiment 2b - Digester 2 TA and IA:PA

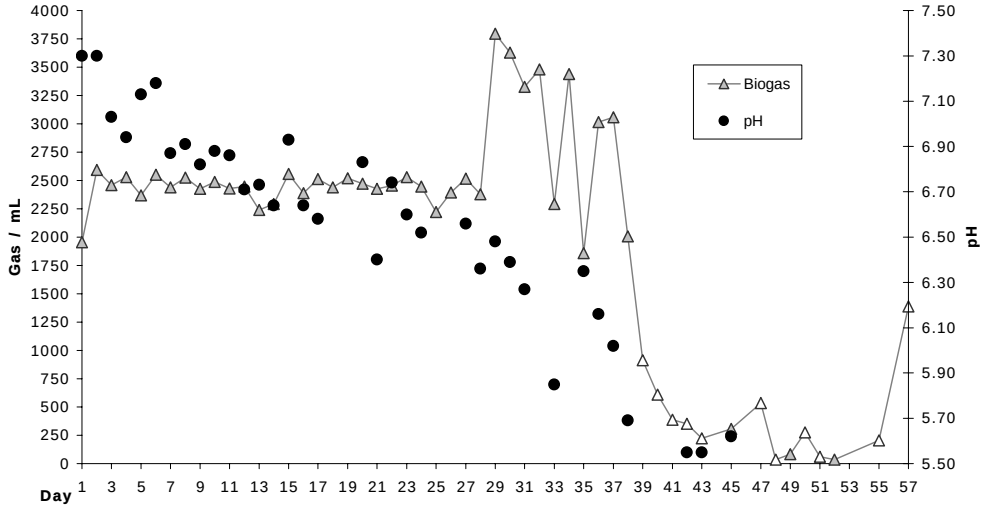


Effect of sludge re-circulation:

Digester 3 showed gas production trends similar to the digester 1 (0.27 m^3 methane kg^{-1} COD added) during the first 28 days. The apparently stable gas production during this period was accompanied by a gradual pH decrease from 7.3 to 6.36 on days 1 and 28 respectively (Fig. 12). The alkalinity decrease was also observed accompanied by a significant increase of IA:PA ratio (Fig. 13). Increasing the loading to 1.5 g COD l^{-1} day^{-1} lead to an expected rapid fall in gas production and pH.

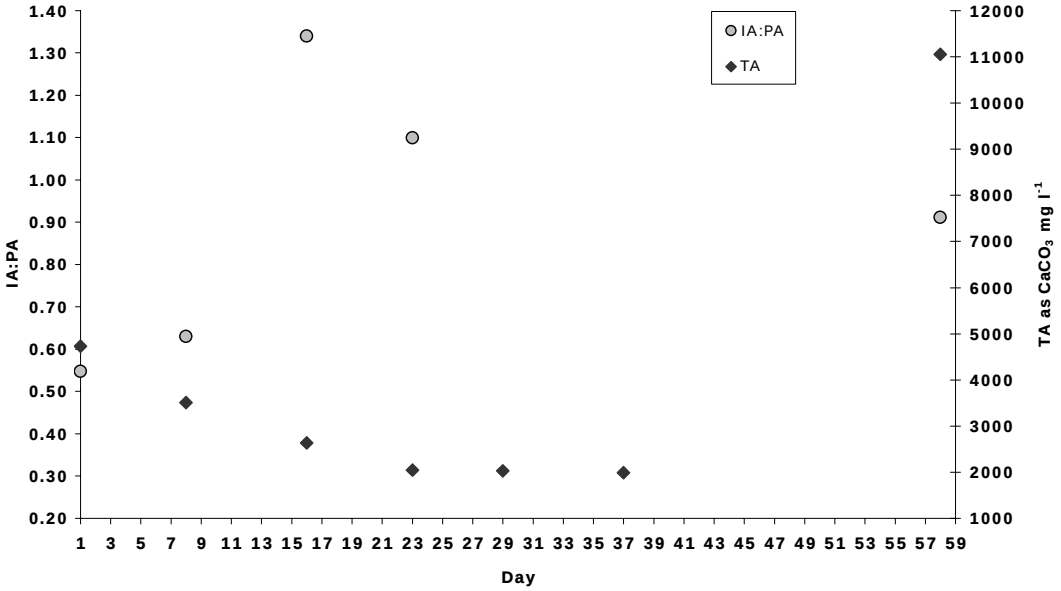
The results of this experiment also suggested that the accumulation of a particular acidogenesis product triggered initial slow down of the methanogenic growth. Re-circulation of sludge in such a case would not improve the situation, as it would promote higher and faster growth of acidogens than methanogens.

Fig. 12 Experiment 2b - Digester 3 Daily gas production and pH change



Clear tiangles indicate no substrate addition on these days.
Days 1-28: loading of 1 g COD l^{-1} day^{-1} used at 16 days HRT.
Day 28-38: increased loading of 1.5 g COD l^{-1} day^{-1}.
Days 45, 49, and 52: loading of 0.75 g COD l^{-1} with 87.5 mg $NaHCO_3$ l^{-1} day^{-1}.

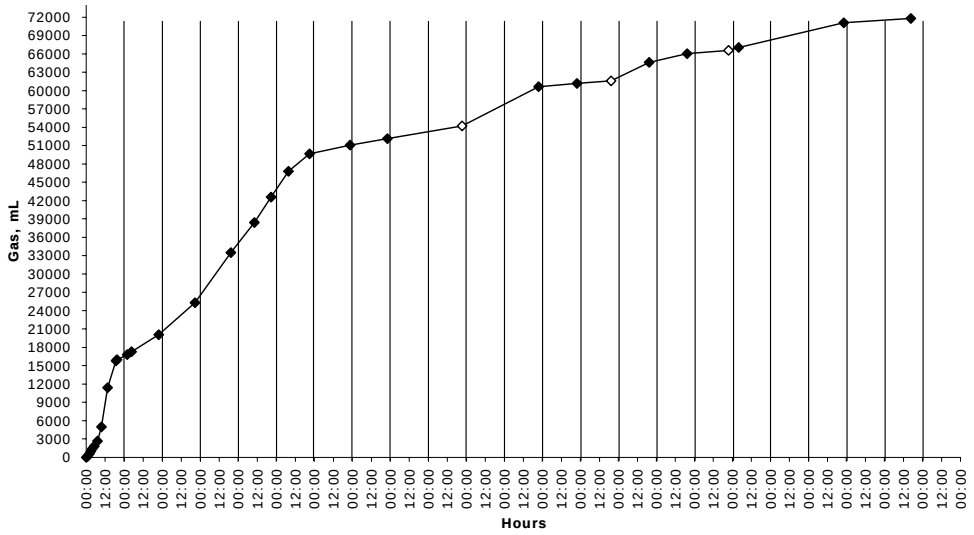
Fig. 13 Experiment 2b - Digester 3 TA and IA:PA



Digester 4 was set up as a batch experiment with 16 days HRT. The lactose substrate was added in the amount equivalent to 16 g of COD per litre. This run resulted in a rapid digester failure. Total amount of gas produced within the first week was less than 5 litres, with methane content less than 20%, the pH dropped below 6, and the gas production stopped.

The run was then repeated with fresh sludge and the same volume of lactose (equivalent to 14.6 g COD l^{-1} loading this time due to COD variations of the substrate). This time over 52 litres of gas were produced in 10 days with the methane contents starting from 41% and increasing up to 75% by day 6. The reactor pH has increased from 6.14 to over 7 during this period. Extra substrate was added on days 10, 14, and 17 at 2.8 g COD l^{-1} loading. The digester was coping with these loadings successfully.

Fig. 14 Experiment 2b Digester 4 Cumulative gas production.



Clear squares indicate addition of substrate at 2.8 g COD l^{-1}.

This run suggested that shock-loading the digester with this type of substrate does not necessarily result in digester failure. This approach was also closer to the plug-flow conditions than daily feeding, so the work moved to Experiments 3, during which this approach was utilised.

Experiment 3a:

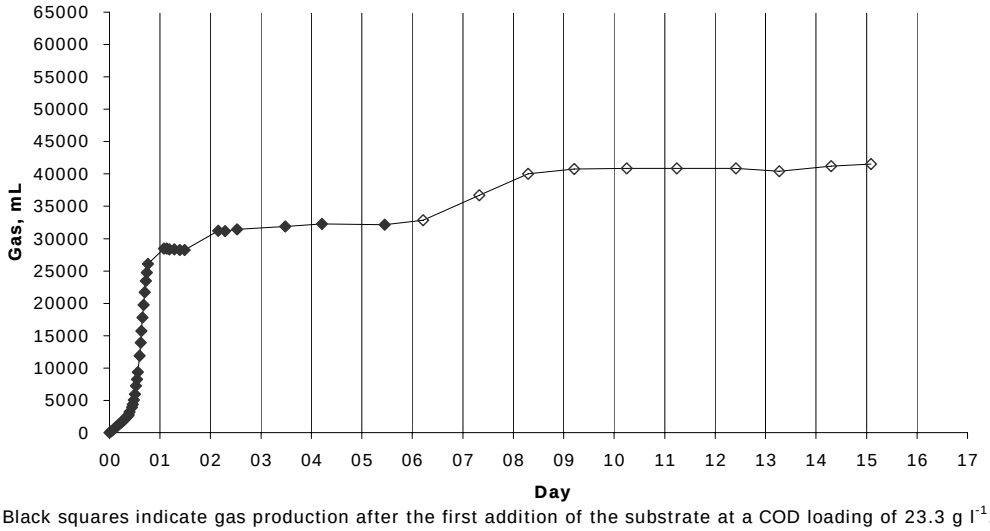
23 days after the first addition of 480 ml lactose substrate, a further amount in the same volume was added to the digester 4. This time 480 ml of lactose added were equivalent to 15.9 g COD l^{-1} loading. No extra seed was added. The substrate was added in the similar way three more times every 14-16 days. The loading over the period of these five runs, with the same digester and no re-seeding, was in the range of 14.5-16.8 g COD l^{-1}. The total cumulative gas production from these runs is shown in Fig. 15.

Digesters 1-3 were set up in a similar way with fresh sewage sludge to investigate lower and higher shock loadings. Digester 1 received substrate in the volume equivalent to 23.3 g COD l^{-1}, digester 2 - 10.8-12.6 g COD l^{-1}, and digester 3 - 7.2-8.4 g COD l^{-1}.

Overall, digesters 2 and 3 showed a better ability to cope with their loadings than digesters 1 and 4. .Six consecutive runs were performed successfully with digesters 2 and 3, and each time the digesters were utilising up to 97% of total VFAs produced (Fig.17, 18, 21, 22). The fast VFA production over the first two days of each run, majority of which were acetic and butyric acids, was accompanied by hydrogen generation and pH drop below 7, whereas VFA utilisation at the later stages of each run was associated with biogas production with up to 75% methane and gradual pH decrease to over 7. The overall specific methane output was between 0.40 and 0.58 m^3 kg^{-1} COD added.

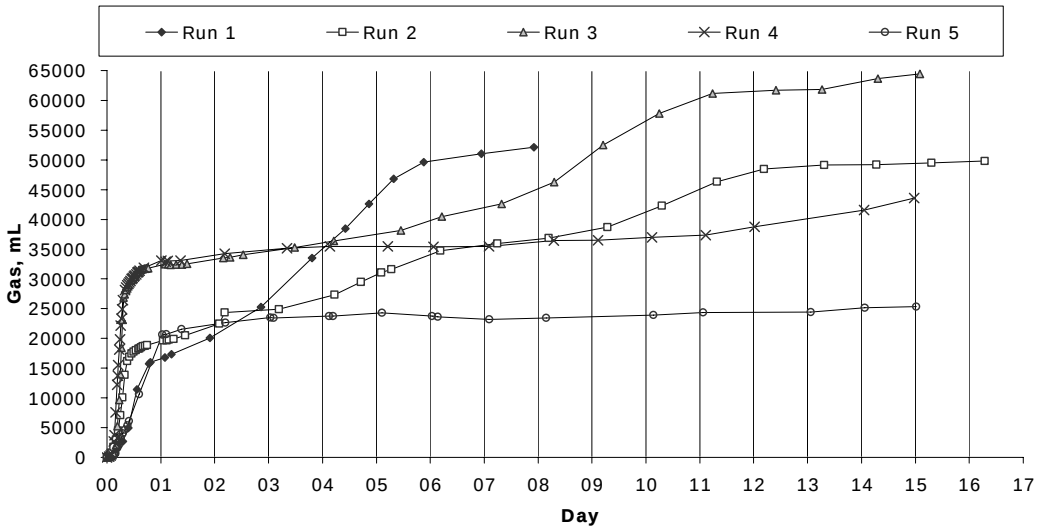
Higher loadings of the digesters 1 and 4 were not utilised as efficiently. A loading of 23.3 g COD l^{-1} was effectively fermented into VFAs (Fig. 19), again mostly into butyric and acetic acids, but this was not accompanied by significant hydrogen output. Instead steady levels of carbon dioxide were observed (50-65%) and less than 0.6 m^3 methane kg^{-1} COD added. The pH dropped to 5.0. A second load of substrate was added to the reactor on the day 6 in an attempt to promote hydrogen production, but this was unsuccessful - after a brief production of 8.6 l of gas with 75% CO_2 contents, the pH dropped to 4.5 and gas production stopped (Fig. 15). The VFA analyses showed high concentrations of propionic acid (5.8 g l^{-1}) as well as acetic (5.8 g l^{-1}), butyric (7.4 g l^{-1}) and valeric (1.8 g l^{-1}) acids. Similar results were obtained from the digester 4 runs. Although the reactor was able to cope with 14.5-16.8 g COD l^{-1} loadings successfully for three times (Fig. 16), the signs of VFA accumulation were observed during run 4 (Fig. 20), and gas production was very low during run 5 with high levels of CO_2 and acetic, butyric and propionic acids accumulation.

Fig. 15 Experiment 3a
Digester 1: Cumulative gas production



Black squares indicate gas production after the first addition of the substrate at a COD loading of 23.3 g l^{-1}. Clear squares indicate gas production after the second addition of the substrate on day 6 at COD loading of 21.8 g l^{-1}.

Fig. 16 Experiment 3a
Digester 4: Cumulative gas production over five runs with substrate loading of 14.5-16.8 g COD l^{-1}



Run 1: data shown is for 10 days only as extra substrate was added on day 10.

Fig. 17 Experiment 3a
Digester 2: Cumulative gas productin over six runs with substrate loading of 10.8-12.6 g COD l^{-1}

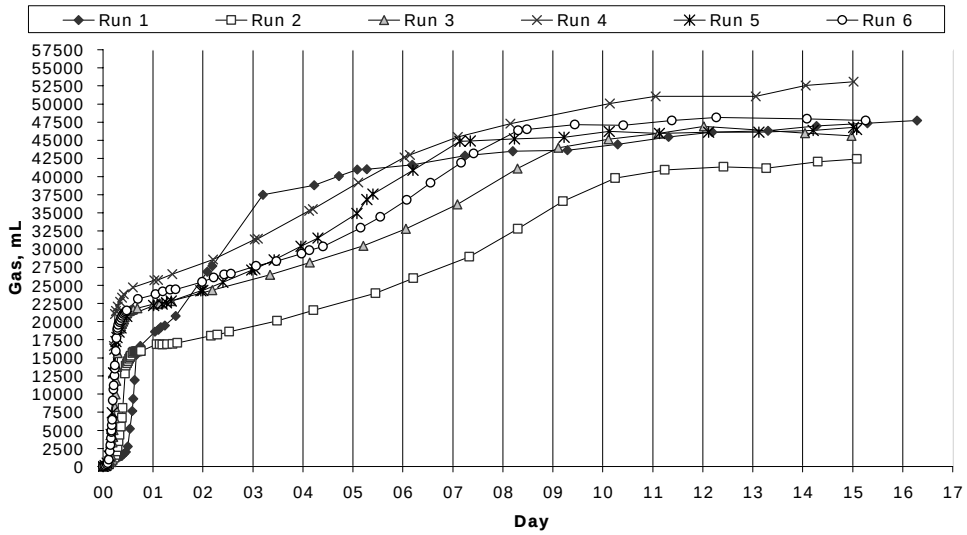


Fig. 18 Experiment 3a
Digester 3: Cumulative gas production over six runs with substrate loading of 7.2-8.4 g COD l^{-1}

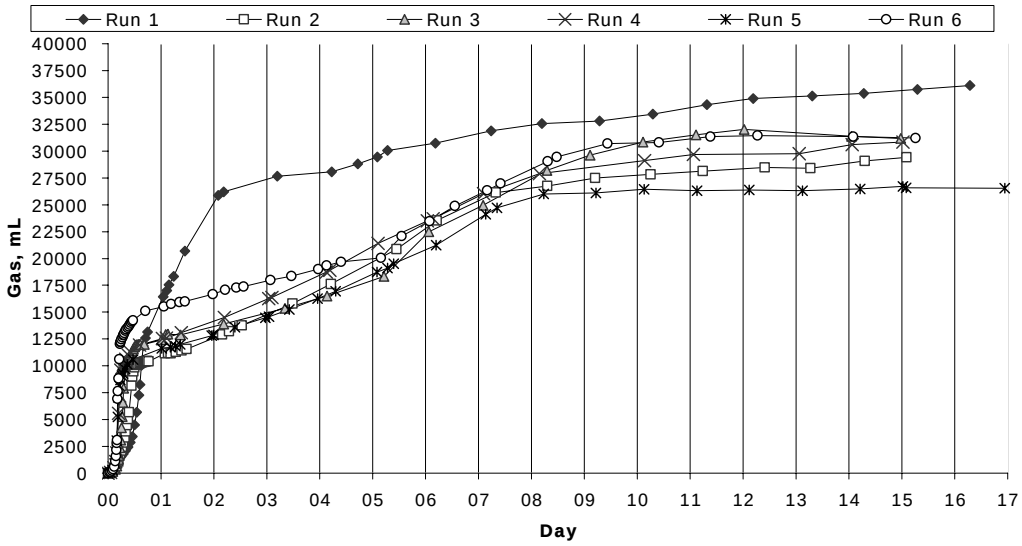
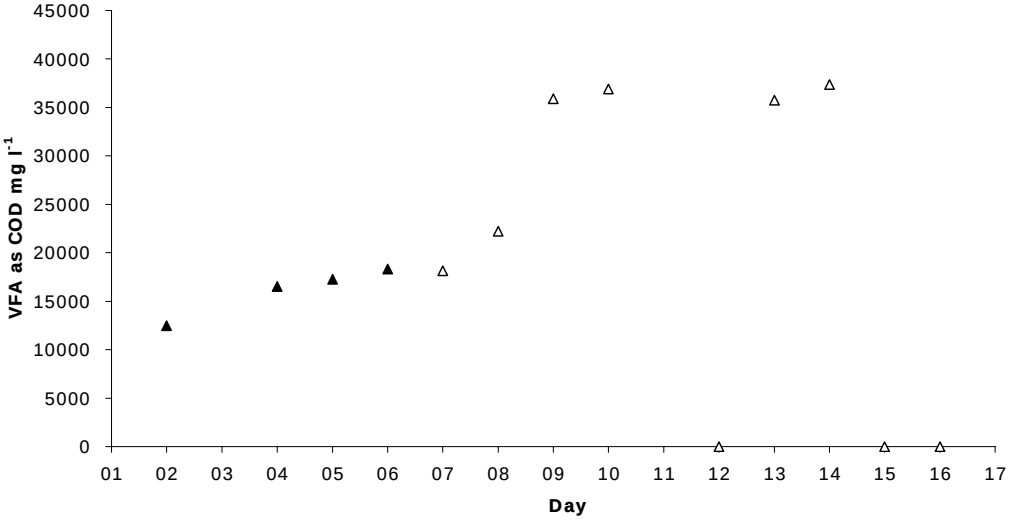
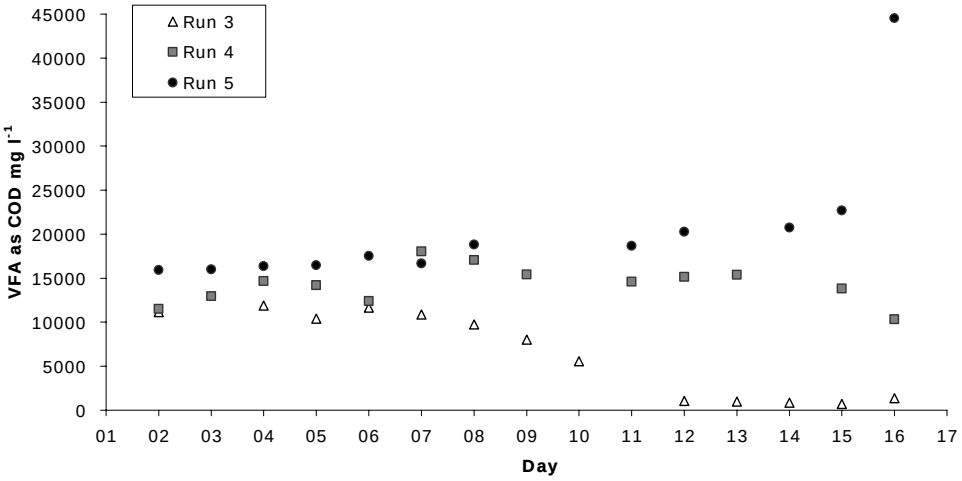


Fig. 19 Experiment 3a
Digester 1: VFA production



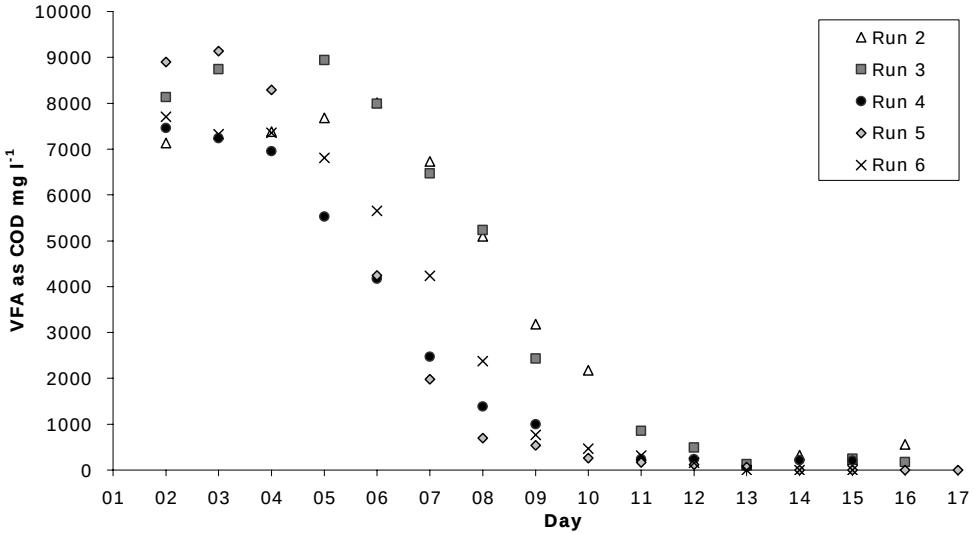
Samples for VFA were not taken during day 1 to avoid disturbing the processes after feeding. Black triangles indicate VFA production after the first addition of substrate at a COD loading of 23.3 g l^{-1}. Clear triangles indicate VFA production after second addition of feed at COD loading of 21.8 g l^{-1}.

Fig. 20 Experiment 3a
Digester 4: VFA production over three runs with substrate loading of 14.5-16.8 g COD l^{-1}



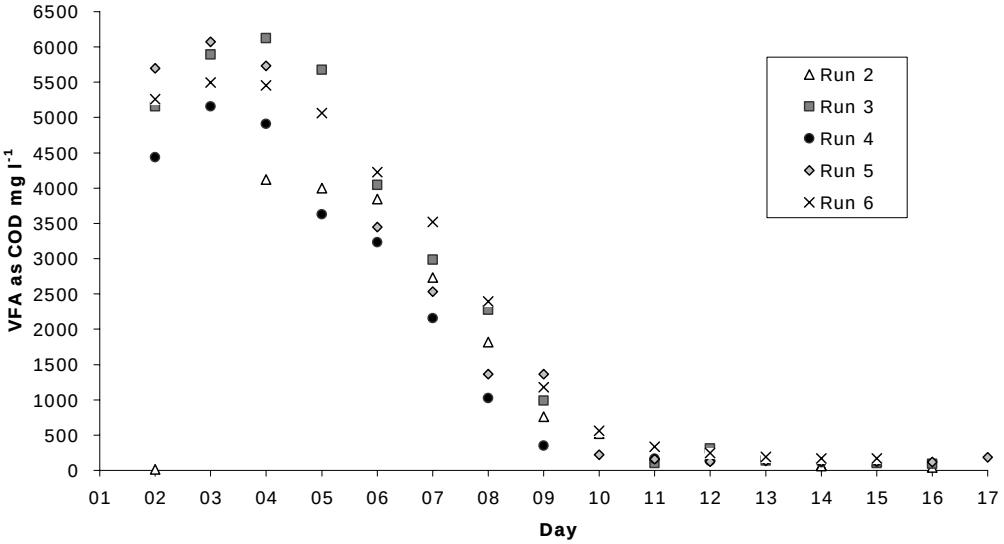
Samples for VFA were not taken during day 1 to avoid disturbing the processes after feeding.

Fig. 21 Experiment 3a
Digester 2: VFA production over five runs with substrate loading of 10.8-12.6 g COD l^{-1}



Samples for VFA were not taken during day 1 to avoid disturbing the processes after feeding.

Fig. 22 Experiment 3a
Digester 3: VFA production over five runs with substrate loading of 7.2-8.4 g COD l^{-1}



Samples for VFA were not taken during day 1 to avoid disturbing the processes after feeding.

The results obtained allowed splitting the processes within the digesters into four distinct stages:

1 Hydrogen fermentation stage. This stage was observed during the first 20 -24 hours of each run. Up to 50% of total biogas was produced during this stage with hydrogen contents between 27-34% of total volume.

2 VFA production stage. This stage was observed between 2nd and 4th days of each run. The VFA concentration rose very quickly and was at its highest during this period, gas production slowed down, hydrogen contents dropped and methane contents increased. This step can also be referred to as a lag phase between hydrogen and methane production.

3 Methanogenic stage - 4th - 7th days of the run. The gas production rates accelerated again and high contents of methane were observed, accompanied by a steady overall decrease in VFA levels. Up to 35% of the total gas volume can be produced during this stage.

4 Trailing-off stage - 7th - 14th days. The gas production during this period was slowing down, but the methane percentage of the total biogas was still high. This stage is also can be looked at as a stabilization stage: alkalinity was found to improve during this

stage and the IA:PA ratio decreased. This step is necessary as it provides enough buffering capacity for the next load of substrate.

Although the loadings used in these trials were referred to as shock-loads, in terms of industrial scale, these are inefficient, as the volume of substrate added successfully each time was maximum 10% of the total reactor volume. In an attempt to investigate if repetitive loadings provided the digesters with necessary acclimatisation, the loadings of the digesters 2 and 3 were increased on their eighth run to 18.2 and 13.7 g COD l^{-1} respectively. Higher biogas production was observed during the first stage, associated with high levels of VFAs (22.5 and 13.8 g as COD l^{-1} respectively), which reduced only slightly by the end of day 14. Digester 3 was then loaded with another portion of substrate, which resulted in a further fast VFA generation with high hydrogen contents. This time the equipment and developed calibration methods allowed estimation of total hydrogen produced. This was 15.8 l, or 0.24 m^3 g^{-1} COD added (previous runs of similar loading were estimated to produce around 8 l of hydrogen). VFA levels increased further. In an attempt to investigate if a stable hydrogen production could be achieved by regular addition of similar loadings, another portion of substrate was added. However, this slowed down the gas production, and CO_2 was the major gas. This could be due to significantly higher VFA levels (35 g as COD l^{-1}) with significant contribution from hexanoic as well as acetic, butyric, propionic and valeric acids, which could have an inhibitory effect on hydrogen fermentation. Digester 2 was left unfed, and the methane production was observed after 24 days lag-phase. This experiment showed that VFA production during stages 1 and 2 of each run was a necessary and also limiting factor for the methanogenic acitivity. Although the products of this stage were the acids that are preferred by methanogens as substrate, the concentration of these acids was rising very quickly, which had an inhibitory effect on methanogenic activity due to lowering of pH. However, a slow return to the acceptable pH levels during the later stages allowed the methanogens to grow and utilise the available substrate effectively.

Five more runs were performed with completely re-seeded digesters and loadings 9.1-17.4 g COD l^{-1} as a method for measuring hydrogen contents of the biogas became available. The results from these runs allowed estimation of energy potential from the produced gas as the primary aims of the CROPGEN project is utilisation of crops and agro-wastes for energy production. On this basis, the average energy production was estimated. Overall, a maximum of 0.4 l CH4 g^{-1} COD added, and 0.2 l H_2 g^{-1} COD added were produced during Experiment 3a. In terms of energy, this equals to over 1000 kJ and 170 kJ for CH_4 and H_2 respectively, or around 1200 kJ total energy production, whereas the total maximum energy that can be obtained from the substrate at this feeding rate (88.4 g lactose) was 1383.5 kJ (lactose calorific value is 15.65 kJ /g). Overall, this represents 85% energy recovery, but quite a low specific energy yield of approximately 16 kJ l^{-1} of the reactor day^{-1}. This is mainly due to the lag phase that is experienced after the hydrogen fermentation and before the start of methanogenesis. During this phase the gas production rate is very low as although plenty of suitable products are generated (mainly butyrate and acetate), the pH is too low and does not allow the methanogenesis to proceed at a full capacity rate.

Sustainable hydrogen production would create a suitable substrate for the methanogenesis, on the condition that it would take place in a separate compartment or, ideally, in a high-performance type reactor as the VFA production is very quick when lactose is used as substrate. A plug-flow simulation in a single stage CSTR showed that hydrogen fermentation results in a low pH and as consequences, a lag phase for the methanogenic stage. The two populations of micro-organisms required for these processes can co-exist together, but the switch between the activities of the two communities is long. In a series of tanks, such as the Maltin® System, the substrate would be expected to travel in a plug-flow manner through the series of the digesters. The conditions in each tank concerning the micro-organism population might result in an effective natural separation between the hydrogen producers and methane producers. However, it is important to understand how the switch between the processes takes place, and how the lag phase between the hydrolysis and the methanogenesis can be reduced.

In terms of the plug-flow investigations the results from the Experiment 3a therefore, provided valuable information about the processes that require control between each step. In the plug-flow simulation of Experiment 3a the substrate was allowed to undergo a full

anaerobic treatment from acidification to acetogenesis and on to methanogenesis. Therefore, it was important to find out that the products of the first two processes were suitable and optimal for utilisation at the last stage.

The results also suggested that most of the lactose was acidified during the first day of each run, which was supported by rapid hydrogen evolution and high levels of VFAs observed on the next day. This suggestion is supported by the results published by Fang and Yu (2001)[15]. The results from this paper also suggest that pH levels in our digesters during the first day could be lower than the levels observed on the second day (5.9-6.3).

Experiment 3b:

To establish a more detailed pH and VFA profiles (particularly during the hydrogen production stage) and to allow closer monitoring of the conditions that were created in the CSTR digesters after addition of the substrate, the Experiment 3b was set up with equipment available for on-line gas collection, pH monitoring, frequent sampling that did not require stopping the stirrers and opening the digesters, more accurate estimation of hydrogen produced, and elemental analysis.

Five digester runs were performed during the experiment 3b. Four 5 litre digesters were used, seeded with 4.5 litre sieved sewage sludge. 500 ml of lactose substrate containing dissolved macro and micro nutrients were added to each digester. The data shown is only from runs 2-5, as equipment was still tested during the first run and it was regarded as acclimatisation period for the digesters. Digesters 1 and 2 were fitted with a sampling port and pH probe port. As only one pH probe was available, the on-line pH monitoring was performed on digester 1 during the run two, and then on digester 2 during the last three runs. Digesters 3 and 4 were fed during runs 1-3 as in the experiment 3a. Digester 4 was also used in a similar way in runs 4 and 5. Not all the data from these digesters is shown as it was similar to the experiment 3a, and these digesters were kept as control/spare ones. The data from the digesters 1 and 2 is more detailed and is discussed below.

In Run 2, the 500 ml lactose addition to each digester was equivalent to 19.8 g COD l^{-1} loading. All four digester coped with this loading successfully. The pH profile showed that pH dropped as low as 5.8 during the first day of the run (Fig. 23). It was also confirmed that this pH drop is accompanied by high rate of VFA production (9500 as mg COD l^{-1}), primarily butyric (C_4) and acetic (C_2) acids and ethanol (Fig. 24). The pH monitoring showed that during the lag phase from day 2 to day 7 the pH was steadily rising accompanied by slow VFA consumption. When pH 7 was reached the VFA consumption increased significantly and within three days most of the VFAs were utilised (Fig. 23 and 25).

Fig. 23 Experiment 3b Run 2
Digester 1 VFA productin and pH profile

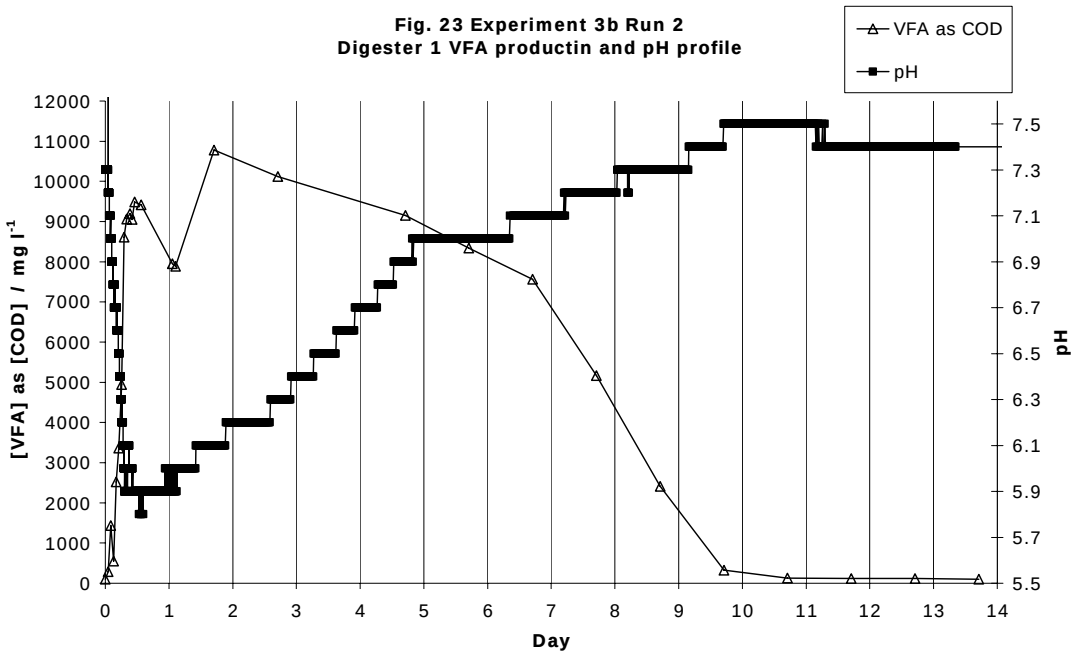


Fig. 24 Experiment 3b Run 2
Digester 1 VFA profile and gas production
Day 1

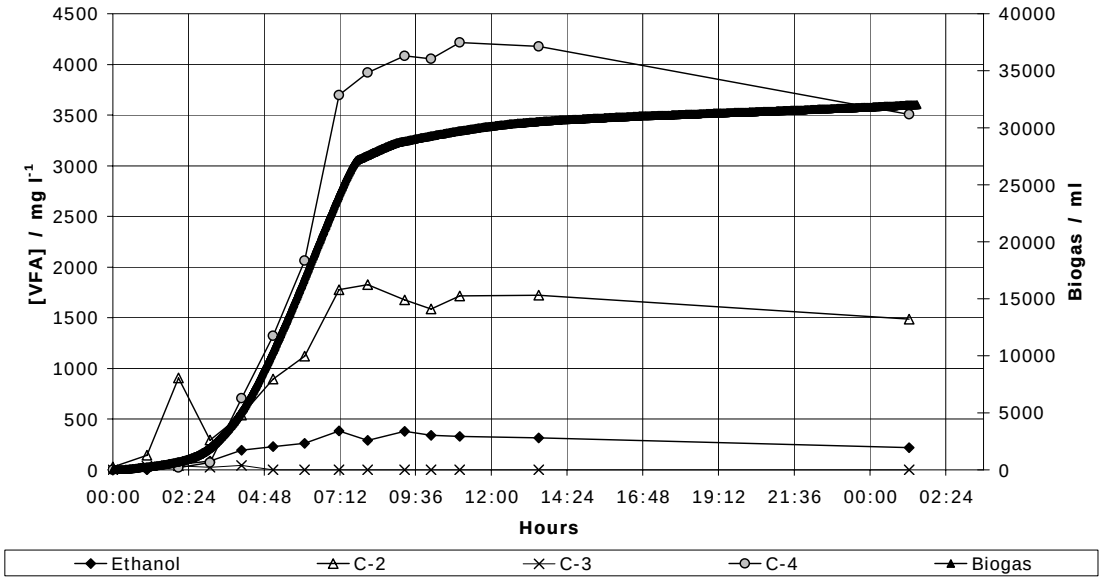
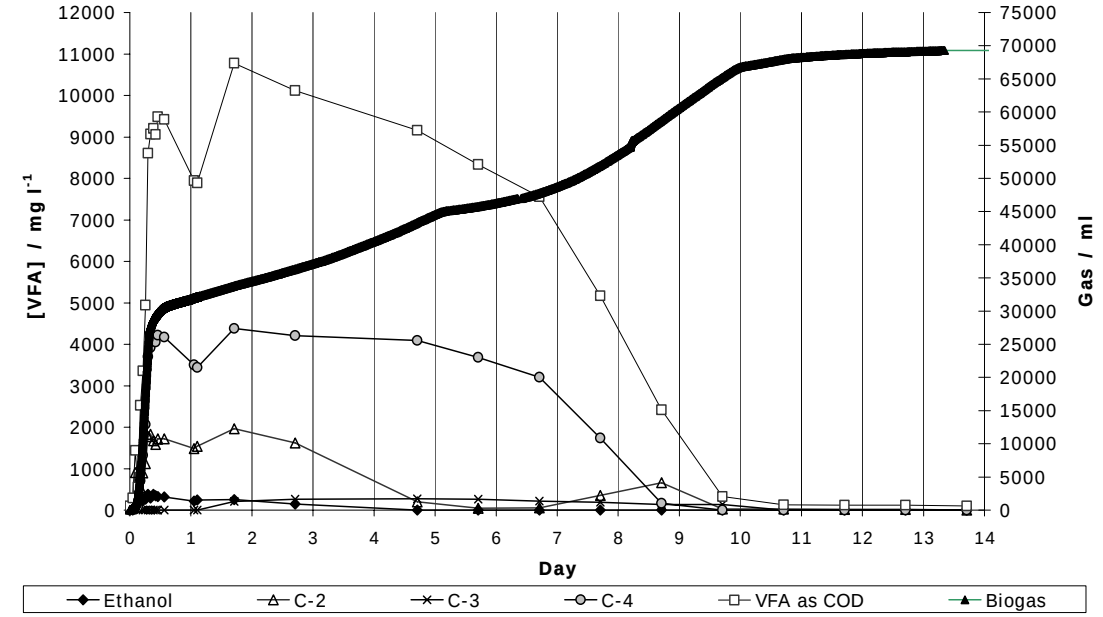


Fig. 25 Experiment 3b Run 2
Digester 1 Cumulative gas production and VFA profile



In Run 3 the pH was monitored in digester 2. The loading of 500 ml of lactose was equivalent to 17.1 g COD l^{-1}. All reactors again coped with this loading. The gas production and VFA profile of the digester 1 was similar to the Run 2.

Digester 2 produced 18.4 litres of biogas within 13 hours from the start of the run (Fig. 26). Over 50% of this (9.8 litres) was hydrogen. The VFA concentration by this stage was 11.6 g COD l^{-1} and increased to 12.1 g COD l^{-1} by day two. A 2500 mg l^{-1} drop in VFA concentration was observed after this time point. Similar drop in VFA production was also noticed around the same time point in this digester during run 2 and in the digester 1 during runs 2 and 3. This brief decrease in the VFA levels was not associated with any sudden increases in gas production. The pH change from 6.1 to 6.2 was noted around the time point of this VFA drop (Fig. 27). The VFA production increased from that point onwards to 12.7 g COD l^{-1} by day 3. Fast VFA utilisation was again observed between days 7-10, which was associated with pH increase to 7.0 similar to the digester 1 during run 2. Alkalinity was measured daily in all four digesters over the period of each run starting from day 2 or 3. The total alkalinity did not vary significantly (data available but not shown here): the range was between 6800 and 8000 mg as $CaCO^3$ l^{-1} for the digester 2. This can be explained by the high intermediate and low partial alkalinity levels during days 3-8, and then the reverse levels (low intermediate and high partial alkalinities) were observed at the

later stages of the run. The overall effect of this was that the total alkalinity did not seem to change much. The IA:PA ratios were, therefore, more informative in terms of stability of the processes within the digesters.

Fig. 26 Experiment 3b Run 3
Digester 2 VFA profile and gas production

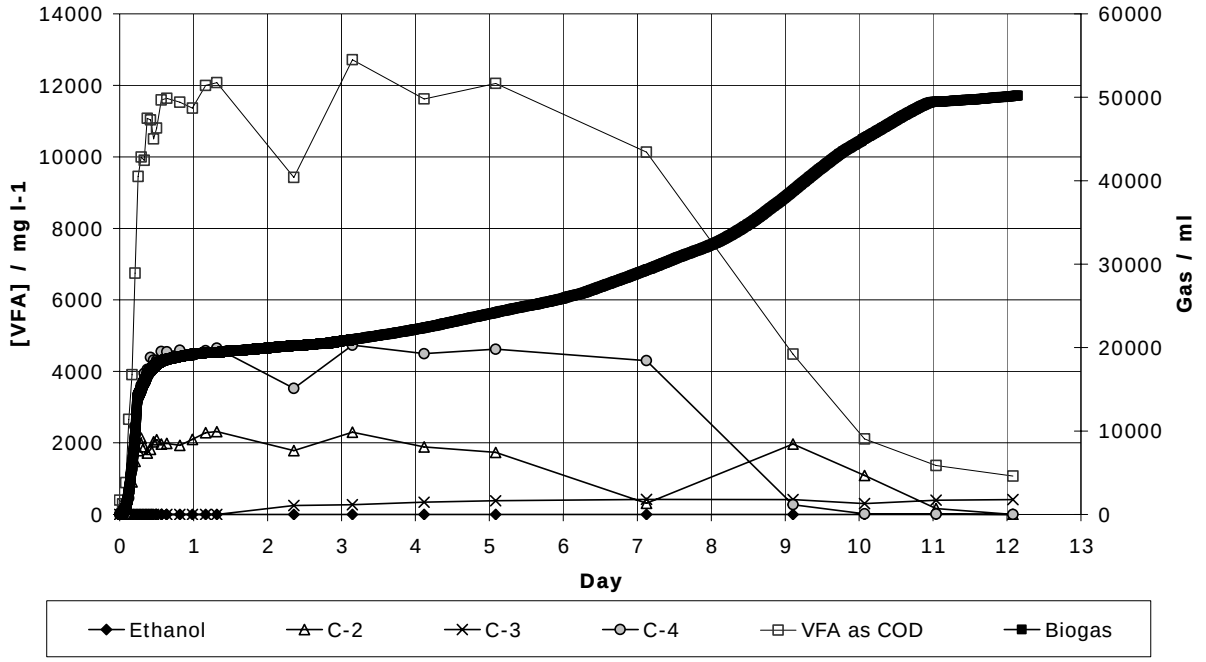
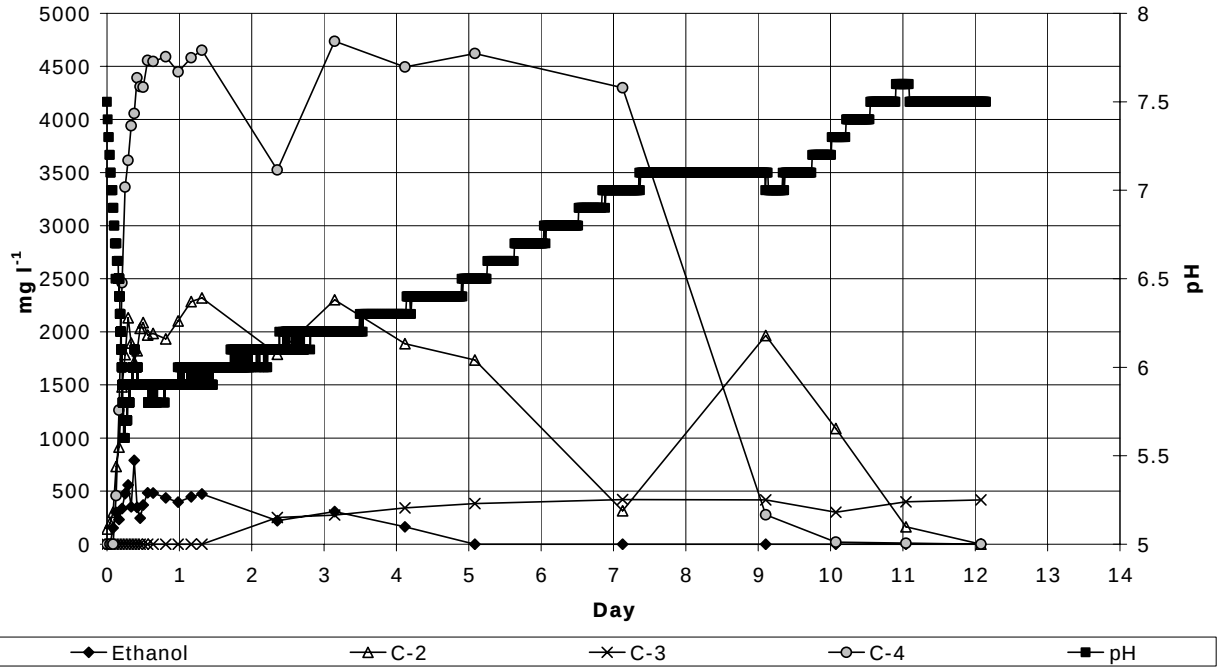


Fig. 27 Experiment 3b Run 3
Digester 2 pH and VFA profile



The data obtained from this experiment also allowed to perform a mass and an energy balances. Elemental analysis of the lactose substrate was performed and this stage as reported in Materials and Methods (Substrate section). The results suggested that the main component of the substrate was dissolved lactose ($C_{12}H_{22}O_{11}$, M_w = 342.3 g/mol, theoretical carbon content 42%, hydrogen content 6.4%). The calorific value of this substrate was previously measured (at the time of the Experiment 2) and was found to be 15.65 kJ/g. This analysis was repeated and the results were confirmed.

The following equations of theoretical reactions were used:

For the energy balance

$C_{12}H_{22}O_{11} + 3H_2O = 5CH_4 + 7CO_2 + 4H_2$
Or 1 g lactose → 0.33 l CH_4 + 0.26 l H2

For mass balance:

$C_{12}H_{22}O_{11} + H_2O = 2CH_3CH_2CH_2COOH + 4CO_2 + 4H_2$
Or 1 g lactose → 0.91 g COD + 0.0234 g H_2

The results are summarised in the Table 3. On average the VFA utilisation was 86-99%. The energy efficiency varied between 58-93%. 72-80% of COD was accounted for, and in most of the runs over 50% of H_2 was also recovered. These results suggested overall good energy efficiency. However, specific energy production was low and varied between 11.5-19.4 kJ l^{-1} day^{-1}. The hydrogen recovery also suggested that the hydrogen production stage could be optimised for higher recovery. But improving the hydrogen stage could not be achieved within the conditions of our experimental set-up. Constant feeding regime and pH control at 5.5 and complete anaerobic conditions are necessary factors for stable hydrogen production.[9] These conditions cannot be achieved if the methanogenic stage is expected from the same digester. The limiting factor for the specific energy production was the lag phase between hydrogen and methane producing stages. A way of reducing this lag-phase would offer a solution for improvement of the overall performance.

Table 3. Experiment 3b Summary of the mass and energy balance for runs 2 and 3

Digester	1	2	3	4	1	2	3	4
Run	2				3			
Total COD added, g	78.4	78.4	78.4	78.4	85.7	85.7	85.7	85.7
Methane output, m^3 per kg COD added	0.31	0.35	0.21	0.27	0.35	0.38	0.22	0.35
Hydrogen output, m^3 per kg COD added	0.15	0.15	0.15	0.15	0.16	0.05	0.11	0.13
Final VFA, as mg COD l^{-1}	101.7	204.1	1393.3	147.9	1095.1	1071.6	1767.8	951.5
Max VFA, as mg COD l^{-1}	10779.5	10848.7	11197.2	11336.9	12809.3	12715.6	12541.8	12230.1
VFA utilisation	99%	98%	88%	99%	91%	92%	86%	92%
Total lactose added, g	85.1	85.1	85.1	85.1	88.4	88.4	88.4	88.4
Energy from CH_4, kJ	958.3	1080.5	650.7	836.0	1171.2	1273.8	733.5	1190.9
Energy from H_2, kJ	157.7	157.7	157.7	155.0	184.7	62.0	130.8	148.3
Total energy, kJ	1116.0	1238.3	808.4	991.1	1355.9	1335.8	864.3	1339.2
Energy, kJ l^{-1} day^{-1}	15.9	17.7	11.5	14.2	19.4	19.1	12.3	19.1
Energy efficiency	79%	88%	58%	71%	93%	91%	59%	92%
Mass balance: COD accounted	70%	70%	72%	73%	80%	79%	78%	76%
Mass balance: H_2 accounted	53%	53%	53%	52%	60%	20%	42%	48%

Runs 2 and 3, therefore, confirmed the results obtained in the Experiment 3a and provided more detailed data on VFA range and the effect of pH.

Runs 4 and 5 were set-up with digester 2 (as variable) and digester 4 (as control) to investigate if the control of pH by addition of NaOH could shorten the lag phase. During the run 4 the solution of NaOH (1 and 2*N*) was added to the digester 2 when pH reached 6.6 to try and keep the pH at that level. However, it was difficult to achieve this as addition of pH provided only short-term stabilisation and the pH kept dropping despite of the addition of further amounts of NaOH until it reached 5.8. It should be noted that the NaOH addition was done manually, which further complicated the procedure. In all published sources, the

pH control is performed automatically using pumps that keep the level in the reactor the same and provide safer means of handling the NaOH at higher concentrations.

Adding NaOH continuously during hydrolysis did not improve digester performance significantly. The control (digester 4) showed, on average, slightly better performance (Table 4). Addition of NaOH to digester 2 during the hydrolysis stage helped to reduce the lag period between hydrolytic and methanogenic stages, but propionic acid was observed at higher levels than in the previous runs, which indicated possible instability (Fig. 28).

Fig. 28 Experiment 3b Run 5
Digester 2 pH and VFA profile

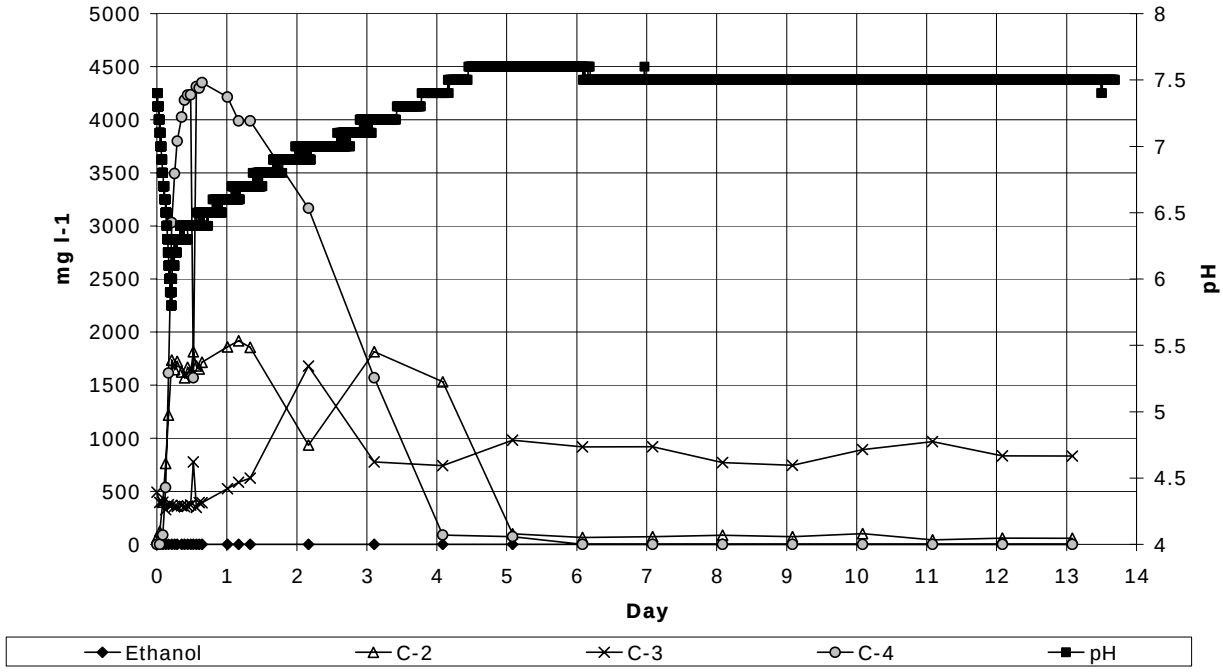
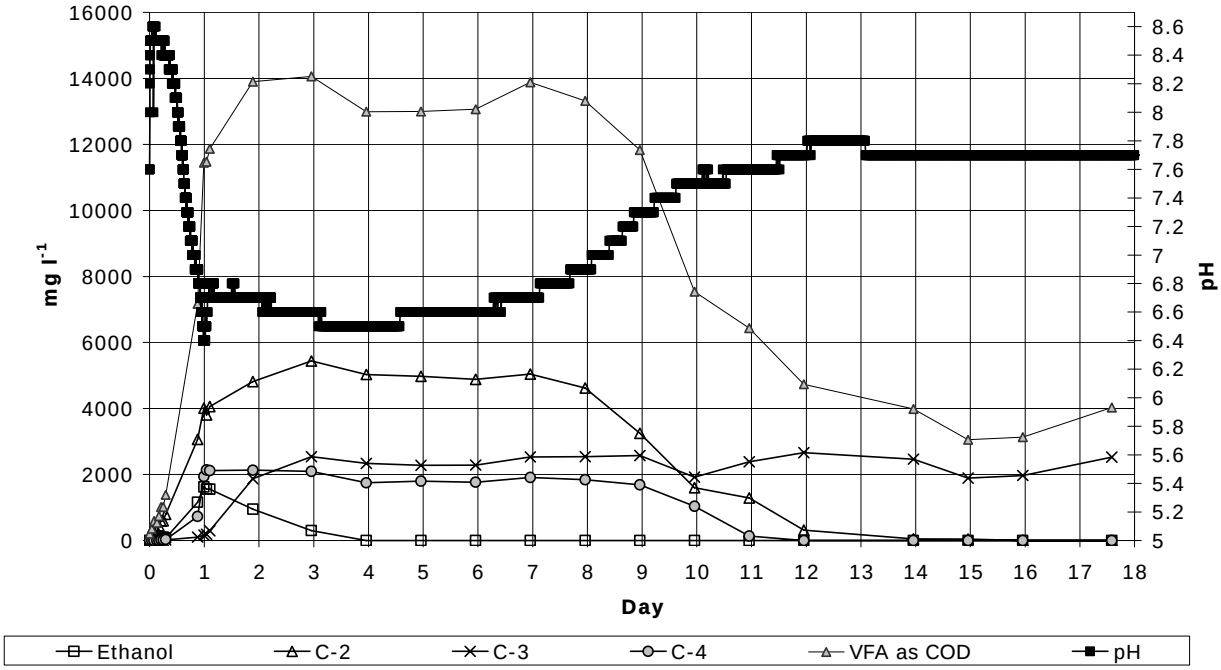


Table 4. Experiment 3b Summary of energy and mass balance for runs 4 and 5

Digester	2	4	2	4
Run	4		5	
Total COD added, g	82.5	82.5	82.9	82.9
Methane output, m^3 per kg COD added	0.37	0.40	0.24	0.25
Hydrogen output, m^3 per kg COD added	0.12	0.16	0.04	0.21
Final VFA, as mg COD l^{-1}	1321.2	1310.9	3986.0	2093.8
Max VFA, as mg COD l^{-1}	11993.5	11967.8	14061.9	14049.2
VFA utilisation	89%	89%	72%	85%
Total lactose added, g	82.0	82.0	83.5	83.5
Energy from H_2, kJ	130.8	173.9	40.4	237.3
Total energy, kJ	1345.4	1483.2	821.3	1041.8
Energy, kJ l^{-1} day^{-1}	19.2	21.2	11.7	14.9
Energy efficiency	99%	109%	60%	76%
Mass balance: COD accounted	80%	80%	93%	92%
Mass balance: H_2 accounted	45%	60%	14%	81%

Run 5 was set up in a similar way, but NaOH was added to the substrate prior feeding the digesters. The amount of NaOH added was estimated from small-scale trials (not described here) to overcome all the possible acidity generated by the hydrolysis step. This time the start of fermentation as well as methanogenic stage were delayed all together until the pH dropped below 8 (Fig. 29). High and stable levels of propionic acid (above 2000 mg/l) indicated unfavourable conditions for both hydrogen and methane producers as these normally produce and utilise acetic and butyric acids, as well as ethanol, in preference to propionic.[17]

Fig. 29 Experiment 3b Run 5
Digester 2 pH and VFA profile



These trials, therefore, did not improve the overall performance of the digester 2. Adding NaOH, either continuously during hydrolysis or as a one-off supplement with the feed, did not improve reactor performance significantly. Adjusting pH at the end of hydrolytic stage could offer a possible improvement and a quicker switch to methanogenic stage. This however could also trigger propionate production unless all of the hydrogen was removed by sparging the reactor with nitrogen.

Conclusions

This work provided detailed information on the processes that take place during the digestion of the lactose substrate, which has high soluble COD and dissolved solids contents. The results from this work show that if this substrate to be utilised in a plug-flow system, the chemistry of the bioconversion should be carefully considered. One important consideration is the hydrolysis step. As this work showed, lactose substrate is very easily hydrolysed. It was shown that the soluble products of the hydrolysis were mainly butyric and acetic acids and ethanol. These products are reported as the most preferable for the methanogenic activity. It was also found that hydrolysis was accompanied by relatively high levels of hydrogen, which is a valuable product on its own. With a mixed culture inoculum, rather than a pure cultures, which did not undergo any heat treatment, and no continuous N_2 sparging, hydrogen production was observed within 3-4 hours after the addition of the substrate, which is a much shorter lag-phase than reported by other sources[9]. Specific H_2 production achieved was 4.4 mol/ mol lactose, or 2.2 mol/ mol hexose, which is higher or comparable to those reported in the literature and suggests butyrate-type fermentation. However, it was also shown that because the substrate is so easily hydrolysed, it results in low pH levels of 5.6-5.8, which inhibit and delay the methanogenic stage. It was shown that the pH control during the hydrolysis stage was not effective. So, if pH control is implemented, it should be considered during the identified lag-phase between hydrogen and methane production to avoid propionic acid accumulation. However, the pH control in a plug-flow system such as the Maltin® System may not be required as the stage separation may occur naturally due to the series of digesters that ensure no cross-contamination between them. This could also offer a possibility of more efficient sustainable hydrogen production in the hydrolysis tank, and higher loadings and overall improvement to the digester energy efficiency. Wash-out of micro-organisms should also be considered if the lactose substrate is to be fed into the system without continuous addition of seed. Feeding with a mixture of the substrate and the seed does not provide an effective way of operating such a system, as the micro-organisms that acclimate to the substrate are constantly being washed out and replaced by an un-acclimated

population. A supporting medium for the micro-organism growth could offer a possible solution. This, however, should be tested on a smaller scale before starting the full-scale system.

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