
Treatment of typical South African milking parlour wastewater by means of anaerobic sequencing batch reactor technology

by

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Declaration

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

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Abstract

Due to the growing demands for fresh and clean water, the investigation into the treatment, reuse and recycling of wastewater from all industries are becoming more of a priority, both globally and in South Africa where as much as 62 % of the total water used per year is used by the agricultural sector. The investigation into the recycling and reuse of wastewater in the agricultural sector (especially the dairy farming industry) is therefore no exception. The water usage in five typical South African milking parlours was estimated in this study and ranged from 15 to 51 L.cow⁻¹.day⁻¹. However, the water used for the cleaning in place (CIP) washing of the milking equipment were rather similar in all five milking parlours and ranged between 4.9 and 6.4 L.cow⁻¹.day⁻¹. The possibility of handling and treating the CIP wastewater separately from the rest of the milking parlour wastewater has been considered in the past by other researchers.

Anaerobic digestion, as a means of treating wastewater from the dairy industry, has been employed successfully in both full scale and laboratory scale projects. The wastewater from equipment washing of milking parlours is assumed to have similar characteristic to that generated from dairy factories. The anaerobic sequencing batch reactor (ASBR) system is gaining popularity as a wastewater treatment technology lately due to its simplicity, ease of operation and compact design and is therefore expected to be a suitable and practical solution for dairy farmers in treating milking parlour wastewater from equipment washing. Investigation into anaerobic treatment at lower temperatures than the conventional mesophilic range is also becoming of interest due to lower energy requirements.

The aim of this study was to determine whether the ASBR technology could be considered as a suitable technology for treating wastewater from the CIP washing of milking parlour equipment. To support this study, the water usage and CIP effluent generated at typical South African milking parlours were firstly verified. Subsequently, laboratory work entailed:

- Assessing the sensitivity of the ASBR process (at mesophilic temperature of 35 °C) to fluctuations in the concentration of the detergents in synthetic CIP milking parlour wastewater; and
- Evaluating the performance of the ASBR process at 22.5 and 35 °C when treating real wastewater from the CIP washing of milking equipment.

Chemical oxygen demand (COD) removal efficiencies between 89 and 98 % were achieved when the synthetic wastewater (similar to wastewater from the CIP washing of milking equipment with COD concentrations ranging between 12 600 and 13 400 mg.L⁻¹) was treated in an ASBR. The results showed that an increase in the CIP detergent concentration up to four times the concentration normally used in milking parlours did not significantly affect the ASBR performance in terms of methane productivity, methane yield and COD removal efficiencies when OLRs between 0.6 and 5.2 g COD.L⁻¹.day⁻¹ were applied.

The results also showed that COD removal efficiencies between 92 and 98 % could be achieved in the ASBR process operated at 35 °C when treating real CIP milking parlour effluent (with COD concentrations ranging between 14 900 and 28 800 mg.L⁻¹) when applying OLRs up to 6.6 g COD.L⁻¹.day⁻¹, without nutrient control. Therefore, the ASBR process is suitable to treat real milking parlour wastewater with OLRs above 6 g COD.L⁻¹.day⁻¹ at mesophilic temperatures.

At an operating temperature of 22.5 °C, the ASBR achieved TCOD removal efficiencies between 86 and 98 % when treating real CIP effluent. Despite these high COD removal efficiencies, the reactor failed at an OLR of 2.9 g COD.L⁻¹.day⁻¹. As such, the ASBR process appears to be susceptible to failure (due to overloading) when the OLR is increased too rapidly at this low operating temperature. This is most probably due to the fact that methanogenic bacteria do not acclimatise and operate as well at temperatures below the mesophilic range. However, during a second attempt at 22.5 °C, the ASBR achieved COD removal efficiencies between 89 and 97 % when the OLR was increased less rapidly, up to 3.3 g COD.L⁻¹.day⁻¹. These results show that the ASBR process can indeed treat real milking parlour wastewater at 22.5 °C without nutrient control at OLRs above 3 g COD.L⁻¹.day⁻¹.

The COD concentration in the effluent from the ASBRs when the maximum OLRs were applied were always below 1 000 mg.L⁻¹. This is notably lower than the South African legal limit for irrigation of up to 50 m³ of wastewater per day. However, this is significantly higher than the South African legal limit of 75 mg.L⁻¹ for safe disposal into a fresh water body.

Opsomming

Die wêreldwye toename in die aanvraag na vars, skoon water het tot gevolg dat die ondersoek in die behandeling, hergebruik en herwinning van afvalwater tans groot aandag geniet. Nie net wêreldwyd nie, maar ook in Suid-Afrika waar tans 62 % van die water wat gebruik word per jaar, aangewend word vir die landbou sektor. Daarom is die ondersoek na besparing van water in landbou aktiwiteite (veral melkboerderye) geen uitsondering nie. Die watergebruik tydens melktyd in 5 verskillende melkerye is ondersoek en dit blyk dat die watergebruik in die 5 melkerye drasties van mekaar verskil. Dit strek van 'n minimum van 15 litres per koei per dag tot 'n maksimum van 51 liters per koei per dag. Die volume water wat gebruik word vir die outomatiese was van die melktoerusting het nie so baie gevarieer nie en het gestrek tussen 4.9 en 6.4 liter per koei per dag. Die moontlikheid om die afvalwater wat gegenereer word tydens die outomatiese was van die melktoerusting apart te hou van die res van die afvalwater, is in die verlede deur ander navorsers oorweeg.

Afvalwater van suiwelfabriekes is in die verlede al deur middel van anaerobiese vertering in 'n groot aantal laboratorium- en volskaalse anaerobiese aanlegte behandel. Daar word aangeneem dat die afvalwater wat gegenereer word tydens die was van melktoerusting min of meer dieselfde samestelling sal hê as die afvalwater van suiwelfabriekes. Die anaerobiese opvolgende lot reaktor (AOLR) word al hoe meer gewild in anaerobiese waterbehandeling as gevolg van die eenvoudige en maklike werking en kompakte ontwerp. Dit word verwag dat hierdie tegnologie 'n gepaste en praktiese oplossing sal wees om die afvalwater van die was van melktoerusting te behandel. Die anaerobiese behandeling van afvalwater by temperature laer as die normale mesofiliese temperatuur word ook al hoe meer gewild as gevolg van minder hitte wat benodig word.

Die doel van hierdie studie was om te bepaal of die AOLR tegnologie 'n gepaste tegnologie is om afvalwater wat gegenereer word tydens die outomatiese wasproses van melkerytoerusting

te behandel. Ter ondersteuning van die doel, is die watergebruik in 'n paar tipiese, Suid-Afrikaanse melkerye eers bevestig. Daaropvolgend, het die laboratoriumwerk die volgende behels:

- The bepaal of die AOLR proses (wat by mesofiliese temperatuur van 35 °C bedryf was) sensitief is vir veranderinge in die konsentrasie van sepe in sintetiese waswater wat na 'n AOLR gevoer word; en
- Om die werking van die AOLR proses te ondersoek wanneer regte afvalwater van melkery by onderskeidelik 22.5 en 35 °C behandel word.

Chemiese suurstof behoefte (CSB) verwydering van 89 to 98 % is bereik toe sintetiese afvalwater wat gelykstaande aan afvalwater gegeneer tydens die was van melk toerusting is (met CSB konsentrasies tussen 12 600 en 13 400 mg.L⁻¹) in 'n AOLR behandel is. Die resultate het getoon dat daar geen aanmerklike verskil in die werking van die AOLR in terme van metaanproduksie, metaanopbrengs en CSB verwyderingseffektiwiteit was met a toename tot en met so hoog as vier maal die normale seepkonsentrasie in die afvalwater was toe organiese ladingstempo's (OLTs) tussen 0.6 en 5.2 g CSB.L⁻¹.dag⁻¹ aangewend was nie.

Die resultate het ook getoon dat die CSB van regte afvalwater van melkerye (met CSB konsentrasies tussen 14 900 en 28 800 mg.L⁻¹) met 92 tot 98 % verminder kan word wanneer dit in 'n AOLR (wat by 35 °C bestuur word) en OLTs tot so hoog as 6.6 g CSB.L⁻¹.dag⁻¹ aangewend word, sonder dat die nutriëntinhoud in die afvalwater beheer was. Hierdie AOLR proses wat is dus gepas om afvalwater van melkery te behandel met OLTs bo 6 CSB.L⁻¹.dag⁻¹ by mesofiliese temperature.

Die AOLR wat by 'n temperatuur van 22.5 °C bedryf was, het CSB verwydering tussen 86 en 98 % behaal. Ondanks die hoë CSB verwydering het die reaktor misluk by 'n maksimum OLT van 2.9 g CSB.L⁻¹.dag⁻¹. Dit het getoon dat die AOLR proses meer geneig is om vatbaar te wees vir mislukking (as gevolg van 'n oerlading) wanneer die OLT te vinnig verhoog word by laer temperature. Dit is moontlik as gevolg daarvan dat die metanogeniese bakterieë nie so goed aanpas en werk by temperature laer as mesofiliese temperature nie. Nietemin, tydens 'n

tweede probeerslag by 22.5 °C, het die AOLR CSB verwydering tussen 89 en 97 % behaal tydens 'n stadiger toename in die OLT tot en met 3.3 g CSB.L⁻¹.dag⁻¹. Hierdie resultate dui aan dat die AOLR proses wat by 'n temperatuur van 22.5 °C bedryf word ook gepas is om afvalwater van melkerye te behandel, sonder nutrient beheer by OLTs hoër as 3 g CSB.L⁻¹.dag⁻¹.

Die CSB konsentrasies in die afvloeiende van die AOLR'e in die studie tydens die aanwending van die hoogste OLTs, was altyd laer as 1 000 mg.L⁻¹. Dit is merkbaar laer as die limiet vir besproeiing van tot en met 50 m³ per dag in Suid-Afrika. Maar, dit was nogtans regdeur hoër as die limiet van 75 mg.L⁻¹ vir veilige storting in 'n varswaterbron.

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Abbreviations and Symbols

Abbreviations

Symbol	Description
AEPA	American environmental protection agency
ASBR	Anaerobic sequencing batch reactor
CARA	Conservation of agricultural resources act
CH ₄	Methane
CIP	Cleaning in place
CO ₂	Carbon dioxide
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactors
DWAF	Department of water affairs and forestry
F:C	Feed-to-cycle length ratio
F:M	Food-to-microorganisms ratio
F:R	Feed-to-react time ratio
FOG	Fats, oils and grease
H ₂	Hydrogen
HRT	Hydraulic retention time
IA	Intermediate alkalinity
IA/PA	Intermediate to partial alkalinity ratio
L/D	Length-to-diameter ratio
LHV	Lower heating value
MPO	Milk producers organisation
NEMA	National environmental management act
NWA	National water act

OLR	Organic loading rate
P	Phosphorus
PA	Partial alkalinity
PFD	Process flow diagram
P&ID	Process instrumentation diagram
N	Nitrogen
R	Removal rate
SCOD	Soluble COD
TA	Total alkalinity
TCOD	Total COD
TDS	Total dissolved solids
TKN	Total Kjeldahl nitrogen
TS	Total solids
TSS	Total suspended solids
UAF	Upflow anaerobic filter reactors
UASB	Upflow anaerobic sludge blanket reactor
USA	United states of America
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
WRC	Water research commission of South Africa
WWF	World wide fund for nature
WC/WDM	Water conservation and water demand management

Symbols

COD_{inf}	COD concentration of the influent wastewater
COD_{eff}	COD concentration of the effluent water
D	Reactor diameter
ε	Removal efficiency

- Abbreviations and Symbols -

E	Efficiency factor
L	Reactor height (length)
$m_{methane}$	Mass of methane
n	Moles
$n_{withdrawals}$	Number of withdrawals
P	Pressure
Q_F	Daily flow rate of wastewater into reactor
Q_{CH_4}	Daily volume methane produced
R	Universal gas constant
T	Temperature
$V_{methane}$	Volume of methane
$V_{reactor}$	Reactor volume
W	Energy
x_{CH_4}	Fraction of methane in biogas
x_{CO_2}	Fraction of carbon dioxide in biogas
Y_{CH_4}	Methane yield

Glossary

Acidogenic bacteria	Bacteria that converts sugars, amino acids and long chain fatty acids to alcohols, ketones and VFAs
Alkalinity	The capacity of the water to neutralise an acid; Expressed in terms of mg/L CaCO ₃ and is measured by means of a titration method; Carbonate, bicarbonate and hydroxides are the major contributors
Anaerobic digestion	Biological process in which complex organic material is converted to hydrogen, methane, carbon dioxide, water, ammonia, hydrogen sulphide and new cells by a close knit bacterial community under anaerobic conditions
Biogas	The gas produced when organic matter is digested by microorganisms under anaerobic conditions
Chemical oxygen demand	The amount of oxygen needed to entirely oxidise organic substances in wastewater
Dairy	Place where milk is processed to dairy products
Effluent	Water leaving the wastewater handling system
Electrical conductivity	Measurement of the total amount of dissolved solids (TDS) or dissolved ions in water
Faecal coliforms	Indicator of faecal contamination in water
Land Application	Discharging wastewater or residual components onto the ground in order to treat or the reuse by means of irrigation or soil amendment
Fats, oils and grease	Measurement of fats in wastewater consist of oils, fats, waxes and other similar compounds

Metallic constituent in wastewater	Metals such as zinc (Zn), lead (Pb), manganese (Mn), copper (Cu), cadmium (Cd) mercury (Hg), nickel (Ni), chromium (Cr) and iron (Fe) are usually present in most waters in trace amounts
Methanogenic bacteria	Anaerobic bacteria that produces methane from CO ₂ or acetate
Milking Parlour	The building on a dairy farm where milk is obtained from cows
pH	Way of expressing the hydrogen concentration in wastewater
Sodium adsorption ratio	Measurement to determine whether the water is suitable to be used for agricultural irrigation
Total Kjeldahl nitrogen	The sum of organic nitrogen, ammonia and ammonium in wastewater
Total dissolved solids	The amount of solids which are still in the filtrate after the sample has been filtered and dried overnight at 105 °C with a filter of pore size of 2µm or less
Total suspended solids	The amount of solids which stays behind in the filtrate after the sample has been filtered and dried overnight at 105°C with a filter with a nominal pore size of 1.58µm
Volatile Solids	Organic matter which will be burned off and volatized once it is ignited at 500 ± 50°C

Chapter 1 - Introduction

We should change our mindset about wastewater. We should stop seeing it as a waste burden but rather as a resource. A lot can be done with wastewater. (Brendon Meulen - Project Manager from a large Dutch wastewater management company)

1.1 Water situation

Water is by far one of the most important natural resources as it is essential to all life on the earth, sanitation and hygiene, agricultural activities, production of most food products and other consumables, power generation and many more. Due to the current growth in the global population, the need for enough clean water is becoming a huge problem both globally and in South Africa. Together with the problem of the growing demand for enough water, the demand for quality of water is also increasing due to pollution caused by industries, mining activities, agricultural activities, energy consumption, urbanisation, deforestation, damming of rivers, destruction of wetlands and accidental water pollution. The following statement was made in a news report on the current water situation in South Africa by the Inter Press Service News agency on the 8th of June 2009 (1):

“South Africa faces chronic water shortages, yet billions of litres are flushed away every year. Being one of the driest countries in the world, the conservation of water resources and managing wastewater should be a top priority for government.”

The report also stated that South Africa is facing great challenges as 98% of its water resources have already been fully utilised (1). To aid to this, the annual rainfall in South Africa is only approximately 500 millimetres, which is almost half of the global annual rainfall of 860 millimetres and the country is therefore said to be a semi-arid country in which water is a scarce resource compared to other non-arid countries (2). According to the World Wide Fund

for Nature (WWF), it was estimated that by 2025, the water deficit in South Africa will be as high as 1.7% if the water resources are not better managed and protected (1).

1.2 Motivation of study

According to water experts from South Africa and Europe who attended a water seminar in Cape Town during May 2009, an approach to reserve and to take care of the country's water resources is to focus on recycling wastewater generated from various industries (1). The investigation into the treatment, reuse and recycling of wastewaters from all industries are therefore of a priority and related investigations in the agricultural industry is therefore no exception. According to the Department of Water Affairs and Forestry (DWAF), the agricultural sector utilises 62% of the total water used in the country annually. A successful water conservation and water demand management (WC/WDM) plan for this sector will make a significant difference in the water demand both in and outside the agricultural sector (3). South Africa is a country rich in a wide variety of climates, soil types and natural vegetation which is ideal for the application of a diversity of farming sectors such as crop production, fruit production, mixed farming, cattle ranching and sheep farming. Dairy farming forms a huge part of the agricultural sector and is practiced in all nine provinces. According to the latest data from the Milk Producers Organisation (MPO), there are approximately 3 551 registered dairy farmers in South Africa with an average of 151 cows per producer (4).

1.2.1 Wastewater from milking parlours

Water usage in a milking parlour is mostly for cleaning purposes in and around the milking parlour during and after milking. The wastewater generated in milking parlours consist of two main wastewater streams, including the wastewater from cleaning and sanitation of milking equipment which consist mainly of water, milk and detergents (this stream is generated from a "cleaning-in-place" (CIP) automatic washing process) and floor washing which consists mainly of water, animal waste such as manure and urine, waste milk and dirt. When these wastewater streams are discharged directly to surrounding water sources such as dams and rivers, the manure and the organic solids from the milk will be broken down by aerobic biological bacteria, which use the oxygen in the water. This will typically cause an oxygen demand in the water

which may lead to the death of fish and other water animals as well as a reduction of plant diversity in the surrounding areas (5). In addition, the phosphorus in the milk, detergents and manure may advance the growth of water plants and algae. Excessive growth of these water plants may lead to the water source being choked (5). Chlorides and ammonia-nitrogen from detergents and manure are poisonous to fish and other water organisms and will also be detrimental to the water source (5). The quality of the surrounding groundwater is also in great danger when the wastewater is discharged directly to the environment as life-threatening illnesses may arise such as “blue baby syndrome” (better known as methemoglobinemia). This is a condition which develops when infants less than six months ingest nitrate which decrease the capability of the blood to transport oxygen, resulting in a lack of oxygen which causes a blue-lavender skin colour (5).

The volume of wastewater generated from milking parlours varies for each milking parlour and depends on the type and size of milking parlour, the amount of cows milked, the milking events per day as well as the location in which the milking parlour is situated. Studies have shown that the daily wastewater generation in milking parlours per cow can range anything between 20 and 70 litres per cow per day (6). According to the latest data, the average number of cows per milk producer in South Africa during 2006 was 151. In 2006 there were 4 184 registered milk producers in South Africa (4). This implies that, on average, the total volume of wastewater generated in South African milking parlours will range from 12 to 44 million litres of wastewater per day.

Currently, a number of handling and disposal systems for wastewater generated in the milking parlour are being used with a certain degree of success globally. This includes liquid manure systems with land application, holding tanks (or ponds) with land application, aerobic lagoons with land application, intensive land application, subsurface disposal and municipal sewage system disposal (7). These are all systems in which the two main wastewater streams from the milking parlour are combined with the manure handling system. In most cases, after wastewater handling, the water is disposed into surrounding water sources, used for irrigational purposes on the farm or reused in the milking parlour to flush the floor of the area

where the cows gathering before entering the milking parlour. In South Africa, this is however, not common practice yet and the majority of dairy farms do not have proper waste handling system in place.

South Africa does have regulations and legislations in place to ensure that the wastewater generated from all industries are managed in the correct and accepted manner. These legislations also holds true for the agricultural sector. The applicable legislations are the National Water Act (NWA) (Act 36 of 1998), the National Environmental Management Act (NEMA) (Act 107 of 1998) and the Conservation of Agricultural Resources Act (CARA) (Act 43 of 1983).

The NWA is written and incorporated to ensure that the water in South Africa are conserved, developed, used, protected and controlled in such a way that sustainability of the environment are of priority and to, amongst many others, reduce pollution and degradation of water resources. It states that it is the responsibility of the landowner or person responsible for the piece of land to prevent any pollution of a water resource by taking reasonable measures continuously. This includes measures to comply with any prescribed waste standard and management practice (8). It also includes regulations concerning the discharging of water containing waste into a water resource through a pipe, canal, sewer, sea outfall or other conduit as well as the disposing of waste in such a manner that it may be detrimental to the water resource (8). Section 39 provides limits to the quality of wastewater which may be used for irrigation purposes. These limits are shown in Table 1-1.

NEMA focuses on the prevention of pollution and ecological degradation, promotion of conservation, sustainable development of the ecology and the use of natural resources while still promoting justifiable economic and social development (9). CARA focuses on the control of the utilisation of natural agricultural resources to conserve the soil, water sources and vegetation. The regulation of the flow of runoff water, erosion, irrigation and mineralisation is also dealt with in this act (10). When considering all these regulations, it is clear that correct and sustainable management of the country's water resources and correct handling of wastewater is an important aspect. Effective wastewater treatment methods or waste

management systems should therefore be employed when considering the amount and the quality of the wastewater generated from milking parlours (be it for the recycling, reuse or safe discharging of the wastewater).

Table 1-1 Limits of irrigation of wastewater (adapted from ref. (8))

Parameter	Units	Irrigate up to biodegradable wastewater per day		
		50 m ³	500 m ³	2 000 m ³
Electrical conductivity	mS/m	< 200	< 200	70 – 150
pH		6 < pH < 9	6 < pH < 9	5.5 < pH < 9.5
COD	mg.L ⁻¹	< 5000	< 400	< 75
Faecal coliforms	100 mL ⁻¹	< 100 000	< 100 000	< 1 000
Sodium adsorption ratio		< 5	< 5	

1.2.2 Wastewater treatment methods

The two main wastewater streams mentioned earlier differ quite significantly in its composition. The wastewater from the equipment washing does not contain cow wastes such as manure and urine and it is proposed that, if treated effectively, the water can be reused in the milking parlour, which aid to the conservation of the water resources. The possibility of treating the wastewater from equipment washing from a few milking parlours in the USA by means of aerobic wastewater treatment methods have been investigated recently (11). Although no work has been done previously in South Africa on wastewater treatment from milking parlours, a few studies have been done for the Water Research Commission of South Africa (WRC) on the treatment of dairy factory effluents which showed that anaerobic wastewater treatment is a suitable wastewater treatment technology for the treatment of dairy factory effluent, which are assumed to have rather similar characteristics than the effluent stream from equipment washing of milking parlours (12).

When considering wastewater generated in the dairy industry, biological treatment methods are mostly preferred above physical and chemical treatment methods, due to the high reagent costs and poor soluble COD removal efficiencies associated with physical-chemical treatment

methods (13). Another reason why biological treatment methods are preferred is due the low health and environmental hazard associated with the process when compared to chemical systems, particularly chemical treatment systems in which chlorine is used. Chlorate and chlorite (products which forms from the chlorination of water) have caused haemolytic anemia in laboratory animals in the past and are considered for regulation by the AEPA (American Environmental Protection Agency) (12). Biological treatment of wastewater can either be done under aerobic or anaerobic conditions or a combination of the two. Table 1-2 shows a summary of the comparison of aerobic and anaerobic wastewater treatments.

Anaerobic treatment methods are commonly preferred over aerobic treatment methods due to the high energy requirements and high sludge production which are associated with aerobic treatment methods. Another advantage that anaerobic processed have over aerobic processes is the formation of biogas during anaerobic digestion which can be used as an alternative energy source. Various types of anaerobic reactor designs have been used in the treatment of dairy factory effluents including anaerobic filter reactors, upflow anaerobic filter reactors (UAF), upflow anaerobic sludge blanket reactors (UASB), anaerobic sequencing batch reactors (ASBR), rotating biological contact reactors, upflow packed-bed reactor and anaerobic continuous stirred tank reactors (CSTR) (13).

When anaerobic wastewater treatment technologies for agricultural wastewater are considered, it is important to use equipment which is simple to use, but which also have good value. If problems arise, the system must be simple enough that the farmer can render a hand himself. The ASBR process is said to be such a simple system which would be suitable for the treatment of agricultural wastewater (16). This process is a recently developed process and was patented in 1993 by Richard Dague and his fellow workers at Iowa State University and has gained a lot of interest due to its simple design, ease of operation, improved retention of solids, simple process control requirements, increased COD removal efficiency and high biogas production (17). It is a batch system which is a modification of the conventional anaerobic contact process. All the steps in a conventional anaerobic process happen in one single reactor which operates on a fill and draw principle consisting of four distinct steps namely feed, react,

Table 1-2 Comparison of aerobic and anaerobic wastewater treatment methods (13; 14; 15)

Parameter	Aerobic Treatment	Anaerobic Treatment
Start-up period	<ul style="list-style-type: none"> Relatively Short start-up periods 	<ul style="list-style-type: none"> Longer start-up periods
Energy requirements	<ul style="list-style-type: none"> High energy requirements due to aeration 	<ul style="list-style-type: none"> Lower energy requirements
Sludge production	<ul style="list-style-type: none"> High excess sludge production 	<ul style="list-style-type: none"> Low excess sludge production
Biological nutrient removal	<ul style="list-style-type: none"> Removal of nitrogen and phosphorus possible 	<ul style="list-style-type: none"> No significant removal of biological nitrogen and phosphorus
Required reactor volumes	<ul style="list-style-type: none"> Larger reactor volumes required as lower organic loading rates can be processed (0.5–3.2 g COD.L⁻¹.day⁻¹) 	<ul style="list-style-type: none"> Smaller reactors required as higher organic loading rates can be processed (3.2-32 g COD.L⁻¹.day⁻¹)
Need for additional alkalinity	<ul style="list-style-type: none"> Not of such great importance 	<ul style="list-style-type: none"> Total Alkalinity requirements between 2000-3000 mg.L⁻¹ CaCO₃ required to maintain pH level
Further treatment	<ul style="list-style-type: none"> Post-treatment not needed 	<ul style="list-style-type: none"> Post-treatment required to remove residual organic material
Carbon Balance	<ul style="list-style-type: none"> 40-50% of carbon converted to biomass, while 50-60% of carbon converted to CO₂ 	<ul style="list-style-type: none"> 5% of carbon converted to biomass while 95% of carbon converted to biogas

settle and decant. The ASBR process have previously been used in the lab scale treatment of various dairy effluents such as dairy manure (18), non-fat dry milk (19; 20; 21; 22) and a combination of landfill leachate and dairy factory wastewater (23), but has not been used in the treatment of wastewater generated from the CIP washing of the milking equipment. Due to its simple operation this anaerobic treatment method is thought to be an effective, inexpensive method of treating wastewater generated from the CIP washing of milking parlour equipment. Several studies have shown that the ASBR process is suitable to treat wastewater at lower temperatures than at the conventional mesophilic temperature range of 30 to 37 °C (20; 24; 25). This makes the ASBR treatment technology even more attractive for application in the treatment of milking parlour wastewater on dairy farms as efficient treatment at lower temperatures will decrease the operating costs.

1.3 Research objectives and hypotheses

1.3.1 Objectives

The main objective of this study was to investigate the possibility of treating the wastewater generated from equipment washing in the milking parlour by means of an ASBR. This was done by:

- Evaluating the water usage and wastewater handling systems in a few milking parlours in South Africa in order to verify typical effluent volumes and COD concentrations;
- Designing and constructing laboratory-scale ASBR reactors to treat wastewater generated from equipment washing in a milking parlour;
- Assessing the sensitivity of the ASBR process to varying CIP chemical concentrations by treating synthetic wastewater similar to milking parlour CIP effluent at normal mesophilic temperature (35 °C);
- Evaluating the performance of the ASBR process treating real wastewater from milking parlour equipment washing at normal mesophilic temperature (35 °C) and at a lower temperature of 22.5 °C;

The achievement of these objectives was instrumental in proving or disproving the hypotheses of the study. These hypotheses have been developed after a thorough literature survey and are therefore specifically stated at the end of Chapter 3. However, it is also provided below as guidance to the reader.

1.3.2 Hypotheses

- COD removal efficiencies greater than 95 % can be achieved when treating wastewater generated from the CIP washing of milking parlour equipment in a laboratory-scale ASBR operated at 35 °C when OLRs larger than 5 g COD.L⁻¹.day⁻¹ are applied.
- COD removal efficiencies greater than 95% can be achieved at lower temperatures (between 20 and 25 °C) when OLRs up to 3 g COD.L⁻¹.day⁻¹ are applied.
- Approved CIP chemicals used at milking parlours do not affect the performance of the ASBR process significantly.

1.4 Thesis structure

Chapter 2 of this thesis focuses on the background of the dairy farming industry in South Africa, basic principles on activities in the milking parlour, the water usage in milking parlours, wastewater characteristics of milking parlour wastewater according to literature as well as the volume of water used in various milking parlours visited in South Africa. **Chapter 3** is the main literature chapter focussing on anaerobic wastewater treatment and the ASBR process. It starts by giving background on the anaerobic digestion process, including the principles of anaerobic digestion (microbiology, biochemistry and environmental factors and inhibition). Some advantages and disadvantages of anaerobic wastewater treatment technologies are then discussed. An overview of the different types of anaerobic wastewater treatment technologies employed in the treatment of dairy wastewater are also given. The ASBR process is discussed in detail, including the history of the process, the principles on which it operates, factors that influence the performance of the system as well as previous studies on this specific process. **Chapter 4** explains the overall experimental approach followed in this study including the apparatus used, experimental procedure and analytical methods. The results obtained from the experiments conducted are presented and discussed in **Chapter 5**. In **Chapter 6**, the conclusions

and recommendation are discussed as well as areas where further research is needed. The references used are then listed, followed by the appendices which include a recommended sizing for a typical ASBR process for a typical South African milking parlour (see Appendix E).

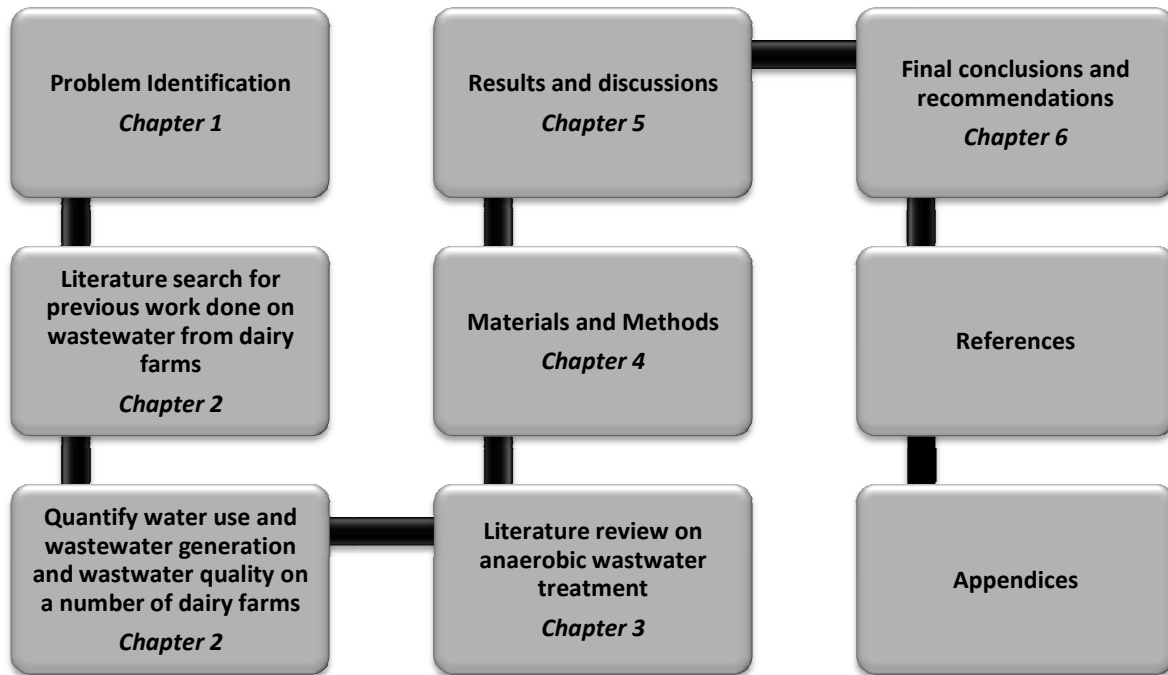


Figure 1-1 Schematic diagram of thesis content

Chapter 2 - Water usage in milking parlours: A review and case studies

2.1 Background on dairy farming

Up until the late 1800's, cows were milked by hand one after the other every morning and every evening. This was very hard, time consuming work as one person was not able to milk more than 12 cows per day. The first attempts of developing a milking device that works with pressure was started during the 1870, but this development seized as the milking was not faster than when milked by hand and the system was very complex. The Royal Agricultural Society of England presented a £50 reward for the development of a successful milking machine. William Murchland from Scotland was the first to develop such as device which he patented in 1889 (26). This device worked by continuously sucking milk from the teats (by means of vacuum) but was however, not very successful as it caused the teats of the cows to swell and the process was too labour intensive. The success of machine milking became more prominent during the time of World War I, but the economical depression during the 1920s caused the downfall of many milking machine companies (26).

The Surge Bucket Milker was developed during 1922, which was an invention that created a natural surging action as the milker moved forward and backwards, creating a tug-and-pull movement which is the same as the tug-and-pull movement of a calf when sucking milk from its mother (27). Following this, the milk pipeline was invented which reduced the size of the milking device significantly as well as the physical labour as the farmer did no longer have to carry the milk buckets to and from each cow. The milk was simply transported from the cow to

the milk storage tank. The milking parlour was then invented which enabled the milking process to become a fully automated process, leading to the expansion of the dairy farming industry worldwide. Since then, various types of milking parlour systems have been successfully implemented and used worldwide.

2.1.1 Types of milking parlours

Five main types of milking parlour configurations are most commonly used on dairy farms, including tandem, herringbone, rotating, side-by-side and swing-over milking parlours (28). The choice of milking parlour depends on a number of factors such as number of cows being milked, milking times per day, number of labourers' available, flow pattern and personal preferences of the farmer (29).

a) Tandem milking parlours

Tandem milking parlours usually consist of a pit for the milkers with stalls situated around the pit in which the cows stand head to tail. If it is not possible to have a pit in the parlour, the stalls in which the cows stand is elevated appropriately (see Figure 2-1). The tandem configuration can either be a walk-through type or a side-gate type. In side-gate type, a side passage is present in which the cows enter and exit their stalls, while in the walk-through type, the cows simply enter the stalls at one end and walk through to the other end after being milked. An advantage of this type of milking parlour is that one whole side of the cow is visible for inspection while it is being milked (30). Another advantage of this type of milking parlour is that it handles each cow separately and a slow-milking cow will therefore not delay the release from the dairy of other faster milking cows (28).

b) Herringbone milking parlours

Herringbone milking parlours are built in such a way that the cows stand at an appropriate angle to the pit in order for the labourer to easily access the udder (see Figure 2-2). This type of configuration decreases the space between the udders of the cows, and more cows can then be milked simultaneously. It also saves time for the labourers to walk from one cow to the other. A disadvantage is that all the spaces have to be filled and emptied at the same time. The size of

this configuration can vary between 4 and 20 points at each side of the milking pit and is mostly suitable for smaller scale parlours (29).

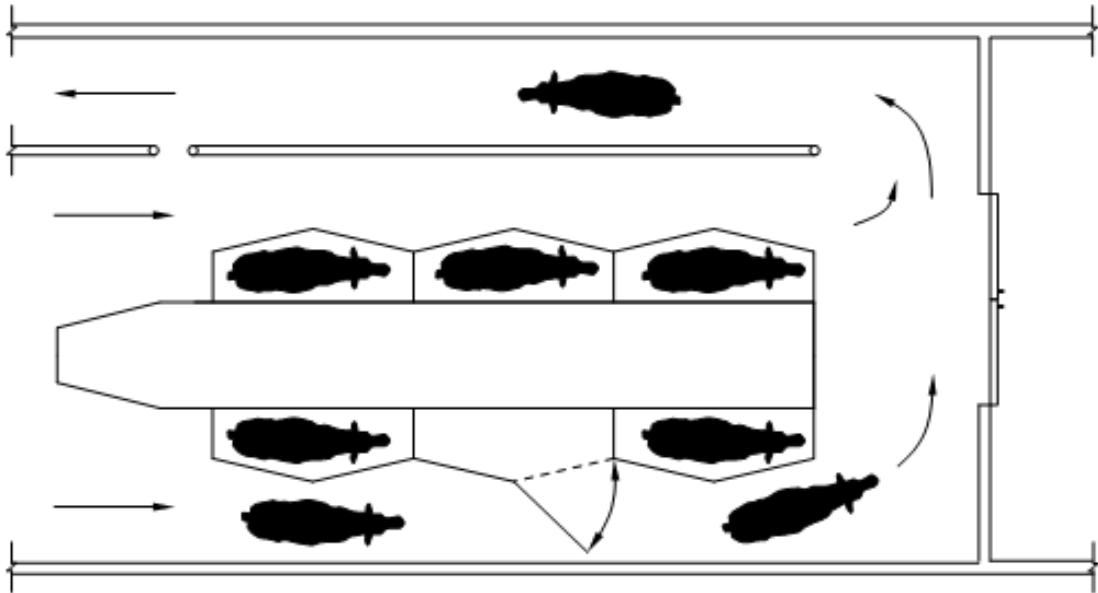


Figure 2-1 Tandem milking parlour (adapted from ref. (28))

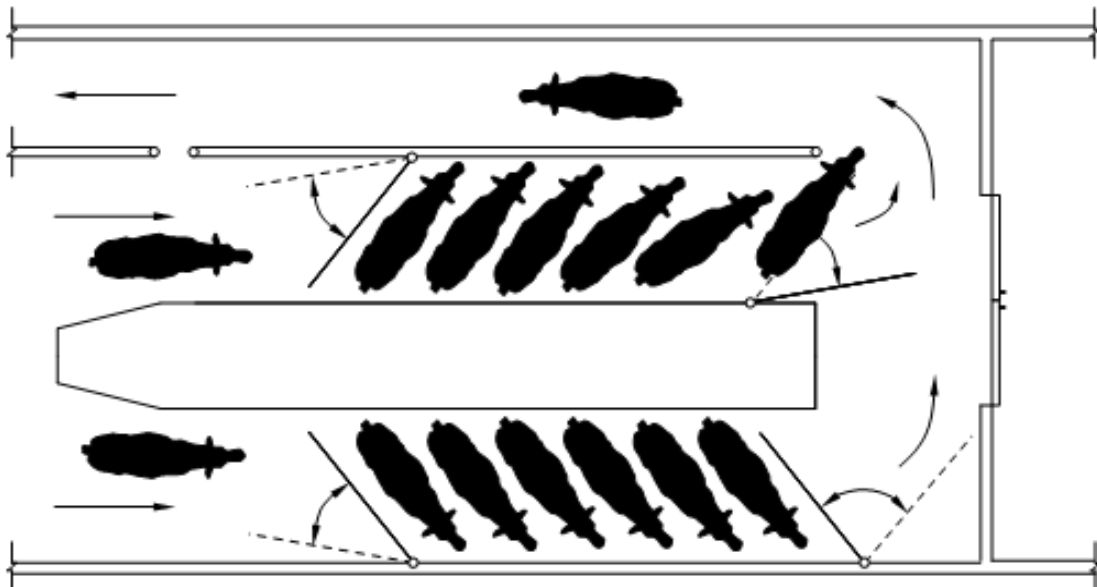


Figure 2-2 Herringbone milking parlour (adapted from ref. (28))

c) Side-by-side milking parlours

Side-by-side milking parlours are built in such a way that the cows stand at an angle of 90 degrees on a platform which is higher than the operating area, facing away from the operating area (see Figure 2-3). The distance that the cows need to walk is shorter than in the case of the herringbone milking parlour, but the visibility of the udder is not that good and the attachment of the milking unit and udder sanitation is more difficult. Each cow is positioned by means of a small gate. Rapid exit stalls and dual return lanes are usually used in this type of milking parlour to aid to the flow of the process (28).

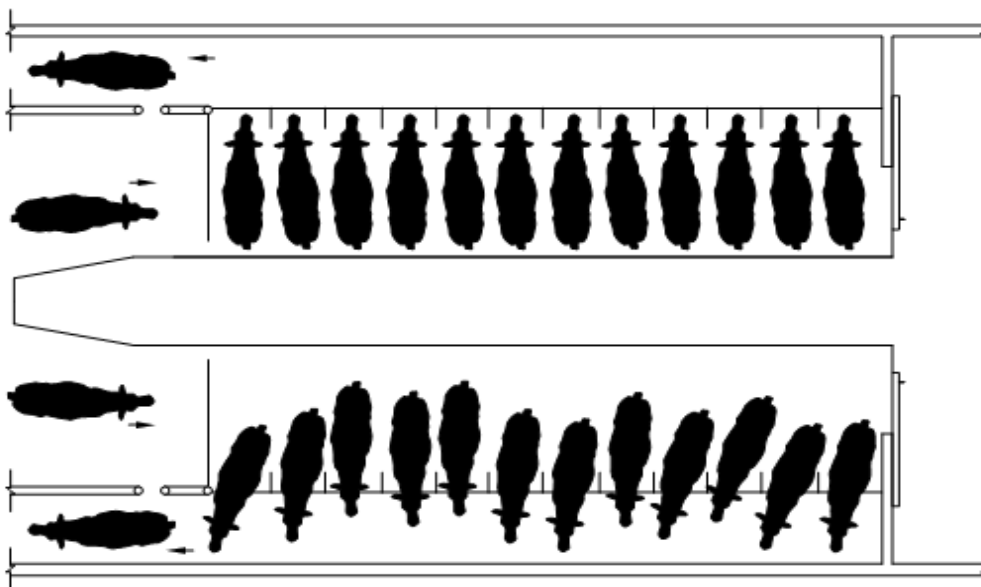


Figure 2-3 Side-by-side milking parlour (adapted from ref. (28))

d) Swing-over milking parlours

In a swing-over milking parlour, cows are positioned at a greater angle to the operation area than in the herringbone parlour and a lesser angle than in the side-by-side parlour (approximately 70 degrees), which eliminates the use of positioners as is the case in side-by-side parlour. The milklane is usually positioned above the operator's head and the cows can either exit at the side or at the front of the parlour. In order to aid to the positioning of the cows to be milked after each other to stand behind each other and to help with flow of the

cows in the milking parlour, the cow platform is usually extended 2-3 cows beyond the operating pit (see Figure 2-4). In order to prevent too many cows from entering the parlour during a loading, a chop gate or pendulum are installed at the entrance of the dairy (28).

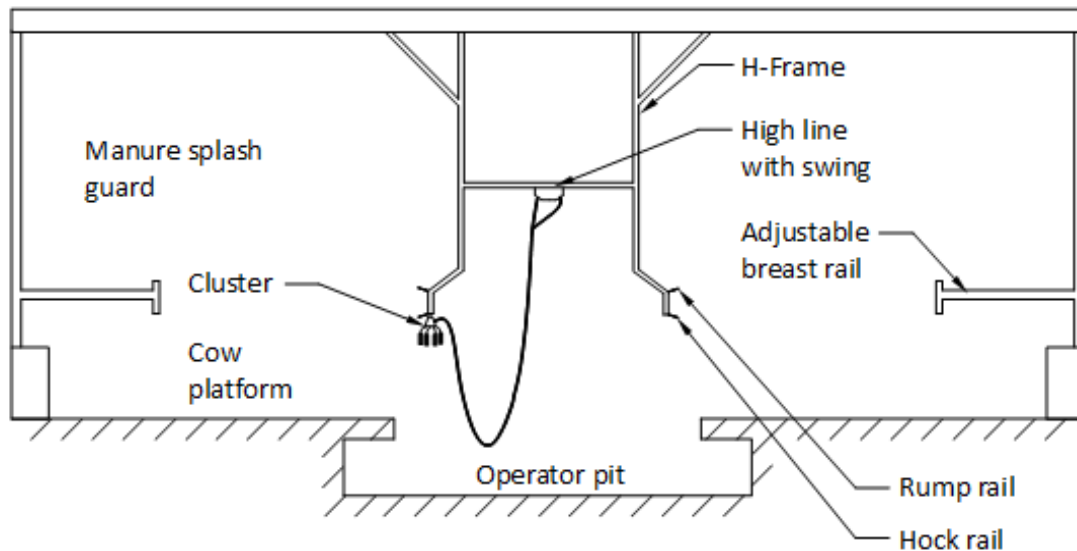


Figure 2-4 *Swing-over milking parlour adapted from ref.(28))*

e) Rotating milking parlours

In a rotating milking parlour, cows are milked on a raised, circular shaped, rotational platform. The arrangement of the cows on the rotational platform can either be herringbone, tandem, abreast facing inside or facing outside (see Figure 2-5). The milker stands at the outside of the rotating platform as the cows move around him on the platform. The speed of the rotating wheel can be adjusted to allow appropriate time for the preparation of each cow. The rotary wheel is driven by an electrical or hydraulic motor. Units from 13 to 60 cows are usually used (30).

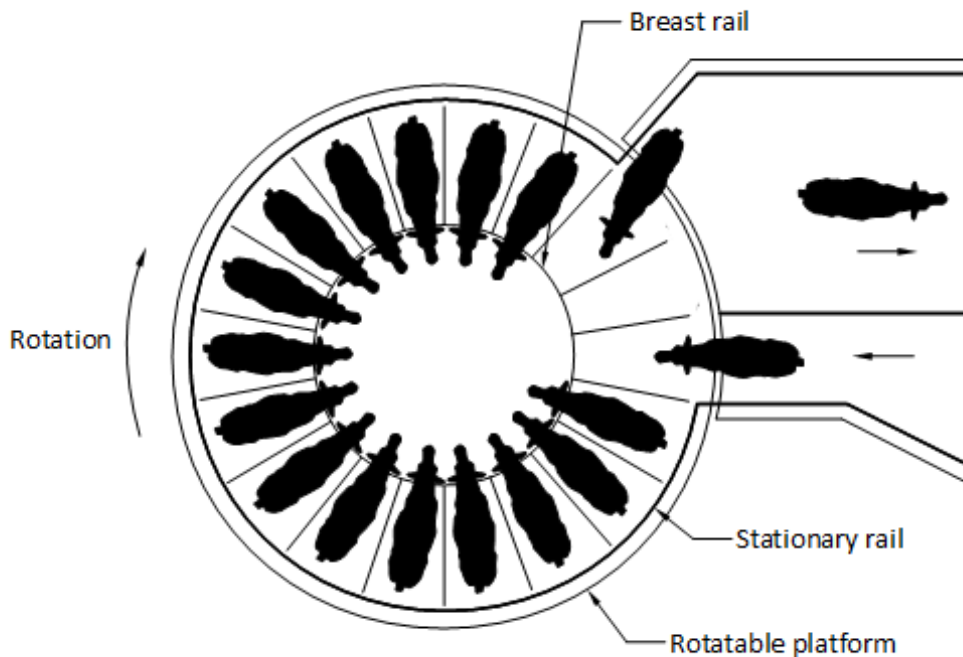


Figure 2-5 Rotating milking parlour (adapted from ref.(28))

2.1.2 The milking process

The main function of the milking machine is to help the milk to flow from the teats of the cows by applying a partial vacuum to the teats. Simultaneously, the teats are also massaged in order to relief the stress that the continuous vacuum causes. Partial vacuum is needed for the milking process. This is created when some of the air in the pipelines is removed by means of a vacuum pump. The milking unit is the most important part of the milking machine and consist of a pulsator, teat cup shells and milk receptacle. A pulsator is situated in the milking machine which directs the air flow from and to the milking unit. As the pulsator provides flow of vacuum and air (at ambient pressure) to the teat-cup, the milk is squeezed out of the teat in the same way as a baby calf would drink milk from its mother (31). The milk unit (receptacle) is either classified as a bucket or a claw-type milker, which is either connected to a milk pipeline system of a floor pail milker by means of a hose. Once the milk is in the pipeline it flows into a receiver jar by means of gravity after which it is sent though a filter before entering the bulk tank in which the milk is stored and cooled.

On a typical dairy farm, milking takes place either two or three times a day. The milking procedure consists of a number of activities before, during and after the actual milking process including preparation of the milker, udder and machines, application of the machine, detachment of the machine, post-milking treatment of teats and cleaning of machines.

Before milking, the equipment should be cleaned, sanitised and checked to see if everything is working properly after which the cows are allowed to enter the milking parlour. The milking process consists of the following steps:

- Firstly, the milker should ensure that his/her hands are clean.
- Once the cows have entered the milking parlour, the udder is washed and examined for mastitis (an infection of the mammary gland).
- The teats are dipped in to pre-milking germicide dip solution which cleans and sanitises the teats.
- Two or three streams of milk are removed from the teats into a beaker by hand to stimulate the milk production.
- Teat cups are then attached to the teats and milking starts.
- Once the cow is milked out, the teat cups are pulled from the teats and rinsed with clean water.
- A disinfectant solution is then sprayed unto the teats to avoid milk from dripping and also to prevent bacteria invasion.
- Cows which have a history of mastitis or which have recently calved are milked last and the milk of these cows is kept separated from the rest of the milk.
- Once the last cow is milked, the milking parlour is cleaned and equipment is washed thoroughly with a sanitising agent.
- The milk produced during the milking process is pumped to the storage tank where it is kept cool (around 3°C) until a milk truck come to collect the milk for further processing.

When the milk truck collects the milk, the milk is pumped from the tank to the truck with a special pump after which the milk will be transported to a dairy processing plant where it is

pasteurised and processed (30). In some cases, pasteurisation is done on the dairy farm itself, while in other cases it is done by another dairy processing company.

The two most common breeds of cows which are milked are Ayrshires and Jerseys. Ayrshires are mostly milked for the volume of milk that they can produce while Jerseys are mostly milked for the fat content in their milk (the milk from Jersey cows are mostly used to make cheese). Ayrshire cows are also usually larger and produce more milk than Jersey cows. Most cows take between 3 and 6 minutes to be milked and Ayrshire cows produce an average of 30 litres of milk per day.

2.1.3 The South Africa dairy farming industry

Dairy farming is practiced throughout the whole of South Africa, but the majority of dairy farms situated in the Western Cape, Eastern Cape, KwaZulu Natal and Free State. Figure 2-6 shows the distribution of dairy farmers throughout the country as it was during May 2009.

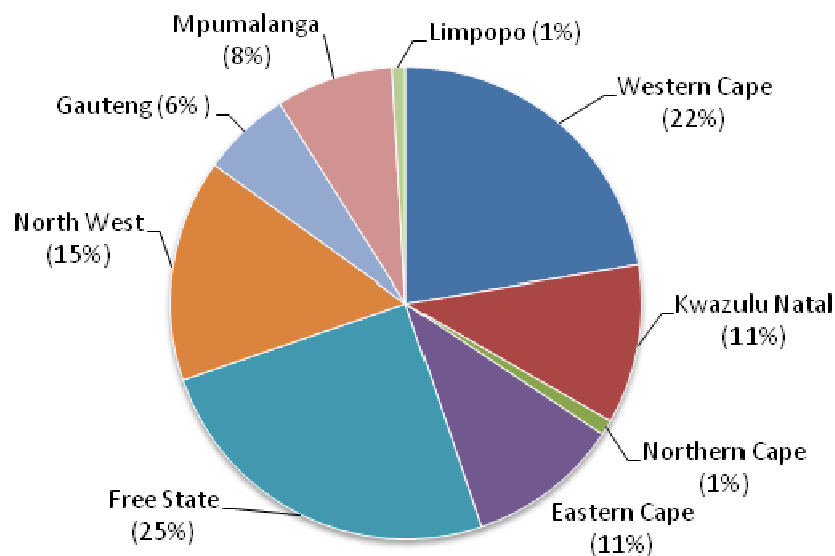


Figure 2-6 Summary of dairy producers in South Africa in January 2009 (adapted from ref. (4))

The dairy industry consists mainly of two sectors, the primary industry (milk producers) and secondary industry (milk processing companies). According to the latest available data concerning dairy farming in South Africa, the amount of registered milk producers by January

2009 was 3 551, which is a 50% decrease from the 7 077 milk producers registered in December 1997 (4). The secondary industry consists of a small number of large milk processing companies which are distributed throughout the country, a great number of smaller milk processing companies which operate only in particular regions as well as a few milk producers who sell their milk or dairy products directly to the consumers or traders (called producer distributors). The number of milk buyers in South Africa during January 2009 was 127, which is a decrease of 53% from 2003, while the number of producer distributors was 153 (4).

2.2 Wastewater from milking parlours

2.2.1 Main water usage

The main usage of water in and around the milking parlour is for the following activities:

- Cleaning of the cows during each milking session when entering the milking parlour;
- Washing of the milking equipment to ensure the system is clean and free of pathogens and bacteria after each milking session (usually an automated CIP washing system);
- Washing of the milk storage tanks;
- Flushing of the floor the holding area as well as the floor of the milking parlour;
- Pre-cooling of the milk in a heat exchanger before entering the milk storage tanks;
- Water softening (if applicable); and
- Other miscellaneous activities such as hand and boot washing, manual equipment cleaning, milk discharge cleaning.

The bulk milking parlour wastewater (if everything is combined) therefore usually contains water, milk, cleaning chemicals, manure, urine, wasted feed, bedding and grit and dirt from the floor (5).

a) Cleaning of cows during each milking session

Once the cows have entered the milking parlour, the udders are first rinsed to get rid of any dirt or mud on the udder. This is done either by an automatic sprayer which is open during the

duration of the milking process or it is done manually with a hose by a person standing at the place where the cows enter the milking parlour.

b) Cleaning of the milking parlour

The floor of the dairy and the area where the cows gather before entering the dairy is usually filled with manure and should be cleaned after each milking session. This is usually done by means of flushing a large amount of water over the area with a high-pressure hose and allowing the manure to run off with the water.

c) Milk storage tank washing and milking equipment washing

The washing of milk storage tanks and milking equipment is usually an automated process which is done by means of a clean-in-place (CIP) system. This CIP system is a very common washing system used in the majority of milking parlours and generally consists of five distinct steps (32):

- First rinse;
- Second rinse;
- Detergent wash;
- Rinse;
- Sanitising rinse or acid rinse (an acid rinse is done every 3-5 washes in stead of a sanitising rinse)

First and second rinse

The first rinse follows directly after the milking process is finished. Its main purpose is to get rid of the excess milk which stayed behind in the pipes and is usually responsible for removing 92 % of the suspended solids in the system. The water temperature for this cycle should be around 40°C (32). The wastewater from this step has a very high concentration of milk. The first rinse is followed by the second rinse which is the same as the first rinse. The wastewater from this step does not have such a high concentration of milk as the first rinse.

Detergent wash and rinse

The detergent wash follows directly after the second rinse and it removes the organic material attached to the equipment. This cycle is usually performed with warm water. The water temperature at the start of this cycle should be around 70°C and should not drop below 43 °C during the 5 to 10 minutes cycle. The detergent usually consists of around 100 mg.L⁻¹ chlorine and the pH of the solution (detergent and water) should be around 11 (the detergent-water ratio depends on the hardness of the water) (32). Once the detergent rinse is finished, the equipment is rinsed again with clean water.

Sanitising rinse

The last cycle in the washing process is the sanitising rinse which is done only before the next milking process. This is done to guarantee that the equipment and pipes do not contain any microorganisms which could have developed after the acid rinse. This cycle is usually performed with water at 40°C and a sanitising agent which contains 200 mg.L⁻¹ chlorine (32).

Acid rinse

An acid rinse is also done 2-3 times a week in order to remove inorganic material attached to pipelines, neutralise the detergent residues and to lower pH in order to prevent development of bacteria. For this cycle, the maximum temperature of the water should be 38°C (32).

2.2.2 Wastewater volumes generated in the milking parlour according to literature

The volume of wastewater generated during the washing of the milking equipment and milking parlour depends mostly on the size of the milking parlour, the number of cows milked in the specific milking parlour and the type of milking system used and will therefore differ in every milking parlour. In recent studies done by the University of Minnesota, the water usage on 16 milking parlours in the USA (in which less than 130 cows are milked in each milking parlour), were investigated in order to get an approximation of the volume of a typical wastewater treatment unit for milking parlour wash water. The authors estimated that the volume of wastewater generated in these 16 milking parlours typically ranges between 7.6 and 35 litres per cow while most milking parlours did not use more than 19 litres (33). Earlier studies in the

USA estimated that the total amount of wastewater generated from milking parlours range from 11 to 32 litres per cow per day (34). Another study conducted in Ontario, Canada showed that the amount of wastewater generated from the washing of milking equipment and milk handling equipment (i.e. wastewater from CIP washing and milk storage tanks) is approximately 14 litres per cow per day (35). One author proposed the that the volume of wastewater generated in milking parlours ranges between 20 and 70 L.cow⁻¹.day⁻¹ but depends greatly on the type of milking parlour and the total wastewater generated (see Table 2-1) (6).

Table 2-1 Wastewater generated from milking parlours (adapted from ref. (6))

Area of Milking Parlour		Wastewater [litres.cow⁻¹. day⁻¹]
Holding area	Multi-lane pit with a central tube for washing	20 - 40
	pith with inclined floor	12 – 18
Milking room	Herringbone or tandem	12 -18
	Circular	16 – 20
Other areas	Teat washing	0 – 3
	Toilets and milk room	3 – 4
Total		20-70

The best way to determine the volume of wastewater generated during the milking process and during the washing of the milking equipment and milking parlour is by measuring the volume of water entering the milking parlour and to subtract the volume not entering the drains. This is not always possible as most milking parlours have more than one single water source and installing measuring equipment is expensive. The University of Wisconsin-Extension (36) has proposed a worksheet to estimate the daily volume of wastewater generated in the milking parlour. This worksheet is can be found in Appendix A.

2.2.3 Milking parlour wastewater characteristics according to literature

A few studies have characterised the wastewater generated in the milking parlour and are shown in Table 2-2 (37; 38; 6; 39; 40; 41; 33; 42). All of these studies were done abroad and no study as far as could be ascertained has been done on the characteristics of wastewater from milking parlours in South Africa. The volume of wastewater generated per cow per day is also given in the table for those available. From the table it is clear that the characteristics of milking parlour wastewater generated on different farms vary quite significantly. The most probable reason for this is due to the fact that each farm set-up differs in terms of volumes of water used in the milking parlour, wastewater handling as well as the quality of water used for the different activities in the milking parlour. The wastewater characteristics also differ due to seasonal variations and geographical setting of farms.

2.3 Wastewater handling and water usage in a few South African milking parlours (MP)

Five milking parlours were visited to evaluate and compare the water usage and wastewater generation in a few milking parlours on dairy farms in South Africa. The total amount of water used per day in each of the five milking parlours was estimated according to the worksheet proposed by the University of Wisconsin-Extension. Three of the farms visited are situated in the Western Cape, while the other two are situated in the Free State. It must be mentioned that this is not a statistical viable study and the purpose of the study was not to gather data to represent all dairy farms in South Africa, but simply to verify typical water usage in a variety of milking parlours.

2.3.1 Wastewater handling

a) MP 1

MP 1 is also situated on a farm in the Eastern Free State, approximately 15 km from Ficksburg. In this parlour, 410 Jersey cows are milked twice a day in a herringbone milking parlour which

Table 2-2 Summary of some wastewater characteristics from dairy farms from literature

Description of wastewater	Location of milking parlour	pH	COD [mg.L ⁻¹]	TS [mg.L ⁻¹]	TSS [mg.L ⁻¹]	P [mg.L ⁻¹]	N [mg.L ⁻¹]	Volume [L.cow ⁻¹ .day ⁻¹]	Source
Equipment and floor washing of 8 different milking parlours	Kentucky, USA	6.3 - 9.3	6200 - 21 100	6600 - 15 800		62.0 - 175.1			(37)
Milking parlour and milk processing plant wash water & water from office and staff accommodation	Dubai, UAE	7.9	1541			20			(38)
Wastewater from washing of milking room, milking machine & milking pit (areas not trodden on by cows)	Casina, Italy	6.86 - 8.95	858 - 2312		0.41 - 1.54	6.5 - 27.4	44.7 - 173.9	25-40	(6)
Parlour and dairy wash water	England		6690	6144		89.3	0.54	62.5	(39)
Parlour and dairy wash water from 20 dairy farms	England & Wales	6.38 - 7.6	6550 - 17 300	0.57 - 1.65 %		340 - 490		6.05 - 60.8	(40)
Milking parlour wastewater from 5 different dairy farms	Wisconsin, USA		616 - 5611	1721 - 4872	73 - 1705	25.75 - 112.9		11.2 - 20.94	(41)

Table 2-2 Continued

Description of wastewater	Location of milking parlour	pH	COD [mg.L ⁻¹]	TS [mg.L ⁻¹]	TSS [mg.L ⁻¹]	P [mg.L ⁻¹]	N [mg.L ⁻¹]	Volume [L.cow ⁻¹ .day ⁻¹]	Source
Washing of milking equipment	Netherlands	2.6 – 11.8	625 – 4390			11 – 320			(42)
Washing of milking parlour	Netherlands	3 – 4.5	2800 – 12000			31 – 54			(42)
Washing of milking tank	Netherlands	9.5 – 10.5	580 – 1330			26 – 110			(42)
Washing of milking equipment	Minnesota, USA	6.2 - 8		200 – 1000		20 – 100	30 – 100	7.6 - 35	(33)

can milk 34 cows at a time. The total daily milk production is approximately 9500 litres which averages about 23 litres per cow per day. The main water source in the milking parlour is borehole water. Currently, no wastewater handling system is installed on the farm and the wastewater generated in the milking parlour during milking, equipment washing and floor flushing simply flows away into the field. The farmer is however, in the process of installing a wastewater management system in which the water is going to be collected in a pit in which the solids will be allowed to settle out. The water will then be used for irrigational purposes. Figure 2-7 shows a flow diagram water in and from MP1.

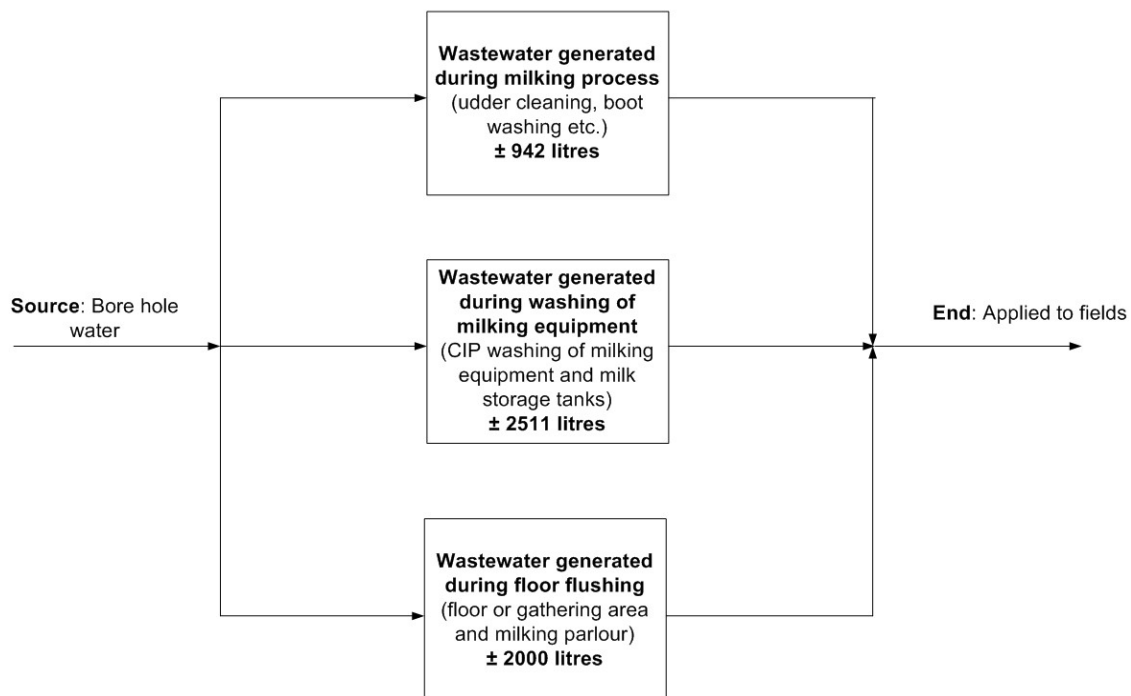


Figure 2-7 Flow diagram of water in and out of MP 1

b) MP 2

MP is situated on a farm in the Eastern Free State approximately 30 km from Ficksburg. In this milking parlour, 250 Ayrshire cows are milked twice a day in a 24 unit rotating milking parlour. Approximately 7500 litres of milk are produced daily in this milking parlour. The main water sources for the milking parlour are bore hole and natural spring water and water from the local farm dam. Currently, no real wastewater handling system is incorporated at this specific dairy.

The water from the washing of the milking equipment as well as water from the flushing of the floor flows through a natural wetland of approximately 500m, back to the local farm dam which has a circumference of approximately 2 km. The water from the dam is used for irrigation purposes as well as for drinking water for the animals on the farm and the dam is fed with rainwater and another dam upstream from this dam. Figure 2-8 is a flow diagram of the water flow in and from MP 2.

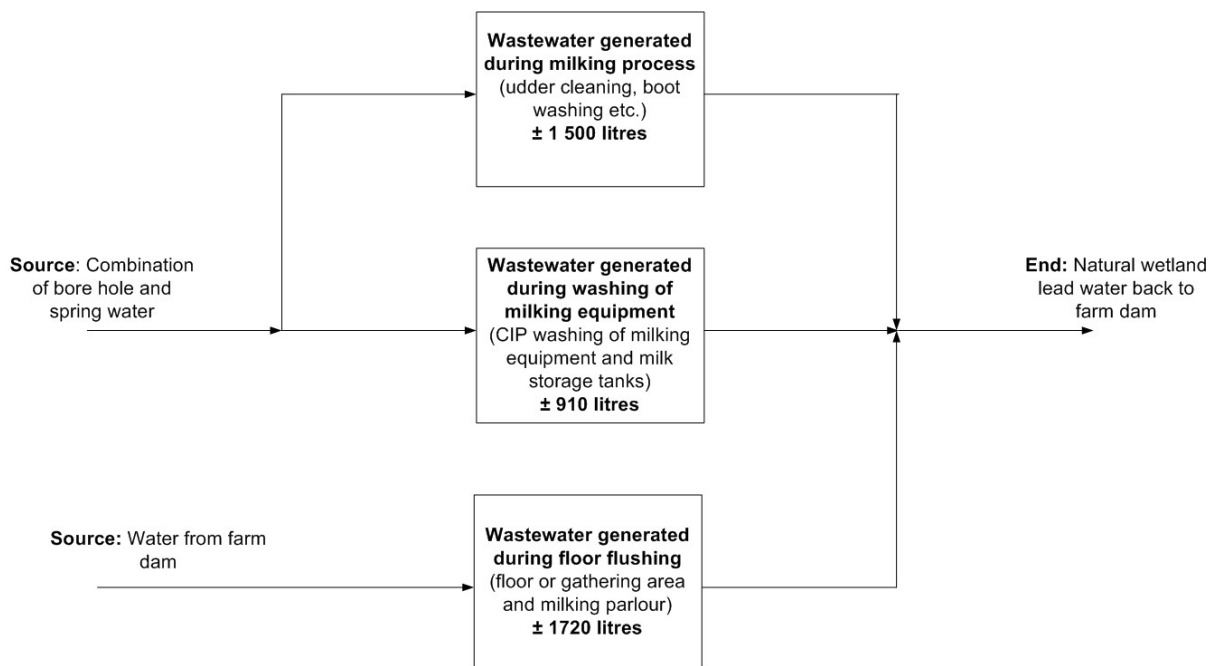


Figure 2-8 Flow diagram of water in and out of MP 2

c) MP 3

MP 3 is situated on a farm near Malmesbury in the Western Cape. 1100 Ayrshire cows are milked 3 times a day in a 60 unit rotating milking parlour. The farm produces an average of 34 000 litres of milk per day. The main water source in the dairy is brackish water from a borehole which is treated with some chlorine before being used for washing purposes. The borehole water is used for the washing of the milking equipment, milk storage tanks and for miscellaneous purposes inside the dairy. All the wastewater generated is transported to a wastewater collection basin after which it goes through a press in order to separate the solids from the liquid. The water then enters a wastewater dam. A pump is situated in the dam which

circulates the water inside the dam every few hours. Some of the water is then recycled back to the dairy to flush the floor of the area where the cows gather before entering the milking parlour, while the rest of the water is used for irrigation purposes on the farm. The solids from the press are used as fertiliser on the farm. Figure 2-9 shows a flow diagram of the water in and around the milking parlour.

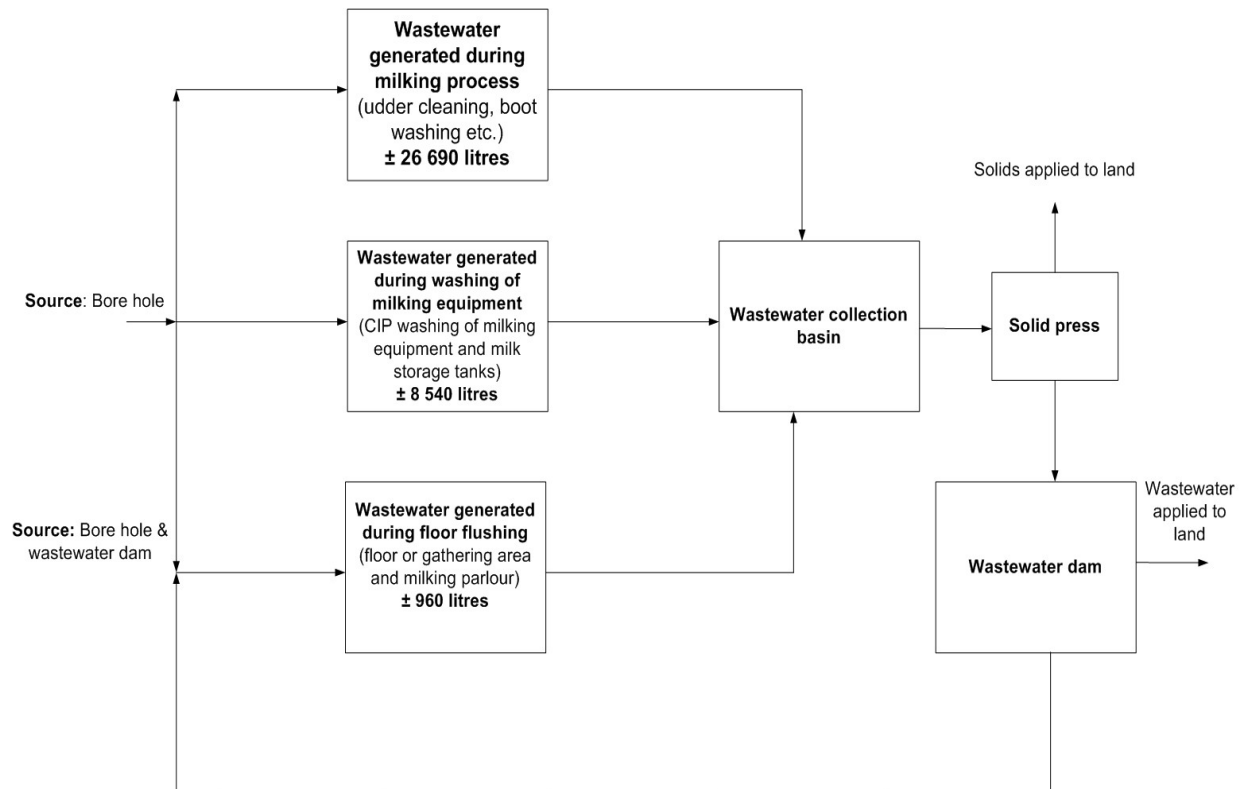


Figure 2-9 Flow diagram of water in and out of MP 3

d) MP 4

Farm 4 is situated near Malmesbury in the Western Cape. In this milking parlour, 400 Ayrshire cows are milked 3 times a day in a 40 unit rotating milking parlour. Approximately 13 000 litres of milk are produced daily and the milk are collected every day by a local milk processing company. The main water source on this farm is borehole water. This farm is situated close to farm 1 and the borehole water used is therefore also rather brackish. Currently, all the wastewater generated in the parlour flows to a sedimentation pit where the solids are allowed to settle out. The solids are then used as fertiliser on the farm, while the wastewater overflows

into another dam from where it is used for the irrigation of roll-on grass. Figure 2-10 shows a flow diagram of the water in and around MP 4 on Farm 4.

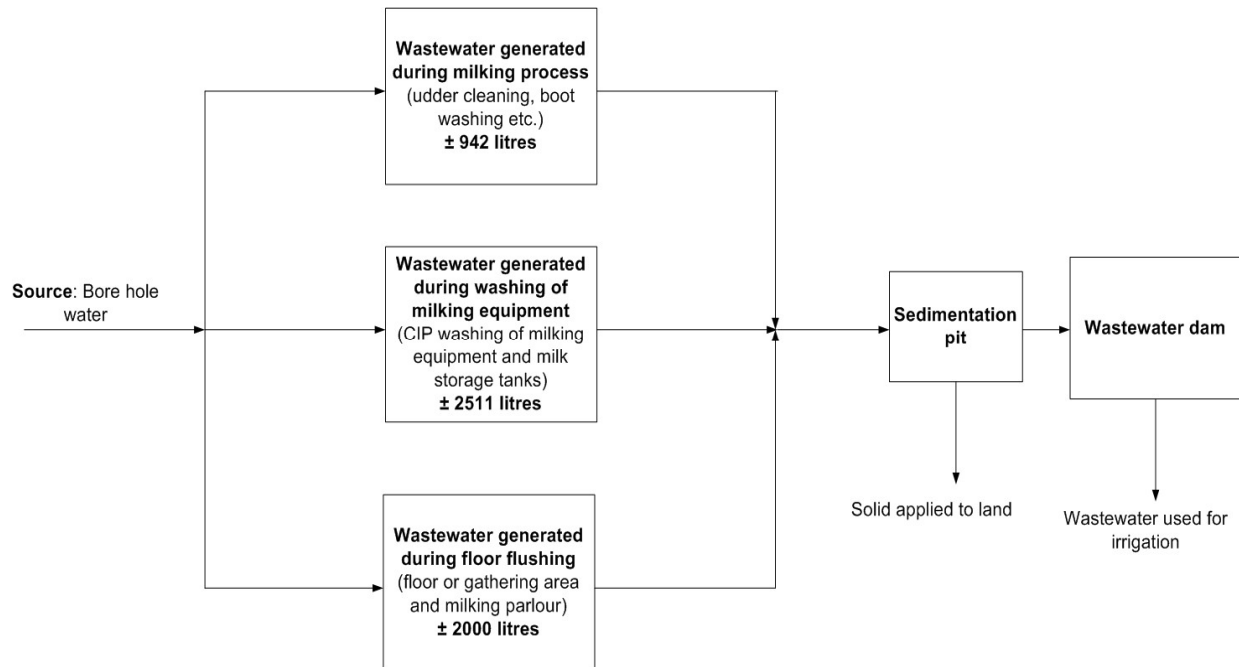


Figure 2-10 Flow diagram of water in and out of MP 4

e) MP 5

MP5 is situated and a farm near Klipheuwel in the Western Cape. In this milking parlour, 650 Ayrshire cows are milked three times a day in a rotating milking parlour. The daily milk production is approximately 24 000 litres. The main water sources on the farm are municipal water as well as borehole water. The wastewater generated during the flushing of the floor flows to a sedimentation pit in which the solids are allowed to settle out after which is allowed to pass through a press in which the majority of the solids still left over are separated from the water. The water from the press, together with the wastewater generated during the washing of the milking equipment flows to a wastewater collection basin. When the water level in this basin reaches a certain level, the water is pumped to a reservoir situated next to the dairy. The wastewater in the reservoir are then reused daily for the flushing of the floor of the area where the cows gather before entering the milking parlour, while the excess water is used for irrigation purposes on the farm. The solids which were separated from the water in the

sedimentation pit and the press are used as fertiliser on the farm. Figure 2-11 shows a flow diagram of the water in and around MP 4 on Farm 5.

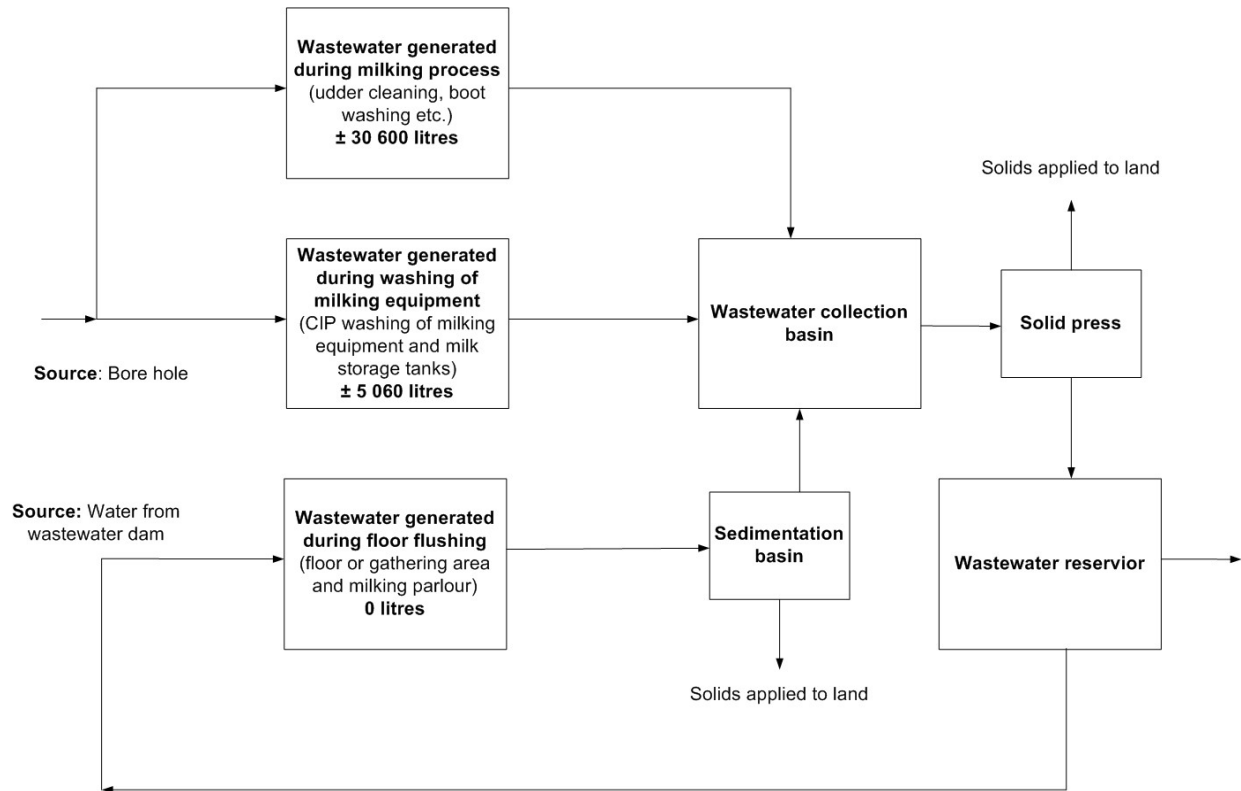


Figure 2-11 Flow diagram of water in and out of MP 5

2.3.2 Volume of water used in each milking parlour

The volume of water used per day in each milking parlour was estimated by means of the worksheet developed by the University of Wisconsin-Extension (see Appendix A). The average water used per cow per day was then calculated for each milking parlour. Figure 2-12 shows the volume of water used for the different activities in each milking parlour.

a) Milking system and bulk tank cleaning

From Figure 2-12 it can be seen that the volume of water used per cow for equipment washing is more or less constant in all 5 milking parlours (4.9 to 6.4 L.cow⁻¹.day⁻¹). The bulk milk storage tanks are usually washed either by hand or by means of an automated CIP washing system each time it is emptied.

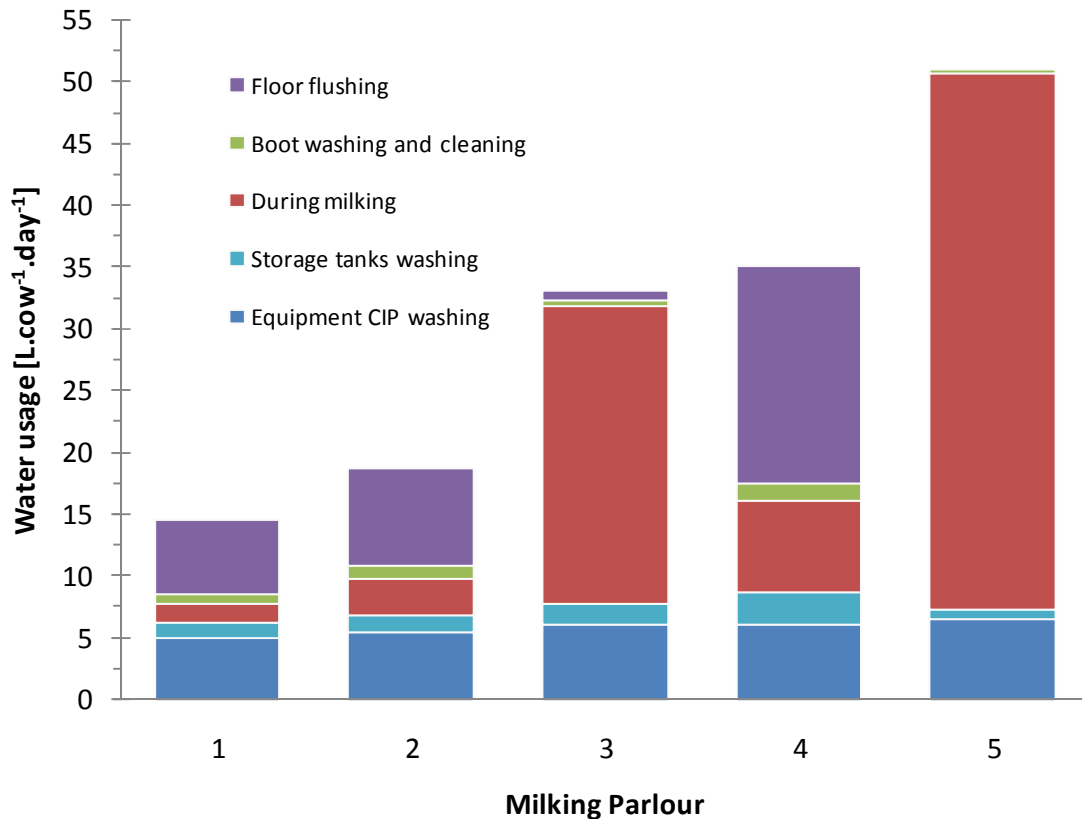


Figure 2-12 Volume water used in 5 South African milking parlours

From Figure 2-12 it can be seen that the amount of water used for milk storage tank cleaning differs more significantly than the water used for equipment washing. The reason for this is the difference in the number and size of milk storage tanks in each milking parlour as well as the frequency at which each tank is emptied (i.e. the times per week that the tanks are washed). Milking Parlour 4 uses the most water (per cow) for milk storage tank washing per cow while milking parlour 5 uses the least amount of water.

b) Water used in milking parlour during milking

The main use for water in the milking parlour during milking is to clean the udders of the cows when they enter the milking parlour and to clean the floor of the milking parlour while milking. The amount of water used during milking in the five different milking parlours can also be seen in Figure 2-12. The milking parlours on farms 3 and 5 use by far the most water per cow during the milking time. The reason for this is the fact that a spraying hose is installed at the place

where the cows enter the milking parlour. The purpose of the hose is to clean the floor surface on which the cows stand while being milked as the rotating milking parlour rotates. This hose is open throughout the whole duration of the milking operation and therefore uses high volumes of water (6000 litres per milking time in milking parlour 5 and 4300 litres per milking time in milking parlour 3).

c) Milk pre-cooling and water softening

In all the milking parlours except those in MP 2 and MP 5, a heat exchanger is used to pre-cool the water before entering the milk storage tanks. The water used in the case of MP 1, 3 and 4, the water used in the pre-cooler is recycled and no wastewater is therefore generated from milk pre-cooling.

d) Milking parlour and holding area wash-down

The activities that generates the most of wastewater, is the flushing of the holding area floor. This area is full of manure which needs to be flushed with a hose with a high flow rate. In MP 5, wastewater from the milking parlour is recycled to flush the floor of the holding area. The holding area is built at a slight angle which helps to flush the floor quicker as the water flows down to the sedimentation pit easier. The floor is flushed by opening a valve with approximate diameter of 30 cm situated at the top of the holding area to allow the water from the wastewater collection dam to flush the manure to the sedimentation pit situated at the bottom end of the holding area. No extra water is therefore used in MP 5 for the washing of the holding area. However, a drawback of this method is the odour arising from reusing the wastewater. The volume of water used for the flushing of the holding area in MP 5 was not recorded.

In the milking parlour on farm 3, some of the wastewater is also recycled to flush the floor of the holding area. Two hoses with diameter of 32 mm are used to flush the floor, but only one of the hoses uses recycled water while the other hose uses borehole water. On farms 1, 2 and 4 fresh water is used for the flushing of the holding area floor. The volumes of wastewater generated during flushing of the floor of the holding area can also be seen in Figure 2-12.

e) Total volume of water used

From Figure 2-12 it can be seen that the total volume of water used per cow per day in the 5 different milking parlours visited ranges from 15 (MP 1) to 51 L.cow⁻¹.day⁻¹ (MP5). This proves the statement made earlier that the water usage in milking parlours varies significantly from milking parlour to milking parlour. When the volume of water used per day is converted to the volume of water used per litre of milk produced, MP 5 still uses the most water (1.49 L.(L_{milk produced})⁻¹), while MP 2 uses the least amount of water (0.55 L.(L_{milk produced})⁻¹). Although MP 5 uses the most water, it is interesting to note that it is also the only milking parlour that uses fully recycled wastewater to flush the floor of the gathering area of the cows. The udders of the cows are not cleaned before entering the milking parlour before milking in MP5 as the cows are kept under a roof and is therefore not exposed to so much mud and dirt as in the case on the other farms. The total volume of water used in the few milking parlours visited corresponds well to that found in literature (see Table 2-2). Table 2-3 shows a summary of the volumes of water used for each activity in each milking parlour visited. The water used for the CIP washing of the milking equipment in each milking parlour visited is indicated in bold.

2.4 Summary

A number of waste handling and disposal systems have been used successfully in the past on a variety of dairy farms globally. In most of these systems, the two main wastewater streams (from equipment washing and floor flushing) are combined with the manure handling system on the farm. These systems include liquid manure systems with land application, holding tank (or pond) with land application, aerobic lagoon with land application, intensive land application, subsurface disposal and municipal sewage system disposal (7). Although the two main wastewater streams are combined, these applications focus primarily on the handling of the manure.

In a recent study, the wastewater generated from 8 different milking parlours in Kentucky (USA) was characterised (37). The milking parlours varied in size (number of cows) and milking times per day. It was found that the volume and composition of the wastewater from each milking parlour varied from day to day. The amount of water used for the equipment washing did,

Table 2-3 Summary of water usage in 5 different South African milking parlours

Parameter	Units	Milking Parlour				
		1	2	3	4	5
Number of cows		410	220	1100	400	700
Number of milking events per day		2	2	3	3	3
Type of milking parlour		Herringbone	Circular	Circular	Circular	Circular
Size of milking parlour		34	24	60	40	60
Daily milk production	L.day ⁻¹	9500	7500	34000	13000	24000
Source of water used		Borehole	Borehole, Spring & dam	Borehole	Borehole	Borehole & Municipal
Water for CIP equipment washing	L.day⁻¹	2000	1200	6641	2400	4500
	L.cow⁻¹.day⁻¹	4.88	5.45	6.04	6.00	6.43
Water for tanks washing	L.day ⁻¹	511	279	1900	1050	560
	L.cow ⁻¹ .day ⁻¹	1.25	1.27	1.73	2.63	0.80
Water used in parlour during milking	L.day ⁻¹	662	660	26346	2940	30334.82
	L.cow ⁻¹ .day ⁻¹	1.61	3.00	23.95	7.35	43.34
Water for boot washing & cleaning	L.day ⁻¹	280	250	543	555	270
	L.cow ⁻¹ .day ⁻¹	0.68	1.14	0.49	1.39	0.39
Water used for floor flushing	L.day ⁻¹	2500	1720	960	7080	0
	L.cow ⁻¹ .day ⁻¹	6.10	7.82	0.87	17.70	0
Total Water used	L.day ⁻¹	5953	4109	36421	14025	35665
Ave per cow	L.cow ⁻¹ .day ⁻¹	15	19	33	35	51
Ave per litres milk produced	L.(L _{milk produced}) ⁻¹	0.63	0.55	1.07	1.08	1.49

however, remain constant in all of the milking parlours. It was estimated that the amount of water from the CIP washing was approximately one third of the total amount of wastewater generated. Although the wastewater from equipment washing is usually combined with the livestock slurry (manure), this practice is under re-evaluation as it dilutes and increase the volume of the manure, causing an increase in the cost of manure storage facilities as well as increased cost of distribution and land application of manure (43). The wastewater from equipment washing is can be responsible for up to 30% of the total amount of wastewater generated from a dairy farm (44). Keeping the equipment wash water separate from the manure handling system and treating it in an appropriate manner may have the following benefits (45):

- Reduction in the storage facility volume and handling cost by reducing the amount of water being applied to croplands;
- Reduction of the volume of water stored together with the farm slurry, causing an increase in the storage capacity of existing slurry storage systems, resulting in a thicker, more concentrated slurry with higher nutrient concentrations which will not so easily cause pollution due to run-off to surface waters.; and
- When the wash water is treated, it may be re-used in the milking parlour to wash machinery as well as the areas where the cows are handled, decreasing the water cost.

Although the characteristics and volumes of wastewater generated on the different farms will differ quite significantly, the characteristics of wastewater from CIP washing would be more or less similar in different milking parlours on the different farms as the CIP process is a standard process used in milking parlours. The wastewater generated from CIP washing consists mainly of water, milk and detergents and will therefore have very similar characteristics than wastewater generated from dairy processing plants.

The possibility of treating the equipment wash water separate from the rest of the wastewater was already investigated in a study in which the aerobic treatment of wash water from milking parlours (11). They proposed to keep the wastewater generated from equipment washing

separate from the rest of the wastewater. Five different aerobic treatment methods have been evaluated and they found that all of the systems showed great potential of reducing the pollution from milking parlour wash water (11). The COD removal efficiency of the 5 different aerobic treatment methods ranged between 41 and 75 %. However, as mentioned in Chapter 1, anaerobic treatment methods have shown to have many advantages over aerobic treatment of which the formation of biogas which can be used as an energy source is probably one of the most important advantages.

Several previous studies have shown that anaerobic wastewater treatment is suitable for the treatment of wastewater from the dairy industry (13). It is therefore expected that wastewater from the CIP washing from milking equipment in a milking parlour would be successfully treated with a suitable anaerobic wastewater treatment method. The rest of this study will therefore focus on the design, construction and operation of a suitable lab scale anaerobic wastewater treatment method to treat wastewater generated from the CIP washing of milking parlours.

Chapter 3 - Literature Review: Anaerobic Wastewater Treatment

3.1 Anaerobic digestion

Anaerobic digestion is a biological process in which complex organic material is converted to hydrogen, methane, carbon dioxide, water, ammonia, hydrogen sulphide and new cells by a close knit bacterial community under anaerobic conditions (46; 47; 48). One of the unique features of the anaerobic digestion process is that the organic material or carbon dioxide produced serves as an electron acceptor and the process can therefore work without the addition of an electron acceptor (such as nitrate or oxygen) to the system (46).

3.1.1 History of anaerobic digestion

The anaerobic digestion process is a very old process which has been used for biological wastewater treatment since the 1860's. It was however,, already discovered in 1776 by Count Alessandro Volta after he demonstrated that the "combustible air" formed from organic material (mostly plants) in the sediment of streams, lakes and ponds were actually methane (46). But, it was only nearly 100 years later, in 1856, that Reiset discovered that methane could be liberated from piles of manure which was decomposing. He then suggested that this decomposing process should be investigated further to obtain a better understanding of the process, which lead to the first full-scale anaerobic wastewater treatment plant in 1860 which had a lot in common with the configuration of the septic tank (46; 49). Another early full-scale application of the anaerobic process in wastewater treatment was in Exeter, England, during the 19th century with the development of the septic tank by Donald Cameron in 1895. He was

awarded a patent and due to the success of his development, the city of Exeter decided to use this method to treat the city's wastewater (46). After this, the septic tank became a popular way of treating wastewater, but the effluent quality was frequently poor, being black in colour and containing indigestible matter, which led to the development of a two-stage process in which the suspended solids could settle out in a separate vessel.

During the 1920s and 1930s many studies were conducted on the anaerobic sludge process, which led to wide applications of the process worldwide (46). It was, however, only in the 1950s that anaerobic treatment gained popularity due to the development of the anaerobic contact process (which was similar to the activated sludge process with recycle stream) and since then, many variations in the process have been developed and successfully employed in the anaerobic treatment of wastewater. Some of these processes include the conventional digester, digester with recycle, upflow fixed film reactor, upflow anaerobic sludge blanket reactor and fluidized bed reactor (50).

3.1.2 Microbiological and biochemical aspects

Anaerobic digestion is a complex biochemical process consisting of four main reaction steps, namely disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis (48). In each step, a variety of microbial groups which differs in their physiological behaviour work together to successfully digest the complex organic wastes. The various metabolic pathways are shown in Figure 3-1.

a) Disintegration and hydrolysis

During anaerobic digestion, the composite material first needs to be broken down into soluble matter as the microorganisms are not capable of digesting particulate organic matter. This is done via disintegration and hydrolysis (48). Disintegration entails the breakdown as well as the solubilisation of composite particles to soluble substrates such as carbohydrates, proteins, fats as well as inert particulate and inert soluble material (47). Hydrolysis follows disintegration and is the process in which hydrolytic bacteria break down complex organic molecules (such as carbohydrates, proteins and fats) formed during disintegration into smaller monomer molecules such as amino acids, monosaccharides (sugars) and long chain fatty acids. The

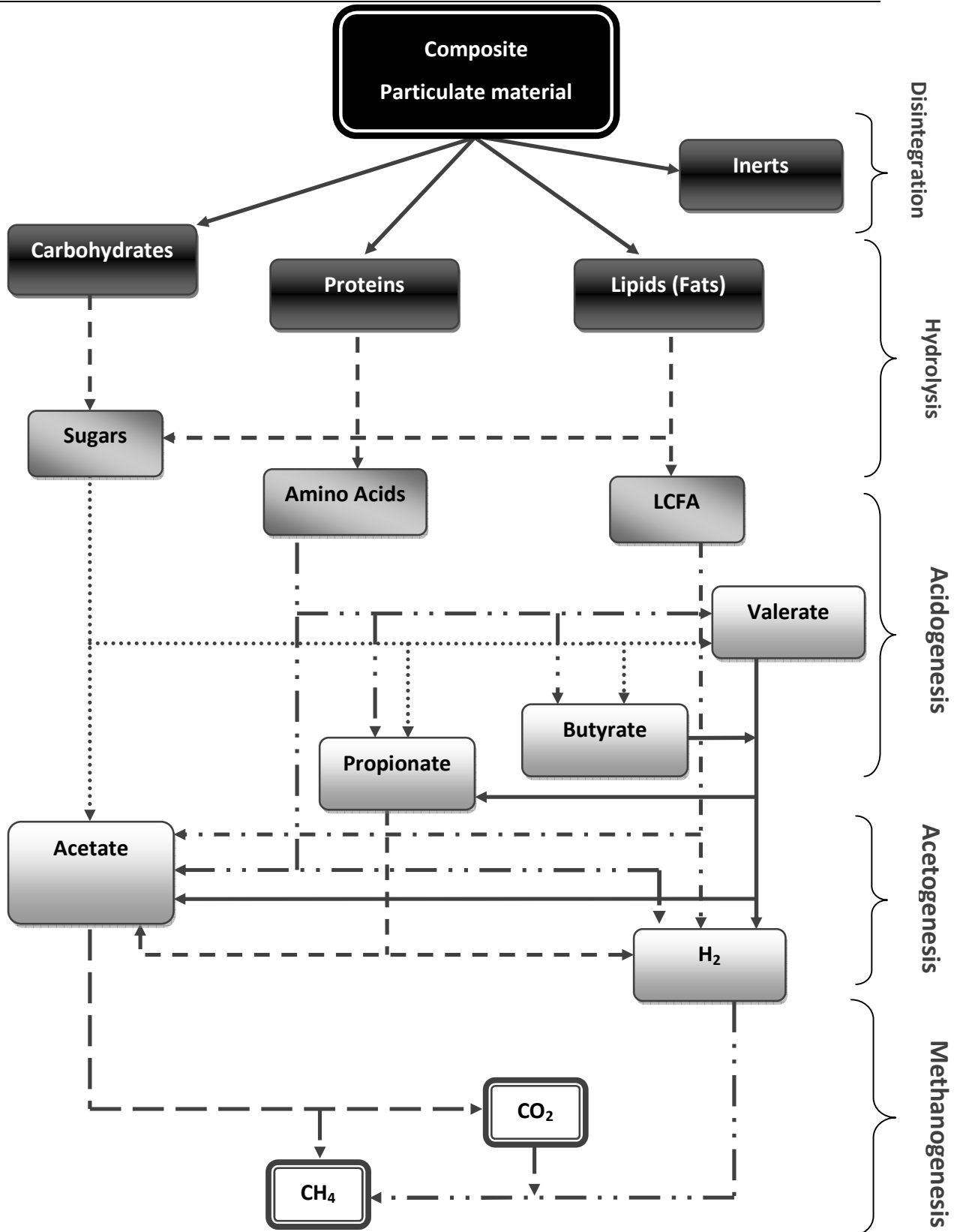


Figure 3-1 Conversion processes during anaerobic digestion from the Anaerobic Digestion Model No. 1 (adapted from ref. (47))

hydrolysis reactions of each of these complex molecules are catalysed by specific extracellular enzymes such as lipases, proteases and cellulases (51). Under anaerobic conditions, the rate at which hydrolysis typically takes place is relatively slow and will therefore limit the overall rate of the anaerobic process. Several factors that have shown to influence the rate of hydrolysis include the pH of the medium, the temperature inside the reactor, the residence time of the substrate inside the reactor, the size of the particles inside the reactor, the concentration of $\text{NH}_4^+\text{-N}$ as well as the hydrolysis product concentration (such as volatile fatty acids) (48).

b) Acidogenesis

Hydrolysis is followed by acidogenesis which is an acid-producing reaction in which the sugars, amino acids and long chain fatty acids produced during hydrolysis, are converted to alcohols, ketones and volatile fatty acids (such as acetic acid, propionic acid, formic acid, butyric acid or succinic acid) by means of acidogenic bacteria. A wide variety of bacteria are involved during hydrolysis and acidogenesis which result in the formation of various kinds of alcohols and organic acids (14). The type of fatty acid produced during acidogenesis depends on the type of bacteria as well as the operating conditions such as temperature and pH (51). Acidogenesis is usually the fastest reaction step during anaerobic digestion and during steady state operations, the main pathway of this step is via acetate, hydrogen and carbon dioxide, which can be directly used by methanogenic bacteria (14). The different reactions that glucose can undergo during acidogenesis together with the main product formed and the Gibbs free energy associated with the reaction are shown in Table 3-1.

Table 3-1 Different products formed during acidogenesis under different conditions (adapted from ref. (47; 51))

Reaction	Main Product	ΔG [kJ]
$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2$	Acetate	-206
$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+$	Propionate	-358
$C_6H_{12}O_6 \rightarrow 2CH_3CHOCOO^- + 2H^+$	Lactate	-198
$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2HCO_3^- + 2H^+$	Ethanol	-206

c) Acetogenesis

The third step during the anaerobic digestion process is acetogenesis. The bacteria which are responsible for this step is acetogenic bacteria (hydrogen and acetate producing bacteria), which converts the fatty acids formed in the acidogenesis step to substrate appropriate for the methanogenic bacteria to metabolise. Even though acetogenesis follows acidogenesis, a clear distinction between the two reaction steps cannot always be made as both acidogenesis and acetogenesis reaction yield hydrogen and acetate which are substrate for methanogenesis (52).

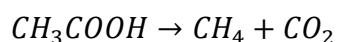
Acetogenic bacteria are sensitive to organic loading rate variations and environmental changes. They have relatively slow growth rates which require long lag periods to adjust to their environmental conditions (14). These bacteria require a relatively low hydrogen partial pressure to convert these fatty acids to acetate, hydrogen and carbon dioxide. Under high hydrogen partial pressures, the fatty acids will be reduced to propionate, butyrate and ethanol, resulting in unfavourable conditions for the anaerobic process as these longer chained volatile fatty acids can be toxic to the methanogenic bacteria in unionized form (51). It is therefore important that a syntrophic association between the acetogenic bacteria and the hydrogen-utilising methanogenic bacteria are established. This syntrophic association refers to a coupled association between the hydrogen-producing bacteria (acidogenic or acetogenic bacteria) and the hydrogen consuming bacteria (hydrogenotrophic methanogens). This coupled association is especially important when the reactions are not thermodynamically favourable as in the case of the conversion of propionate, butyrate and ethanol to acetate (see Table 3-2). In this case, in order for acetogenesis to take place, the concentrations of the products have to be kept low, which is made possible by the methanogenic bacteria which consumes hydrogen and acetate formed during acetogenesis (52). A wide range of acetogenic bacteria are present in anaerobic digesters, including *Syntrophomonas wolfeii* (valerate- and butyrate-decomposing acetogenic bacteria), *Syntrophobacter wolinii* (propionate-decomposing acetogenic bacteria) and homoacetogenic bacteria (which converts products formed during acidogenesis to acetate, carbon dioxide and hydrogen) (14).

Table 3-2 Free Gibbs energies of acetogenic and methanogenic reactions at 1atm, pH 7 and concentration of reactant and products 1M (adapted from ref. (52; 14))

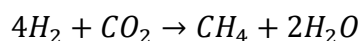
Substrate	Reaction	Product	ΔG [kJ]
Acetogenic Reactions			
Lactate	$CH_3COHCOOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 2H_2 + H_2O$	Acetate	-4.2
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	Acetate	+9.6
Butyrate	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	Acetate	+48.1
Propionate	$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$	Acetate	+76.1
Methanogenic Reactions			
Carbon Dioxide + Hydrogen	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	Methane	-135.6
Acetate	$CH_3COOH + H_2O \rightarrow CH_4 + CO_2 + H_2O$	Methane	-31.0
Syntrophic Reaction			
Carbon dioxide	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	Methane	-135.6
Butyrate	$2CH_3CH_2CH_2COOH + 4H_2O \rightarrow 4CH_3COOH + 4H_2$	Acetate	+96.2
Net Reaction			
Butyrate	$2CH_3CH_2CH_2COOH + 2H_2O + CO_2 \rightarrow CH_4 + 4CH_3COOH$	Acetate	-39.4

d) Methanogenesis

Methanogenesis is the final step during anaerobic digestion. During this step, methane is produced from acetate in two ways for which two different types of bacteria are responsible (47). The primary route is by the fermentation of acetate to carbon dioxide and methane (see Eq. 3-1) by the acetoclastic methanogens (acetate-using microorganisms). The other alternative way in which methane is produced, is by the reduction of carbon dioxide to methane by hydrogenotrophic methanogens (hydrogen-using microorganisms) (see Eq. 3-2).



Eq. 3-1



Eq. 3-2

Both the hydrogenotrophic and acetoclastic methanogenic bacteria are obligate anaerobic organisms which are very sensitive to small changes in the environmental conditions. The acetoclastic methanogens forms a very important part of the anaerobic process as they are responsible for as much as 60-70% of the total methane production (48). Two types of acetoclastic methanogens can produce methane from acetate, which includes the *Methanosarcina* genera which dominates at acetate concentrations above 10^{-3} M and the *Methanosaeta* which dominates at acetate concentrations below 10^{-3} M (48). *Methanosarcina* have higher yields, is less pH-sensitive, have higher growth rates and require shorter solid retention times when compared to *Methanosaeta*. *Methanosaeta*, on the other hand can operate at lower acetic acid concentrations and have a higher affinity with acetate and are the most flexible methanogens as they are able of utilising both hydrogen and methylamines (48; 53; 54). The hydrogenotrophic methanogens can produce methane from carbon dioxide and hydrogen and the three types which are the most common in anaerobic reactors are the *Methanobacterium*, *Methanospirillum* and the *Methanobrevibacter* (48). Both the acetoclastic and the hydrogenotrophic bacteria are essential in maintaining a balance in the anaerobic digestion process, as the hydrogen which is produced in the earlier steps are consumed. This causes a lowering in the hydrogen partial pressure, producing a favourable environment for the acidogenic and acetogenic bacteria to work optimally (48).

3.1.3 Environmental factors and inhibition

The growth rate of the anaerobic bacteria is greatly influenced by the environment inside the reactor in which anaerobic digestion takes place. The success of the anaerobic process therefore, depends greatly on how well the anaerobic bacteria can adapt to their environment and how well and accurate the ecological balance can be maintained.

The acidogenic bacteria and the methanogenic bacteria differ greatly regarding their nutritional needs, physiology, growth kinetics as well as their sensitivity to the change in the environmental conditions (55). When the right balance between these two bacteria groups is not maintained, reactor instability is unavoidable (13). The concentration of VFA such as formic

acid, butyric acid, propionic acid and acetic acid is an important parameter which can give an indication whether an imbalance is occurring inside the reactor and to evaluate the ecological balance. The reason for this is due to the fact that VFA are formed during acidogenesis as intermediates from which methane is eventually produced by means of methanogenic bacteria (48). As the VFA are produced by the acidogenic bacteria during acidogenesis, the methanogenic bacteria will convert it to methane and hydrogen. The rate at which this conversion takes place is dependent on how favourable the conditions inside the reactor are for the methanogenic bacteria and whether there are enough methanogenic bacteria present to the VFA to methane and hydrogen. If the methanogenic bacteria are present in sufficient amount, they will consume the VFA as soon as it is formed and there will therefore be no accumulation of acids inside the reactor as the acids can only accumulate up to the neutralising capacity of the alkalinity usually present in the media. The pH inside the reactor will therefore remain in a high range, favourable for the effective working of the methanogenic bacteria (48). If, however, the methanogenic bacteria are not present in sufficient amount, accumulation of VFA inside the reactor will occur as the amount of methanogenic bacteria will not be capable of converting the VFA at the same rate at which it was formed. This accumulation of VFA inside the reactor will evidently lead to a drop in the pH as the alkalinity usually available in media will be consumed very quickly leaving a high amount of acids un-neutralised, causing the reactor to be “sour” (48).

Strict control on the environmental and operating conditions such as temperature, pH and alkalinity, nutrient concentrations, VFA concentrations, organic loading rates (OLR), hydraulic retention times (HRT) and presence of toxic materials is therefore of great importance in any anaerobic process (48).

a) Temperature

Temperature is one of the most important factors which influence the anaerobic digestion process as it affects the microbial growth in terms of stability of the process, conversion, kinetics, quality of effluent as well as the net amount of energy produced (56). The microorganisms responsible for the anaerobic digestion are not able to regulate and control

their own internal temperature and the ambient temperature inside the reactor will therefore determine the internal temperature of the cells (48).

Even though anaerobic digestion have been reported to occur at a wide range of temperatures ranging from 0°C up to as high as 97°C, the three main temperature ranges which have been used are the psychrophilic range (approximately 4-15°C), mesophilic range (approximately 20 to 40°C) and thermophilic range (approximately 45-70°C) (47; 56). Each one of these temperature ranges have a maximum temperature above which microbial growth cannot occur, a minimum temperature below which microbial growth cannot occur and an optimum temperature at which microbial growth is at its maximum (48). The temperature directly affects the anaerobic reactor kinetics and the degree of anaerobic digestion is therefore dependent on the temperature. The rate at which decomposition is taking place increases as the temperature increases until it reaches an optimum at the optimum growth rate temperature (See Figure 3-2) (49). At temperatures higher and lower than the optimum growth rate temperatures the metabolic activity of the microorganisms will decrease which will result in slower kinetics.

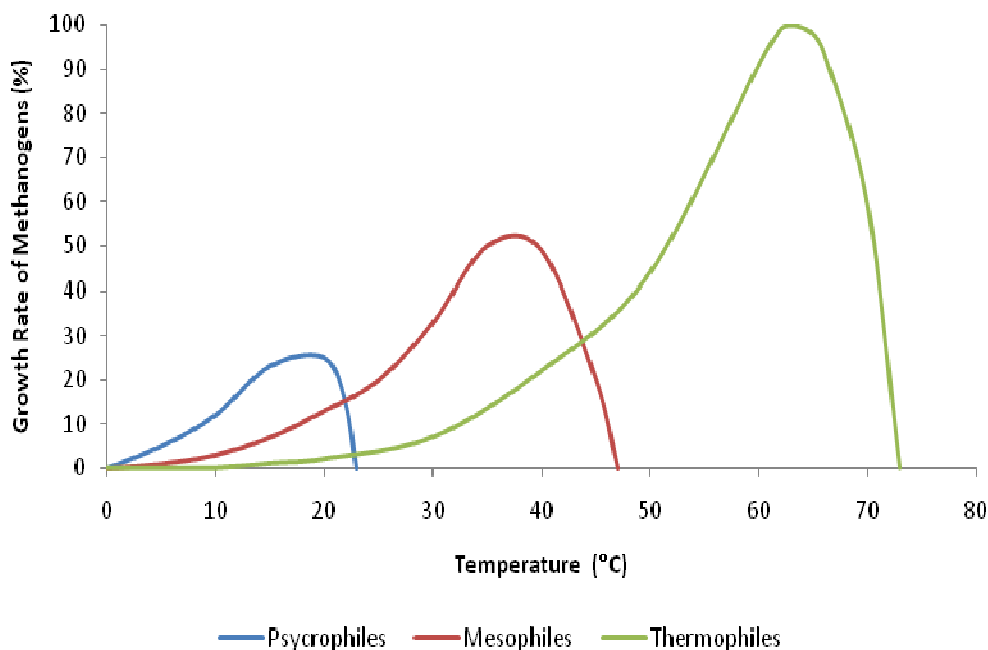


Figure 3-2 Influence of temperature on the growth rate of biomass at psychrophilic, mesophilic and thermophilic temperatures (adapted from Cherinichora (2007))

In general, full-scale anaerobic digesters are operated at ambient temperatures (20-25°C), mesophilic temperatures (30 to 37°C) or at thermophilic temperatures (50 to 55°C) (48). Studies have generally shown an increase in the conversion rates at higher temperatures, but also a decrease in the process stability (56). Two main disadvantages of operating at ambient temperatures and mesophilic temperatures are that the process cannot effectively kill pathogens and long retention times are required (14). Some authors have suggested that operating at thermophilic conditions can overcome these problems as higher organic loading rates can be fed due to a higher growth rate of the microorganisms (14; 18). Operating at thermophilic temperatures does however, affect the digester stability significantly. It was shown that thermophilic digesters could only tolerate a $0 \pm 0.8^\circ\text{C}$ change in the operating temperature while the concentration of the VFA was always more than two times higher than in a mesophilic digester (57; 56). Digesters operated at lower temperatures have lower energy requirements than digesters operated at thermophilic temperatures. The economical viability of operating at higher temperatures depends greatly on the design and questions are still asked whether the additional energy costs for thermophilic digesters justifies the advantages associated with thermophilic operations (14).

b) Alkalinity, pH and VFAs

The pH inside the anaerobic digester gives an indication whether the culture media is acidic or basic of nature. The anaerobic process is highly dependent on the pH due to the variation of optimum growth ranges of the different anaerobic bacteria (14). The pH can affect the anaerobic process directly by affecting the enzymatic activity drastically (when changes in the pH occur) or indirectly by affecting the toxicity of a number of substances in the system (48). Methanogenic bacteria are very sensitive to low pH operations and the pH range at which optimum growth will be achieved is believed to be between 6.6 and 7.4. Even though stability in methane production may also be attained at a wider range (between 6 and 8), pH levels above 8.3 and below 6 should be avoided as it can cause inhibition in the methanogenic bacteria activities (48; 56). The acidogenic bacteria on the other hand experience optimum growth at a pH range of 5 to 6 and can tolerate lower pH levels better than methanogenic bacteria. In order to avoid reactor failure, anaerobic processes are operated at conditions to

minimize or eliminate the inhibition of the methanogenic bacteria by controlling the pH at appropriate levels (48).

The buffer capacity (alkalinity) of the anaerobic system is the capacity of the system to avoid a change in the pH caused mainly by carbonic and volatile acids. Usually, a buffer solution contains a combination of weak acids and their salts. This enables the H^+ and OH^- ions to group in such a way to avoid the pH to increase or to decrease (48). In order to keep the pH in the range of 6.6 to 7.4 in anaerobic processes it is crucial that the system provide enough alkalinity to neutralise any VFA which could have possibly accumulated. In the pH range of 6 to 8, the carbon dioxide-bicarbonate systems is the key chemical system which is responsible for the control of the alkalinity and the bicarbonate alkalinity are the most significant contributor to the total alkalinity and can be represented by Eq. 3-3 (56).



In the anaerobic system, accumulation of volatile acids may sometimes occur and some interaction between the volatile acids and the alkalinity exists which are based on the merit whether the alkalinity in the system is able to neutralise the accumulated acids in order to ensure that the pH does not drop below 6. Volatile acids are stronger than bicarbonates and will therefore, when present in the system, destroy the bicarbonate alkalinity. Consequently, excess alkalinity will be required in order to compensate for the bicarbonate alkalinity lost during its reaction with the volatile acids and to ensure that the stability in the system is maintained by keeping the pH in the appropriate range (48; 56). The main compounds which contribute to the buffering capacity when the pH is around 7 is bicarbonate, hydrogen sulphide, dihydrogen phosphate and ammonia (14).

When choosing an appropriate chemical to add to the system as an alkalinity supplement, it is important to evaluate the applicability and the economical aspects of each chemical compound. There are a number of chemicals which can be used as an alkalinity supplement, including sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), sodium hydroxide ($NaOH$), lime (CaO), hydrated lime ($Ca(OH)_2$), ammonia bicarbonate (NH_4HCO_3) or it can be generated in the system through degradation of proteins to ammonia (56; 48). Of these

chemicals, lime is the most commonly used material, as it is relatively inexpensive and readily available, but serious operational problems may occur when using lime, as the calcium salts is fairly insoluble (58). Another reason why lime can be problematic is the fact that it reacts with carbon dioxide to form calcium bicarbonate. If the carbon dioxide in the system is not enough to consume all the lime, the pH may rise drastically. This may be just as dangerous to the system as in the case of very low pH values. On the other hand, when considering NaOH, NaHCO₃ or Na₂CO₃ as alkalinity supplement, it must be noted that high sodium concentrations should however, be avoided as it may be toxic to the anaerobic process (56).

When monitoring the anaerobic system, it is important that both pH and alkalinity are evaluated and not just the pH. The reason for this is that due to the logarithmic scale of the pH, a small change in the pH of the system will reduce the buffer capacity of the system quite drastically by consuming a large amount of alkalinity (48). The most commonly used method to estimate the alkalinity of the system is to titrate the sample with a standardised HCl solution (0.1N) to a pH of 4.3, which is the point at which 99% of the bicarbonate system will be converted to carbonic acid in (59). When VFA are present in the system it will cause more than 80% of the total VFA to be measured which will lead to an overestimation of the bicarbonate alkalinity when titrating to a pH of 4.3. In order to prevent this, an endpoint titration of a pH of 5.75 should be done in addition to the titration to a pH of 4.3 (60). By titrating to an end point of 5.75, 80% of the bicarbonate system and less than 20% of the VFA being measured (61). The titration to a pH of 5.75 is referred to as the partial alkalinity (PA), while the titration to a pH of 4.3 is referred to as the total alkalinity (TA or MA). The intermediate alkalinity (IA) is the difference between the partial and the total alkalinity, which is related to the VFA concentration. When the partial alkalinity and intermediate alkalinity of the digester content is known, the IA:PA ratio can be obtained. According to literature, IA:PA ratios below 0.3 indicates stable process operations., while an increase in the IA:PA ratio (above 0.3) is an indication of process upset (62). Partial alkalinity measurements below 1200 mg/L CaCO₃ have shown to indicate digester trouble (63).

The VFA concentration in anaerobic reactors is a very important parameter in monitoring the stability of anaerobic processes (14). It can be described as a reflection of a kinetic uncoupling between acid producing and acid consuming bacteria occurring typically under stress situations in anaerobic reactors (64). The build-up of VFA concentrations has therefore been identified as an important parameter for accurate control of anaerobic processes (64; 58). Accumulation of VFA concentrations inside the anaerobic reactor is usually accompanied by a drop in the pH in the reactor. Propionic and butyric acid have been identified to be the most reliable indicators of the metabolic condition of the anaerobic bacteria inside anaerobic reactors (14).

c) Nutrient concentration requirement

In order for anaerobic biological waste treatment to succeed, it is important that sufficient inorganic nutrients required for the growth of the microorganisms are available in sufficient quantities as the regeneration time of the microorganisms is a function of the nutrient concentration in the substrate (65). The specific nutrient requirement for a biological system is rather difficult to determine as it depends largely on a number of factors which is amongst others, the characteristics of the wastewater to be treated, the design of the treatment system and the amount of nutrients already present in the system (49). It is not always possible for ideal nutrient concentrations to be present in the system and some compensation should therefore be made by either lowering the operating loading rates or by reducing the efficiency of the anaerobic system (65). In literature, little is published on the nutritional requirements of the hydrolytic, acidogenic and acetogenic bacteria, but some more work has been done on the nutritional requirements of the methanogenic bacteria. The main nutrients of importance for the stimulation of growth of the methanogenic bacteria are (in decreasing level of significance) nitrogen, phosphorus, sulphur and micronutrients (such as sulphur, potassium, calcium, magnesium, iron, nickel, cobalt, zinc, manganese and copper)(14). Even though these elements are required in very low concentrations, their absence can affect the growth of the microorganisms severely (66).

Nitrogen is the nutrient required in the highest concentration, and under anaerobic conditions the main source of nitrogen is in the form of ammonia and organic nitrogen. The other forms in

which nitrogen can occur, nitrate and nitrite, will not be present for the growth of the microorganisms as it will be reduced to nitrogen gas (48). Phosphorus is also an essential nutrient needed for anaerobic digestion, but has been reported to only be required at concentrations of 14-20% of the required nitrogen concentration and can be integrated into the bacteria cells in the inorganic form as orthophosphate (PO_4^{3-}) by means of the enzyme phosphatases (48). The optimum ratio of carbon, nitrogen and phosphorus (C:N:P) to yield maximum volumes of methane was reported to be 1000:2.5:0.5, while that of COD:N:P is around 350:5:1 (66; 48).

Sulphur is an important nutrient needed for protein synthesis during anaerobic digestion. The methanogenic bacteria depends strongly on sulphur for growth, but when sulphur is present in the substrate, the sulphur reducing bacteria will also compete with the methanogenic bacteria for hydrogen and acetate. This may limit the methanogenic bacteria activity as the sulphate reducing bacteria have a lower half-saturation coefficient than the methanogenic bacteria for acetate and hydrogen, which makes the sulphur requirement for the methanogenic bacteria rather complex (65). If inorganic sulphate (SO_4^{2-}) is present in the substrate, the sulphate will be reduced to sulphide which will then form sulphur and the cysteine amino acid during a reaction with the serine amino acid (48). Sulphide is therefore also regarded as a sulphur source. The requirements for sulphide for anaerobic digestion are often disregarded as many sulphide sinks are present in the anaerobic digestion process, which include the presence of H_2S in the off-gas (biogas), the total amount of sulphides in the effluent, the microbial synthesis of sulphides and the precipitation of sulphides by metals (48; 65).

A large number of micronutrients (trace metals) are also of great importance in the anaerobic digestion process. These metals represent the micro-molecules of the bacteria cells and can comprise up to 4% of the cells' dry weight (48). The micro-nutrients which are of importance are iron, nickel, cobalt, molybdenum, selenium, riboflavin and vitamin B12 (57). The exact amount of these micro-nutrients needed is not easy to determine. This is because formation of sulphide (for the growth of methanogenic bacteria) causes the metals to precipitate, resulting in low equilibrium concentrations of these metals. In order to counteract this, acidified influent

can be added at regular intervals. This will disturb the chemical equilibria causing the metals to be temporarily available for uptake by the methanogenic bacteria (48).

d) Organic loading rate (OLR) and hydraulic retention time (HRT)

The OLR is a very helpful standard which is used to evaluate the performance of anaerobic reactors and can be defined as the organic material fed per unit volume of the reactor per unit time and is usually expressed in terms of $\text{g COD.L}^{-1}.\text{day}^{-1}$ (14). One of the main advantages of anaerobic digestion in wastewater treatment is that it allows for high OLRs and low sludge production when treating organic wastewaters. It has been shown that the OLR is greatly dependent on the type of substrate and type of reactor used as well as the operating parameters such as temperature (14).

The HRT can be defined as the time that the wastewater spends in the reactor and is calculated by dividing the daily influent flow rate by the reactor volume. This is one of the most important factors that influence the economical performance of anaerobic reactors. A shorter HRT will result into a smaller reactor volume which means lower capital cost (18; 14). The required HRT to successfully digest waste depends on the characteristics of the wastewater. Wastewater containing simplistic waste may only require hours to be digested in a high-rate reactor (such as a UASB reactor), but more complex waste (for example animal manure) will require HRTs of even longer than 10 days (14).

e) Toxic Material

The effective degradation of organic material by any biological process relies strongly on the maintenance of the environmental conditions for the microorganisms. This also includes the control and/or removal of material which are toxic to the microorganisms (48). Any substance, when present in high enough concentration, will be toxic to the microorganisms. It is therefore, important to determine at which concentration a specific substance becomes toxic to the microorganisms, whether the effect of toxicity is reversible or antiseptic and whether the microorganisms have the potential to acclimatise when intoxicated (48). The concentration at which inhibition or toxicity occurs differs for different substances and may range from lower than 1 mg/L to more than $1\,000 \text{ mg.L}^{-1}$ (67).

When microorganisms are inhibited by a certain substance, the microbial population and bacterial growth are affected negatively. An indication of inhibition is usually accumulation of organic acids or a decrease in the steady state methane gas production rate (55). There are a number of substances which can be inhibitory to the anaerobic digestion process, which includes ammonia, sulphide, light metal ions (salts), heavy metals and organics (55). At low concentrations, most of these substances will cause an increase in the stimulation of growth of the microorganisms up to a certain stimulation concentration after which a decrease in stimulation will be experienced. Once the rate of the biological activity is noted to be less than when the substance is not present in the system, the substance are then said to be toxic to the microorganisms. When the concentration of the substance is increased even more, the biological activity will approach zero. This can be seen in Figure 3-3 (67).

Most microorganisms do however, have some measure of resistance to the inhibition of toxic substances, providing that the effect caused by the substance is minimised by design parameters such as short retention times of the toxic substance or long solid retention times. Some methods which may be employed to control the effect of toxicity include the removal of the toxic materials from the wastewater prior to entering the bioreactor, dilution of the wastewater below the toxicity threshold, the formation of insoluble precipitate or complex or by addition of antagonistic material (an antagonistic material will decrease or antagonise the toxicity of some compounds) (67; 48).

Toxicity by ammonia

Ammonia is produced when nitrogenous materials (such as proteins or urea) are biologically degraded. The most common forms of inorganic ammonia present in an aqueous solution are free ammonia (NH_3) and the ammonium ion (NH_4^+). Both of these two forms of ammonia can cause inhibition, but the free ammonia is believed to be the most significant inhibitor as it is membrane permeable and is therefore able to diffuse into the cell and cause potassium deficiency and/or an imbalance in the proton (68; 55).

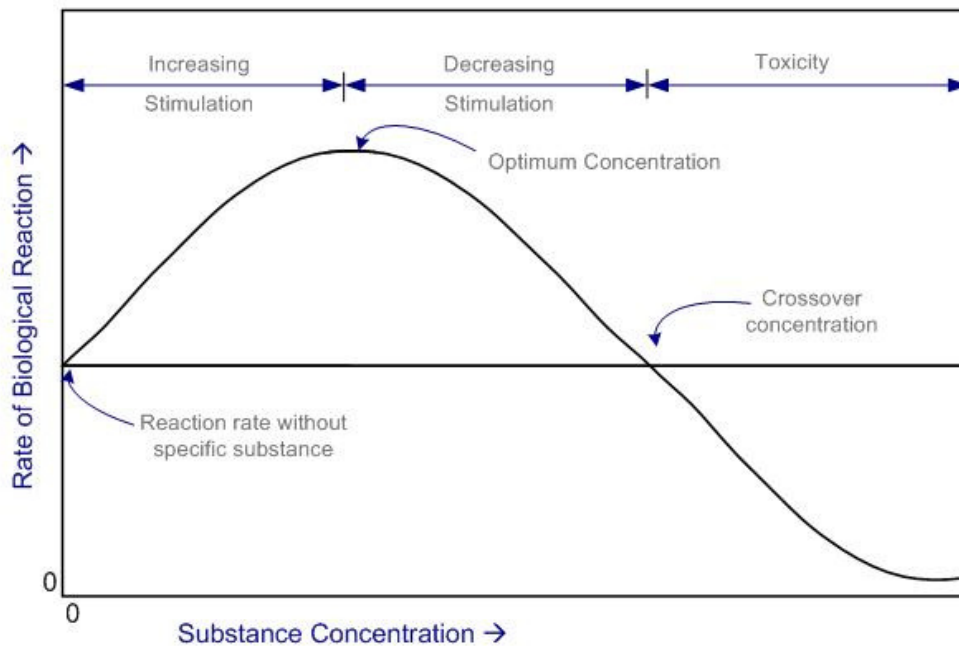
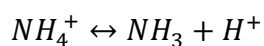


Figure 3-3 Effect of substance concentration on biological reactions (adapted from ref. (67))

A number of factors are believed to control the inhibition caused by ammonia, including the concentration of ammonia, pH, temperature, the presence of other ions and the acclimation of bacteria (55). It is believed that the methanogenic bacteria are much more susceptible to changes in the environment than other anaerobic bacteria as they are very sensitive bacteria and their growth will therefore most likely be inhibited by ammonia (69). This was shown in a study in which the concentration of ammonia was increased from 4 051 to 5 734 mg.L⁻¹ and the population of the methanogenic bacteria decreased by 56.5%, while the acidogenic bacteria population hardly showed any decrease (70; 55).

When high concentrations of H⁺ ions are present, the pH will increase, shifting the reaction shown in Eq. 3.4 to the left and the inhibition will be caused by the increased concentration of NH₄⁺ ions.



Eq. 3-4

An increase in pH has shown to increase the free ammonia (NH_3) to ionized ammonia (NH_4^+) ratio and as the free ammonia is said to be the most significant inhibitor, the increase in pH will cause an increase in toxicity. An accumulation of VFA can occur due to process instability caused by ammonia, causing the pH to drop, which will again cause a decrease in the free ammonia concentration. There is therefore, an interaction between pH, VFA concentration and free ammonia concentration which may eventually result in a condition called an “inhibited steady state”, in which the process is at steady state, but the methane yield is significantly lower than usual (71; 72). Table 3-3 shows the effect of the free ammonia concentration on the anaerobic process as found in literature.

Table 3-3 Effect of NH_3 on anaerobic digestion (adapted from ref. (67))

Concentration (mg/L N)	Effect
50 - 200	Beneficial
200 – 1000	No adverse effect
1500 – 3000	Inhibitor for pH greater than 7.4
> 3000	Toxic

Ways to counteract ammonia inhibition includes air stripping, chemical precipitation, diluting the ammonia in substrate, increasing the biomass retention time, using inert material (such as clay, activated carbon or zeolite) to immobilize microorganisms, adding substances (such as ion exchange or adsorbants) which can remove or decrease ammonia inhibition or adding antagonistic cations such as Mg^{2+} , Ca^{2+} or Na^+ (55).

Toxicity by sulphide

Sulphides can be present in the anaerobic system due to its presence in the wastewater entering the system and due to protein degradation and the reduction of sulphates and other sulphur containing compounds by the sulphate reducing bacteria (SRB) (67). The H_2S which forms during the reduction of sulphate will dissociate in water according to Eq. 3.5 and Eq. 3.6.

This dissociation is dependent on the pH and temperature of the medium. Figure 3-4 shows the distribution of H₂S at different pH values at 25°C.

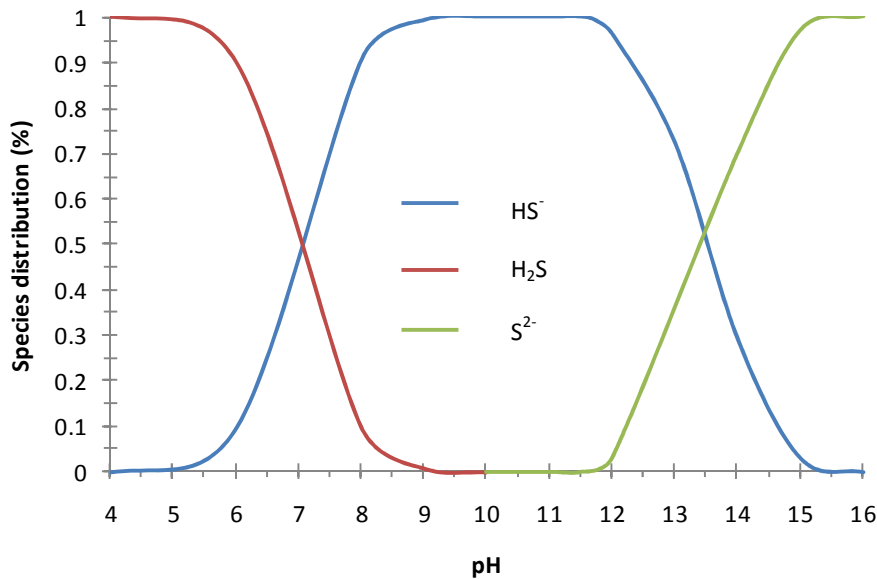


Figure 3-4 Distribution diagram for H₂S at 25°C (adapted from ref. (48))

It can be seen that at pH values lower than 7, H₂S is the main dissolved component. At pH values between 7 and 14, HS⁻ is the main component, while at pH levels higher than 14, S²⁻ prevails, but this concentration range is negligible when considering anaerobic waste treatment (48). As the pH in anaerobic reactors is usually around 6.5-8, inhibition by sulphides depends strongly on the concentration of the H₂S in the medium. Due to the limited solubility of H₂S, some H₂S will be present in the gas phase and will escape with the methane gas formed during the anaerobic digestion. As the methane gas production increases, the amount of H₂S in the gas phase will also increase, causing a decrease in the amount of H₂S in the solution (67). Anaerobic microorganisms can tolerate soluble sulphide concentrations between 50 and 100 mg.L⁻¹ with very little acclimation time, while, concentrations up to 200 mg/L can be tolerated without very

little inhibitory effect on the microorganisms. Sulphide concentrations above 200 mg.L⁻¹ will however, be toxic to the microorganisms (67).

Toxicity by sulphides can be decreased or prevented in a number of ways, which includes the separation of the sulphate and sulphur containing streams from the wastewater stream to be treated, by scrubbing the digester gas, by diluting the wastewater stream to be treated or by precipitation of sulphides by using iron salts (67).

Toxicity by salts (light metals)

Light metal ions are important for the growth of microorganisms (such as Ca²⁺, Mg²⁺, K⁺ and Na⁺) and are usually present in the substrate of anaerobic digesters due to organic matter breakdown or as a result of addition of pH adjustment substances (such as NaOH). When these metal ions are present in too high concentrations, the growth rate can be affected negatively, while very high concentrations can even result in intoxication and inhibition (55). Table 3-4 shows a summary of the concentration ranges at which these metal ions are most likely to stimulate, moderately inhibit and strongly inhibit the anaerobic process.

Table 3-4 Stimulatory and inhibitory concentrations of light metal ions (adapted from ref. (67))

Cation	Stimulatory (mg.L⁻¹)	Moderately Inhibitory (mg.L⁻¹)	Strongly Inhibitory (mg.L⁻¹)
Na ⁺	100 – 200	3500 – 5500	8000
K ⁺	200 – 400	2500 – 4500	12000
Ca ²⁺	100 – 200	2500 – 4500	8000
Mg ²⁺	75 – 150	1000 – 1500	3000

In some cases, the possibility exists that an inhibitory effect of one metal cation can be reduced by another antagonistic metal ion which can either be present in the system or added to the system (67). Potassium and sodium are said to be very good antagonists and best results will be observed when they are present at concentrations which would stimulate growth as shown in Table 3-4. Concentrations higher than the stimulatory concentrations will not be as effective

and will in fact enhance the toxicity. Magnesium and calcium on the other hand, are not good antagonists and will, when present in the system or added to the system enhance the toxicity caused by other light metal ions (67). Usually, antagonistic metal ions are added to an anaerobic system by adding chloride salts, but if this is not economically viable or beneficial to the process, the waste should be diluted (48).

Toxicity by heavy metals

Heavy metal ions are most often present in industrial and municipal wastewater. Due to fact that heavy metals are not biodegradable, accumulation is unavoidable and if these metals are not removed they will be toxic to the anaerobic process. The toxicity effect of heavy metals is said to cause disruption in the enzyme structure and functionality (55). Heavy metals which can occur in wastewater and which are reported to significantly influence anaerobic digestion is chromium, nickel, cadmium, zinc, copper, cobalt and iron (73). The concentrations at which these metals become toxic to the anaerobic bacteria are related to the sulphide concentration in the system. The reason for this is that the heavy metals will react with sulphide to form insoluble sulphide salts which are not toxic to the anaerobic bacteria even though sulphides and heavy metals on their own are toxic when present in to high concentrations (67). It is therefore important that enough sulphides are available in the system when high concentrations of heavy metals are present in order to precipitate these metals. If this is not the case, sulphides can be added to the system in various forms. Table 3-5 provides the concentrations at which certain heavy metals (copper, nickel and zinc) will precipitate when 1 mg.L⁻¹ of different forms of sulphides is added.

Table 3-5 Concentration of heavy metals precipitated (adapted from ref. (67))

Sulphide salt added		Concentration of heavy metals precipitated
[1 mg.L⁻¹]		[mg.L⁻¹]
Sulphides	S ²⁻	1.8 – 2.0
Sodium Sulphide	Na ₂ S	0.75 – 0.84
Sodium Sulphide	Na ₂ S·9H ₂ O	0.24 – 0.27

Toxicity by organic material

A large variety of organic compounds has shown to be inhibiting anaerobic digestion when present in high concentrations. This usually includes organic compounds which are either insoluble or poorly soluble in water and apolar compounds. Examples of these chemicals (stated in literature) include benzenes, phenols, alkanes, alcohols, ethers, ketones, carboxylic acids, nitriles, amides and amines (55). The toxicity of these compounds are affected by operating parameters such as the concentration of the toxicant, exposure time of the toxicant, concentration of biomass, age of cells, feeding strategy, temperature and acclimation (74; 55). Some of these organic materials are only toxic at high concentrations and can therefore be anaerobically degraded at low concentrations if fed to the system continuously. This will cause the organic compounds to be degraded as soon as they are added to the system and therefore, maintaining the concentration of the organic compound low enough (67).

3.1.4 Biogas potential and methane production

As mentioned earlier, biogas is one of the main products formed during anaerobic digestion. The biogas compositions and the biogas volume (or production rate) produced are therefore important parameters in the anaerobic digestion process. The main composition of the biogas is methane and carbon dioxide and trace amounts of hydrogen, ammonia, hydrogen sulphide, water vapour and some other gasses (50). The normal composition of the biogas is usually 60-70% methane and 30-40% carbon dioxide of which only the methane can contribute to the energy content of the biogas. Methane by itself has an energy content of 37 MJ.m⁻³ (at STP), but the presence of carbon dioxide with the methane reduces the energy content of the biogas to between 22 and 26 MJ.m⁻³ (at STP) (50). The methane yield (Y_{CH_4}) is a very significant parameter in anaerobic digestion processes as it gives an indication of how well the process recovers the nett energy and is given in Eq. 3-7. It can be defined as the volume of methane produced for a certain amount of COD removed per volume of wastewater fed to the reactor per day (50).

$$Y_{CH_4} = \frac{Q_{CH_4}}{Q_F(COD_{inf} - COD_{eff})}$$

Eq. 3-7

Under ideal, steady state conditions for a specific carbon substrate, this value is constant. The maximum theoretical methane yield (volume of methane that can be produced per unit COD removed) at STP with pure glucose as substrate is $0.35 \text{ L}_{\text{CH}_4} \cdot (\text{g COD}_{\text{removed}})^{-1}$ (15). If this theoretical maximum methane yield is reached, it will show that that microorganisms use the carbon only for maintenance, growth and respiration. Values in literature for methane yield in general vary between 0.10 and $0.35 \text{ L}_{\text{CH}_4} \cdot (\text{g COD}_{\text{removed}})^{-1}$, but according to Droste (50), a conservative value of $0.2 \text{ L}_{\text{CH}_4} \cdot (\text{g COD}_{\text{removed}})^{-1}$ should be used. This deviation from the theoretical methane yield can be due to gas leakages or due to compounds broken down which cannot be oxidised under the conditions at which the COD test is done (50).

3.2 Anaerobic wastewater treatment technologies

3.2.1 Advantages and disadvantages of anaerobic wastewater treatment

One of the main advantages of anaerobic treatment processes is the low amount of surplus sludge produced during the operation. This is due to the fact that as much as 95% of the biomass can be converted to biogas (methane + carbon dioxide) and the rest are used by the cells for food and maintenance. This is however, not the case for aerobic systems. The energy requirement for anaerobic treatment systems is also generally lower than that of aerobic treatment systems as no aeration is required for anaerobic treatment. The biogas which forms during anaerobic digestion can be used as a heat or power source, which is a great advantage in South Africa currently due to the energy crisis which is currently experienced (75). Another advantage of anaerobic wastewater treatment is the small area required compared to aerobic systems (13). Some disadvantages associated with anaerobic treatment systems include the high initial capital cost as well as the long start-up periods. Anaerobic treatment systems also require stricter operational control, since the methanogens are very sensitive to pH and temperature changes and works optimally at a pH around 7 and temperatures between 33 and 37°C (75). Some more advantages and disadvantages of anaerobic wastewater treatment are listed in Table 3-6.

3.2.2 Anaerobic reactors used in treating dairy industry wastewater

Although various laboratory scale and pilot scale studies have been conducted in the anaerobic treatment of dairy effluents, very few dairy plants actually use anaerobic treatment methods to treat their effluents. Although the application of anaerobic wastewater treatment methods in the dairy industry is limited, the examples that are in fact mentioned in literature show that the anaerobic digestion is a feasible treatment method for dairy effluent (12). It is not always easy to categorise the different wastewater treatment technologies as the definition of different wastewater methods most often overlap. However,, when biomass retention is considered, most anaerobic processes can be categorised into four main types, namely, attached biofilm systems, gravitational settling systems, mechanical separation of solids systems and completely mixed systems (without any biomass retention) (12).

In this literature review, the following 11 digester types are discussed briefly:

- Anaerobic ponds;
- Completely stirred anaerobic reactors (CSTR);
- Anaerobic contact reactors;
- Anaerobic sequencing batch reactors (ASBR);
- Anaerobic filters;
- Upflow anaerobic sludge blanket reactors (UASB);
- Anaerobic fluidised and expanded bed reactors;
- Anaerobic membrane bio-reactors (AMBR);
- Anaerobic hybrid reactors; and
- Two-phase anaerobic reactors.

Each of these anaerobic reactor configurations have been used in the treatment of dairy factory effluent.

Table 3-6 Advantages and disadvantages of anaerobic wastewater treatment (adapted from ref. (48; 49)

Advantages	Disadvantages
Low biomass production	Complex biochemistry and microbiology which are not yet fully understood
Low construction cost	Can produce bad odours
Small area of land required	Microorganisms are inhibited by number of factors and conditions
Low nutrient utilization	Long start-up periods if seed sludge is not present
Low energy utilization	Not as efficient in removal of nitrogen, phosphorus and pathogens
Produces Methane which can be used as energy source	High concentrations of NH_4^+ necessary for best biomass activity
Can handle high organic loads	
Can biodegrade aerobic non-biodegradable substances	
Can reduce toxic levels caused by chlorine	

a) Anaerobic ponds

Anaerobic ponds are deep earthen basins in the ground which are free of any dissolved oxygen in order to create an anaerobic environment and are usually covered to eliminate air and to prevent loss of biogas to the atmosphere (75). The lagoon is usually 5 to 10 meters deep to allow for sedimentation and solids and to allow for anaerobic digestion of some retained sludge and soluble organic matter (15). The raw wastewater usually enters the lagoon at the bottom after which it mixes with the biomass in the sludge blanket. Aeration is sometimes supplied to the surface in order to control bad odours. The wastewater is discharged at the discharge port which is located opposite to the influent port. The lagoon is usually covered with a floating membrane cover which is lined in the inside with a layer of closed-cell polyethylene insulation (15). Anaerobic lagoons are most often used for the pre-treatment of high strength industrial wastewater or the pre-treatment of municipal wastewaters and the effluent is most often not appropriate for release into receiving waters and should therefore be treated further usually by

means of facultative or aerobic lagoons (76). According to literature, typical loading rates should be lower than $2 \text{ g.L}^{-1}.\text{day}^{-1}$ (16).

In the treatment of dairy industry wastewater, anaerobic lagoons followed by a clarifier and an aerated lagoon have been successfully used in New Zealand to obtain an overall COD removal efficiency of 99% at OLRs of $1.5 \text{ g.L}^{-1}.\text{day}^{-1}$ and HRTs of 1 to 2 days (75).

b) Completely stirred tank anaerobic reactor (CSTR)

The completely stirred tank reactor (CSTR) is used extensively in the digestion of high-strength organic wastes and is, after lagoons, the most simple anaerobic reactor configuration. This process consist of an anaerobic reactor system in which the wastewater entering the system is completely mixed with the microorganisms by means of pumps, mechanical stirrers or biogas recycling (77). The main feature of the CSTR process is that the solid retention time (SRT) is equal to the HRT and the biomass is therefore not retained (14). Due to the mixing inside the reactors, the concentrations of solids in the effluent is the same as that in the reactor and a lot if bacteria are therefore lost if the bacterial washout rate exceeds the bacteria growth rate (77). These digesters are usually used for wastes high in organic matter (COD values above $30\,000 \text{ mg.L}^{-1}$) with the majority of the polluted matter being suspended solids. A laboratory scale anaerobic CSTR has been successfully used in the treatment of cheese factory wastewater in which a 78-90% COD removal efficiency was obtained at HRT of 9 days and influent COD of $17\,000 \text{ mg.L}^{-1}$ (78). In another study, synthetic dairy wastewater was treated in a laboratory scale CSTR at an OLR of 0.015 to $0.23 \text{ g VS.day}^{-1}$ with a maximum COD removal efficiency of 60 % (79). Although the CSTR performs well as a lab-scale technology, it is impractical to implement as a full-scale treatment technology due to the biomass washout and HRT limitations (HRT equal to SRT). The working volume of an efficiently operated UASB reactor which operates at an HRT of 1 day will be 9 times smaller than a CSTR operated at a HRT of 9 days when treating the same effluent (12). According to literature, typical loading rates for a CSTR range between 1 and $5 \text{ g.L}^{-1}.\text{day}^{-1}$.

c) Anaerobic contact reactors

The anaerobic contact process is an improvement on the CSTR process as it allows biomass to be separated from effluent and to be recycled back to the completely mixed reactor, which causes the SRT to be longer than the HRT (15). The most common method used to separate the solids from the effluent is by means of gravity separators, but methods such as gas flotation has also been used. The anaerobic contact process uses a degasifier to remove biogas that may have been entrapped and a clarifier that allows the solids to settle out, after which the solids are recycled back to the reactor to increase to solid retention time (80). However, the anaerobic contact process is, rather old-fashioned and not really considered as an effective and economical treatment method (12). According to literature, typical loading rates for anaerobic contact reactors range between 1 and 8 g.L⁻¹.day⁻¹ (16).

d) Anaerobic sequencing batch reactors (ASBR)

The ASBR process is a simple wastewater treatment technology in which wastewater is treated in an anaerobic batch reactor and consists of four operational steps, namely feed, react, settle and decant. This process allows good control on effluent quality, high production of biogas and the high substrate concentration at the beginning of the process and low substrate concentration at the end of the operation. This allows effective separation of sludge (81). This process is gaining a lot of interest lately due to the simplicity of the operation and control of the system and the little space occupied by the reactor compared to conventional systems (13). The ASBR process has been successfully employed in the laboratory treatment of a number of dairy effluents including dairy manure wastewater (18), non-fat dry milk (19; 20; 21; 22), synthetic wastewater (82) and a combination of landfill leachate and dairy wastewater (23). The ASBR process is discussed in more detail in Section 3.3. According to Metcalf & Eddie (15), typical loading rates for ASBRs range between 1.2 and 2.4 g COD.L⁻¹.day⁻¹, but higher OLRs up to 9 g COD.L⁻¹.day⁻¹ have been recorded in recent literature (see Table 3-9).

e) Anaerobic filters

Anaerobic filters can be described as packed biological reactors which are filled with a media such as plastic particles or rocks, which forms channels in which the water can flow and which

enlarge the contact surface area. Wastewater can be introduced either at the bottom or at the top and the water is then allowed to flow over the media. Bacteria are present on the surface of the media as well as in the channels in which the water flows throughout the whole reactor (50). The main disadvantage of this wastewater treatment method is the possibility of clogging of the channels by suspended solids, biomass or precipitated minerals (75). According to literature, dairy wastewater has been treated in various full-scale and pilot plant anaerobic filter reactors of which the reported COD removal efficiency ranged between 60 and 98% for OLR which ranged between 1.7 and 20 g COD L⁻¹day⁻¹ (75). In a study treating ice-cream wastewater, removal efficiencies up to 80% was achieved at OLRs up to 21 g COD L⁻¹day⁻¹ and HRT of 0.5 days (83). According to Deublein and Steinhauser (16), typical loading rates for anaerobic filters range between 10 and 20 g COD.L⁻¹.day⁻¹.

f) Upflow Anaerobic Sludge Blanket Reactor (UASB)

The UASB consists of a reactor of which the lower portion contains a dense “blanket” of activated sludge from granules or flocculants. The wastewater enters at the bottom of the reactor and the waste is converted to CO₂ and CH₄ gas after contact with the sludge blanket. The gas bubbles that form move through the reactor, causing the solids to float to the top. These gas bubbles provide sufficient mixing in the bed so that no external recycling of solids is necessary. A gas-liquid separator is situated at the top of the reactor and the design is essential in order to obtain the highest possible sludge hold-up (50). The success of the UASB reactor depends greatly on the granulation of biomass in the reactor as the presence of granules causes the HRT and the STR to be separated from each other. Some advantages associated with the UASB process is that it occupies little space and can treat high amounts of wastewater from food industries in short periods of time. However, a drawback of the UASB process is, as in the case of most anaerobic process, a long start-up period.

In the dairy industry, UASB reactors have been used successfully for nearly 20 years to treat wastewater (13). Several laboratory scale UASB reactor studies have been employed to treat wastewater from different sectors of the dairy industry such as wastewater from the cheese-producing industry, ice-cream industry of which the COD removal efficiencies ranged between

81 and 99% (84; 85; 86; 87). Literature describes a very successful UASB plant in the UK at South Caernarvan Creameries. The system is a combination of a UASB reactor and an aerobic denitrification system with a capacity of 2000 m³. A 95% COD removal efficiency was recorded, while the process produced enough biogas to meet the electricity need for the whole dairy factory (12; 13). The internal circulation system is a more advanced anaerobic treatment process based on the UASB process which has been developed recently. This process is capable of treating high upflow gas and liquid velocities, making it possible to treat high-strength wastewater at high volumetric flow rates as well as low-strength wastewater at short HRTs (13). Deublein and Steinhauser (16), suggests that typical loading rates for UASB reactors range between 2 and 24 g COD.L⁻¹.day⁻¹.

g) Anaerobic packed-bed reactor (Fixed film reactor)

The anaerobic packed bed reactor configuration consists of an upflow reactor with fixed packing through which the wastewater flows upwards through the porous spaces between the packing materials (15). The surface-to-volume ratio is an important parameter in this reactor configuration and it is important that the optimum ratio is achieved. The optimum ratio differs for each type of packing material. The first anaerobic packed-bed reactors used rock as packing material, but more recently a wide variety of synthetic plastics are used as packing material. This reactor type can either be operated in an up-flow or down-flow configuration, depending on whether the wastewater is introduced at the top or at the bottom of the reactor. In a study treating cheese whey in a fixed film anaerobic reactor, COD removal efficiency of 75 % was achieved at an OLR of 13 g COD.L⁻¹.day⁻¹ and HRT of 4.9 days (88).

h) Anaerobic fluidised and expanded bed reactors

In fluidised bed reactors, bacteria are grown on the media which consist of particles such as sand particles, plastic granules, glass beads, activated charcoal chips or clay particles. Liquid is pumped through the system at a very high rate, causing it to suspend, and thus, enhancing mass transfer. With an increase in the fluid velocity through the reactor, the particles are forced apart and instead of resting on each other, they are supported by the liquid. The movement of the particles are also increased as the fluid velocity increases (50). One of the main advantages

associated with this reactor configuration includes the reduced reactor size due to the large surface area which is available for the bacteria to grow on. Another advantage is that the constant fluid velocity eliminates problems associated with plugging, channelling and gas hold-up (12). In one specific study, dilute dairy wastewater (with COD between 200-500 mg.L⁻¹) was treated in a fluidised bed reactor in a study to determine the effect of HRT and temperature and an 80% COD removal efficiency at an 8 h HRT was achieved (89).

i) Anaerobic membrane bioreactors

The Anaerobic Membrane Bioreactor (AMBR) can be described as an anaerobic bioreactor (any configuration), coupled with a membrane unit which is used for filtration. The anaerobic microorganisms are not washed from the system, allowing them to reproduce inside the reactor and it also separate the HRT and the SRT from each other. This results in a high biomass concentration inside the reactor, making the treatment of high-strength wastewater possible with this treatment method (90).

The treatment of artificial and industrial dairy wastewater with a pre-acidification reactor in series with an anaerobic membrane bioreactor has been studied and it was found that the preacidification reactor performed rather well, but the performance of the AMBR was insufficient with a COD removal efficiency lower than 80% (91).

j) Anaerobic hybrid reactors

The anaerobic hybrid reactor can be described as a combination of anaerobic reactor configurations (for example the combination of an UASB and a packed bed reactor). The advantages of this reactor configuration is that it combines the advantages of the two reactor types, making it very effective in wastewater treatment. In a study to evaluate the feasibility of treating dairy wastewater with a hybrid reactor, synthetic dairy wastewater with influent COD concentration of 10 000 mg.L⁻¹ was treated in a hybrid reactor (which is a combination of a packed bed and UASB reactor configurations). The OLR was varied between 0.82 and 6.11 g COD.L⁻¹.day and the HRT was varied between 4.1 and 1.7 days. Their system achieved a 90 % COD removal efficiency with an effluent pH of 7.2, making the wastewater more acceptable for irrigation. At HRT of 1.7 days, a methane yield of 0.354 L_{CH₄}.(g COD_{removed})⁻¹ was

achieved (12). According to Deublein and Steinhauser (16), typical OLRs for anaerobic hybrid reactors range between 10-20 g COD.L⁻¹.day⁻¹.

k) Two-phase systems

The two-phase anaerobic operation is when the acidogenic (acid forming) and methanogenic (methane forming) bacteria are separated into two separate reactors. The usual operation of anaerobic systems is single-phase reactors in which the acidogenic and methanogenic bacteria live together under a delicate balance. These two types of bacteria do, however, differ significantly in terms of growth kinetics, nutritional needs, physiology and sensitivity to changes in the environmental conditions, making it difficult to find conditions where all the types of bacteria operate optimally. When the OLR fed to a single-phase reactor is too high, it may result in reactor failure as the increased VFA concentration will inhibit to the methanogenic bacteria (92).

Separation of the single-phase anaerobic system into a two-phase system may result in significant increase in the efficiency of the overall process as well as the control and stability of the process (93). It also allows an increase in the allowable organic loading rate as well as the activity of the methanogens in the methanogenic reactor. Shorter start-up periods of high rate systems have also been reported (93). Other potential advantages of the two-phase system are that materials that may be toxic to the methanogenic bacteria can be removed in the acidogenic phase reactor, as well as a potential reduction in total reactor volume (94). Several studies have investigated two-phase anaerobic digestion processes for dairy effluent (95; 94; 96; 92; 97; 18).

Table 3-7 shows a summary of various dairy effluents treated with different anaerobic treatment methods, while Table 3-8 shows some advantages and disadvantages of the different anaerobic treatment methods discussed.

Table 3-7 Performance of several anaerobic reactors treating dairy wastewater (adapted from ref. (13))

Substrate	Reactor configuration	OLR [g COD.L ⁻¹ .day ⁻¹]	Temperature [°C]	HRT [days]	COD removal efficiency [%]	Methane Yield [L _{CH₄} ·g COD _{removed}]	Source
Cheese wastewater	ASBR	1.8±0.78	35	22±13	93	0.17±0.13	(91)
Whey	ASBR	1.6-12.8	50	40-5	68-95	0.122-0.263	(96)
Synthetic dairy wastewater	ASBR	1.5-6.25	35		90-97		(82)
Synthetic dairy effluent	Hybrid reactor	0.82-6.11	35	4.1-1.7	90-97	0.206-0.354	(12)
Cheese whey	UASB				>97		(86)
Wastewater from cheese production	UASB	31.0			90		(87)
Wastewater from cheese production	UASB	2.3-4.5	35	10-20	71-91		(84)
Cheese factory wastewater	CSTR	0.63	35	9	78-90		(78)
Ice-cream wastewater	Anaerobic filter	21		0.5	80		(83)
Cheese whey	Anaerobic fixed film reactor	13		4.9	75	0.28	(88)

Table 3-8 Summary of advantages and disadvantages of several anaerobic reactor configurations (adapted from ref. (12; 15; 77; 14; 16)

Reactor Type	Advantage	Disadvantage
Anaerobic ponds	<ul style="list-style-type: none"> • Ability to handle wide range of wastes such as oils, solids and greases • Simple and economical construction • Large volumes can be handled 	<ul style="list-style-type: none"> • Large amount of land required • Labour intensive as geomembrane cover needs to be maintained • Need for final polishing step • Can only handle low $ODLRs$
Anaerobic contact reactors	<ul style="list-style-type: none"> • SRT controlled separately from HRT • Shorter HRTs required • Reduced reactor volumes 	<ul style="list-style-type: none"> • Requires separate clarifier for solids separation • Solid-liquid separation can be inefficient due to gas formation during separation phase • Solids settling characteristics not always good and other, more expensive separation technologies required
CSTR	<ul style="list-style-type: none"> • Can process wastes with high suspended solids contents • Consistent spreading of microorganisms, substrate and heat throughout the reactor • Thorough mixing prevent formation of scum layer • Can easily be modelled • Mixing sustain minimum concentrations of inhibiting substances at one specific point in reactor • Minimum plugging, gas entrapment and channelling 	<ul style="list-style-type: none"> • Large reactor volumes required • Large energy requirement in order for adequate mixing • Complete mixing difficult to achieve at industrial scale • Volumes of substrate not completely digested leaves reactor sometimes limited optimisation of environmental conditions as separation of acid-forming bacteria and methane-forming bacteria not possible • Microorganisms are lost with digester effluent • No biogas retention

Table 3-8 Continued

Reactor Type	Advantage	Disadvantage
ASBR	<ul style="list-style-type: none"> • Simple operation • Easy to control • Reduced reactor volume since all steps happen in one reactor • Lower investment cost due to smaller reactor volume • No separate clarifier required • Intermittent operations cause high biogas production 	<ul style="list-style-type: none"> • Full-scale operations not yet implemented • Sludge settling may be a problem
Anaerobic Filters	<ul style="list-style-type: none"> • Lower energy requirements as no mixing is required • Reduced reactor volume due to ability to handle high loading rates • High concentration of bacteria improve stability of system • Good ability to maintain stability under load variations • Can rapidly restart after prolong shutdowns 	<ul style="list-style-type: none"> • High operating cost due to packing material • Biomass accumulation causes increased pressure drop inside reactor • Possibility of reactor plugging • Longer start-up periods required
UASB Reactors	<ul style="list-style-type: none"> • Simple reactor configuration and construction with exception of the gas/liquid separator • Can handle very high loading rates • Possible to maintain low concentrations of suspended solids inside the reactor • Lower operating cost requirement as no need for mechanical mixing or support media • Effluent low in suspended solids 	<ul style="list-style-type: none"> • Effective gas/liquid separator is required • When solid loading rate is high, some bacteria may be lost with the effluent • Possibility of losing a portion of sludge bed during toxic upset or a hydraulic surge • Effluent recycling required for bed expansion

Table 3-8 Continued

Reactor Type	Advantage	Disadvantage
Packed Bed Reactors	<ul style="list-style-type: none"> • Can handle high COD loadings • Small reactor volumes required • Simple operation 	<ul style="list-style-type: none"> • High cost of packing material • Possibility of packing plugging • Solids accumulation may cause operational problems and high maintenance
Fluidised and expanded bed Reactors	<ul style="list-style-type: none"> • High specific surface area available for attachment of microorganisms increase amount of microorganisms • Can attain high loading rates • High concentration of minor organisms results in more stable reactor operation • Reduced reactor volume • No clogging, channelling, plugging or gas hold-up problems • Good mixing provides limited mass transfer resistance 	<ul style="list-style-type: none"> • High energy and maintenance cost required for fluidisation • Possibility of washout of support media which may damage pumps and other equipment • High cost associated with recovering of media from effluent • Feed consisting of high concentrations of particulate matter not appropriate • Separation of acidogenic and methanogenic bacteria not possible • Requires degasifier
Membrane Reactors	<ul style="list-style-type: none"> • Higher biomass concentrations can be used which reduces reactor volume and increase possible OLR's • Allows very high SRT's • High quality effluent due to fact that most of suspended solids are captured 	<ul style="list-style-type: none"> • High membrane costs • Membrane fouling possible
Hybrid Reactors	<ul style="list-style-type: none"> • Combines the advantages of both the fixed-bed and sludge blanket reactors • Can maintain high biomass concentrations 	<ul style="list-style-type: none"> • Biogas must pass through both levels • Possibility of plugging • Lower activity of methanogenic bacteria in lower level of reactor

Table 3-8 Continued

Reactor Type	Advantage	Disadvantage
Two-phase Reactors	<ul style="list-style-type: none"> Overcomes stability and control problems associated with single-phase systems 	<ul style="list-style-type: none"> Difficult to implement, operate and control Lack of experience in two-phase process Increased investment cost due to two reactors being used Separating methanogenic and acidogenic bacteria can possibly disrupt the interspecies hydrogen transfer Decrease in methane potential due to CO₂ and H₂ production in acidogenic reactor
	<ul style="list-style-type: none"> Allows for optimisation of both acetogenic and methanogenic bacteria 	
	<ul style="list-style-type: none"> Can treat higher OLRs 	
	<ul style="list-style-type: none"> Biogas contains higher methane concentrations due to higher activity of methanogenic bacteria 	
	<ul style="list-style-type: none"> Shorter start-up periods 	
	<ul style="list-style-type: none"> Acidification reactor can serve as buffer for methanogenic reactor if wastewater composition varies significantly 	

3.3 The anaerobic sequencing batch reactor (ASBR)

When treatment of wastewater generated in a milking parlour is considered, a wastewater treatment technology which is simple to use and easy to control would be the most suitable application. According to the advantages and disadvantages of the different anaerobic processes discussed in Table 3-8, it is evident that the ASBR process is such a process which would be suitable for applications of treating wastewater generated from milking parlours on dairy farms.

3.3.1 The development of the ASBR process

The ASBR process was derived from the anaerobic contact process (also called the anaerobic activated sludge process). The anaerobic contact process is one of the first high-rate anaerobic wastewater treatment processes and has been employed in wastewater treatment since the 1950's which consisted of a (21). It consists of a well-mixed reactor in which anaerobic microorganisms can come into close contact with wastewater, followed by a degasifier in which the dissolved gasses in the wastewater are removed from the wastewater to ensure good solid settling. Once the dissolved gasses are removed, the biomass solids are allowed to settle out to the bottom of a solids separating unit. The solids are then recycled back to the contact reactor tank (21). One drawback of the anaerobic contact process is that each step in the process occurs in a separate unit, resulting in a very high capital investment. Another drawback is that the materials (biomass and wastewater) need to be transferred to each unit for each process step. This may lead to process inefficiencies and also goes hand in hand with relatively high energy requirements (19). These drawbacks led to the development of the ASBR process.

The first laboratory studies conducted in the use of a single, batch-fed reactor with internal solids separation and supernatant wasting was called the anaerobic activated sludge process. These studies were conducted during the 1960's by Richard Dague at Iowa State University in order to increase the microbial population inside the reactors. Although the early studies on the anaerobic activated sludge process showed very good solid separation due to bioflocculation (which enabled the process to achieve short hydraulic retention times (HRTs) and long solid retention times (SRTs)), it was only during the early 1990's that laboratory studies on the ASBR

process have been conducted at Iowa State University (22; 21). In 1993, Dague and his co-workers patented the ASBR process as an application to convert various wastes in wastewater (including the biotechnology industry and the grain and food processing industries) as well as livestock wastes to biogas (carbon dioxide and methane) (19). Since then, the ASBR process has been employed in the laboratory-, pilot- and full-scale treatment of a wide range of wastewater types and animal wastes (see Table 3-9).

3.3.2 Principles of the ASBR process

The principle on which the process operates is very simple and a typical cycle consists of four distinct steps which are feed, react, settle and decant. These four steps are all carried out in one process vessel, making the ASBR process an excellent improvement on the anaerobic contact process discussed earlier (21). Figure 3-5 shows a schematic drawing of a typical cycle in an ASBR.

a) React stage

The react stage is the most important stage in the whole ASBR process for this is the step in which the organic substrate is converted to biogas and it commences once the reactor feeding stage is complete. The anaerobic reactions continue while the reactor content are mixed intermittently or continuously by either recycling the wastewater or biogas (produced during the anaerobic reaction) or by means of mechanical mixers, biogas recycling or liquid recycling. This is done to improve the performance of the bioreactor and to ensure that the substrate is well distributed throughout the reactor. The influent characteristics of the wastewater, the desired quality of the effluent, the concentration of the biomass and the temperature of the waste are factors which influence the duration of the react step. The low F:M ratio at the end of the react step result in low biogas production, producing conditions which will favour biomass flocculation and separation, thus, enhancing settling of biomass during the settling stage (92). In literature, various react times have been employed.

The duration of the react stage varies as the total cycle time varies and will therefore vary for different systems. Timur and Öztürk (98) used a react time of 22.5 hours with a total cycle length of 24 hours (the react-to-cycle time ratio begin 0.9) for treating landfill leachate in an

ASBR. The HRTs and OLRs applied to the reactor varied between 1.5 and 10 days and 0.38 and 9.43 gCOD.L⁻¹.day⁻¹ respectively while the COD of the feed varied from 3800 to 15940 mg.L⁻¹. In another study by Xiangwen *et al.* (17), brewery wastewater with COD values between 22500 and 32500 mg.L⁻¹ was successfully treated in an ASBR (more than 90% COD removal efficiency was observed). The duration of the react stage was 6.35 hours with 8 hours cycle time (resulting in a react-to-cycle time ratio of 0.8).

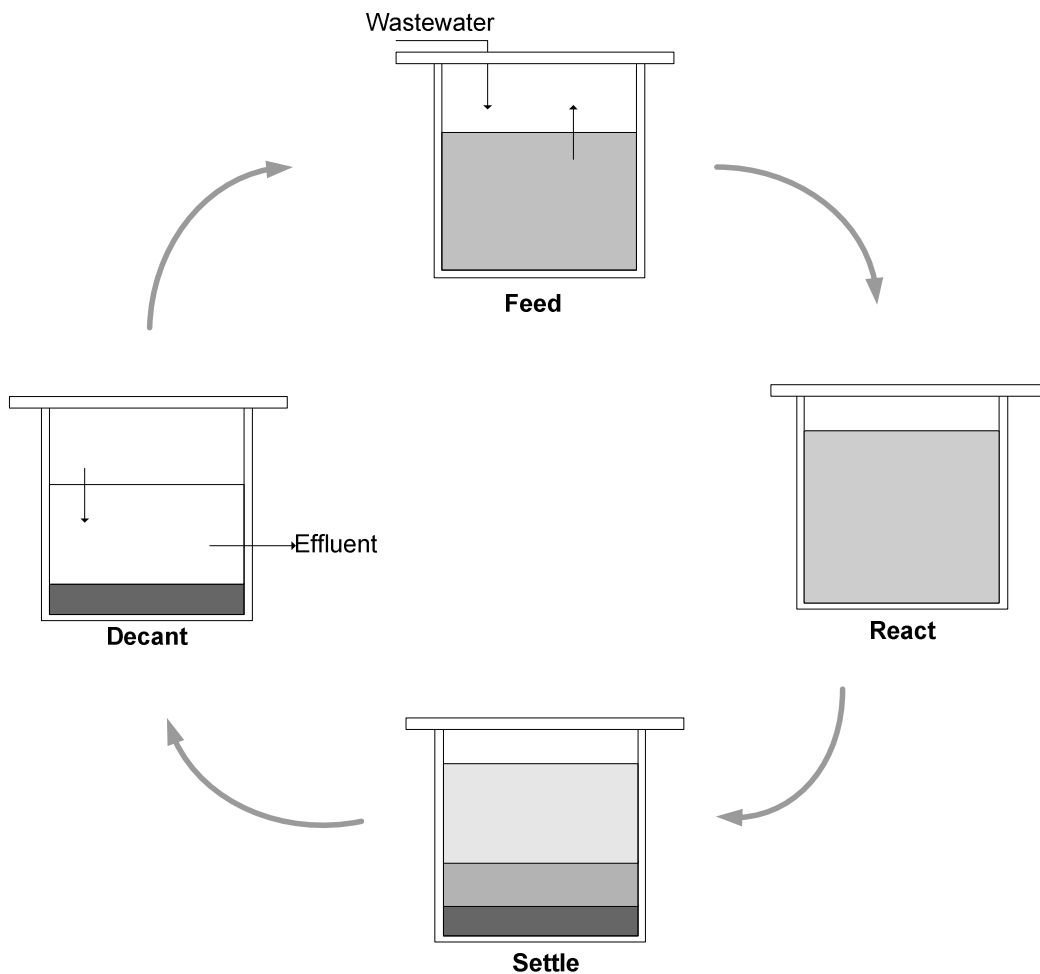


Figure 3-5 Schematic representation of the sequential process steps in an ASBR (adapted from ref. (50))

The operating HRT was set to 1 day, while the OLR was varied between 1.0 and 5.0 gCOD.L⁻¹.day⁻¹ (17). Uppendrakumar *et al.* (92) treated raw cheese wastewater (COD concentrations between 25000 and 51000 mg.L⁻¹) in an ASBR with a total cycle time of 20 hours

and react stage time of 20 hours (the react-to-cycle time ratio begin 0.8) and obtaining a 93% COD removal efficiency. The applied OLR varied between 1.0 and 2.6 gCOD.L⁻¹.day⁻¹.

b) Settling stage

Once the react stage is complete, mixing of the reactor contents is stopped and the biomass and solids in the reactor are allowed to settle out, causing the reactor to act as a clarifier, cancelling the need for an external clarifier. The time required for the settling stage depends on the concentration and settling ability of the biomass in the reactor. It should be long enough to ensure that the solids inside the reactor have all settled out but it should also be short enough to wash out the biomass which settles did not settle out well (92). Sometimes, the blanket-height might rise due to biogas built-up in the system during the settling stage (21). According to Sung and Dague (21), the settling time varies from as short as ten minutes to as long as one hour, but no distinct, fixed time is given in literature as to be the correct settling time for a typical ASBR and it may be changed during the run of the reactor operation. This is confirmed by Zaiat *et al.* (81) who stated that the settling stage may vary from a few minutes to several hours. From a practical, operational viewpoint, it is important that the sludge blanket height after the settling stage is finished, should be below the decanting height to prevent biomass washout. A time should therefore be chosen to obtain a clarified effluent and to retain as much biomass as possible inside the reactor (81).

c) Decanting stage

Once the solids have settled out to a sufficient height, a fixed volume of the effluent is decanted from the reactor. The volume effluent that needs to be decanted depends on the HRT and the total operating volume of the reactor and is usually the same volume that is fed to the reactor during the feeding stage. According to Zaiat *et al.* (81) this step must happen as quickly as possible in order prevent too high concentrations of oxygen to enter the system, which may have influence the anaerobic bacteria negatively. The ASBR process is a closed system and decanting a fixed volume from the reactor will result in a reduction of pressure inside the reactor, unless the biogas formed during the react stage can flow back into the reactor. This is usually done by inserting a gas bag to equalise the pressure inside the reactor (99).

The time which is necessary for the decanting stage is dependent on the rate at which decanting will take place and the volume to be decanted. The volume decanted is dependent on the HRT at which the reactor will be operated (99). Decanting is usually done manually or by means of a peristaltic pump and the decanting rate (and therefore the decanting time) will depend on the reactor design and configuration. In some cases, the decanting times and feeding times have been kept as short as possible to increase the react time as in the case of Sung and Dague (22). Typically, the decanting time is very short compared to the total cycle time. Decanting times found in literature was 9 minutes (17) and 13.5 minutes respectively (99).

3.3.3 Factors influencing the performance of the ASBR system

There are several factors that influence the performance of the ASBR process, including the operating temperature, mixing strategy, substrate-to-biomass concentration ratio, the reactor geometry, the operating temperature, the biomass granulation, cycle times, hydraulic retention time (HRT) and feeding strategy (81).

a) Mixing strategy

Mixing in the reactor during the react stage is vital to ensure sufficient contact between the biomass and substrate as it increases the performance of the bioreactor due to the improved mass transfer fluxes (100). The rate at which mixing is done inside the reactor is of importance, since too intense mixing may cause the granulated biomass to rupture, while insufficient mixing will limit the mass transfer between the biomass and the substrate (81). The three main methods which have been employed to ensure sufficient mixing in the ASBR process are biogas recycling, liquid recycling and mechanical agitation (81). Of these three, biogas recycling is the most commonly used method. However, some problems with gas recycling were found to limit the mixing process, especially when treating low-strength wastewater or during start-up, as the gas production will be inefficient in providing adequate mixing, resulting in dead zones inside the reactor and external mass transfer resistance (100; 81). Two other possible problems associated with biogas recycling is the possible release of volatile matter and foam generation (81; 99; 101). In a study in which the performance of two ASBR systems with different mixing modes (liquid recycling and mechanical mixing) were investigated, it was shown that

mechanical mixing provides a better process stability and organic matter removal efficiency due to the conservation of the integrity of the granular biomass (102).

When using mechanical agitation, the degree of mass transfer will be dependent on the agitation rate as well as the impeller type. In a study on the influence of agitation rate on the performance of the ASBR process, the authors operated the ASBR at four different agitation rates (0, 25, 50, and 75 rpm) with a six-vertical-blade disk impeller. The reactor-diameter to impeller-diameter ratio was equal to a value of 3. The results showed that as the agitation rate was increased, the overall organic matter removal rate also increased due to the improvement in the mass transfer between the bulk substrate and the biomass. It was also found that the methane production rate increased with an increase in the agitation rate, while the time taken to reach the maximum methane production rate decreased (100). Another, more recent study investigated the effect of impeller type and mechanical agitation on the mass transfer in an ASBR. Five different impeller types (including a six-vertical-flat-blade-turbine impeller, a six-vertical-flat-blade-paddle impeller, a six-45°-inclined-flat-blade-turbine impeller, a six-45°-inclined-flat-blade-paddle impeller and a three-blade-helix impeller) were employed at four different agitation rates (50, 70, 75 and 100 rpm). The same type of reactor with the same reactor configurations and reactor geometry was used as in the case of the study by Rodrigues *et al.* (100). It was found that by varying the rotor speed and type of impeller did not significantly influence the performance or stability of the system. The mass transfer was however, increased with an increase in the agitation rate. It was also shown that, when using a helix impeller resulted in the best performance of the ASBR (103).

Mixing in the ASBR process can either be continuous or intermittent. It was shown by Sung, et al. (1992) that reactor performance when intermittent mixing was employed was superior that the reactor performance when continuous mixing was employed. The reactor operated with intermittent mixing have shown to have a higher overall COD removal efficiency as well as improved settling characteristics of the sludge (21). Typical intermittent, mechanical mixing times which have been used by previous authors is in the range of 1-2 min/h (98; 104).

b) Food-to-microorganism ratio (F:M)

When discontinuous, non-steady state processes such as the ASBR process is considered, the initial ratio between the substrate (organic matter which can be used by the microorganism) and the biomass (F:M) is important to consider as it can influence the granulation process in an ASBR process (81). It was shown in a study by Sung and Dague (22) that low F:M ratios can provide optimum granulation causing an improvement in the settling characteristics of the sludge. In another study, the effect of the initial seed concentration on the start-up of an ASBR was investigated. An initial F:M ratio ranging between 0.09 and 1 mg COD/(mg VSS), were used. The best results were obtained when a F:M ratio of 0.5 were used (105). In order to achieve a low F:M ratio, the food concentration can be lowered or the microorganism mass can be increased. During a batch process, initially, the food concentration is high at the beginning of the cycle (just after the reactor is fed) and decreases as the process continues until the reactor is fed again (see Figure 3-6).

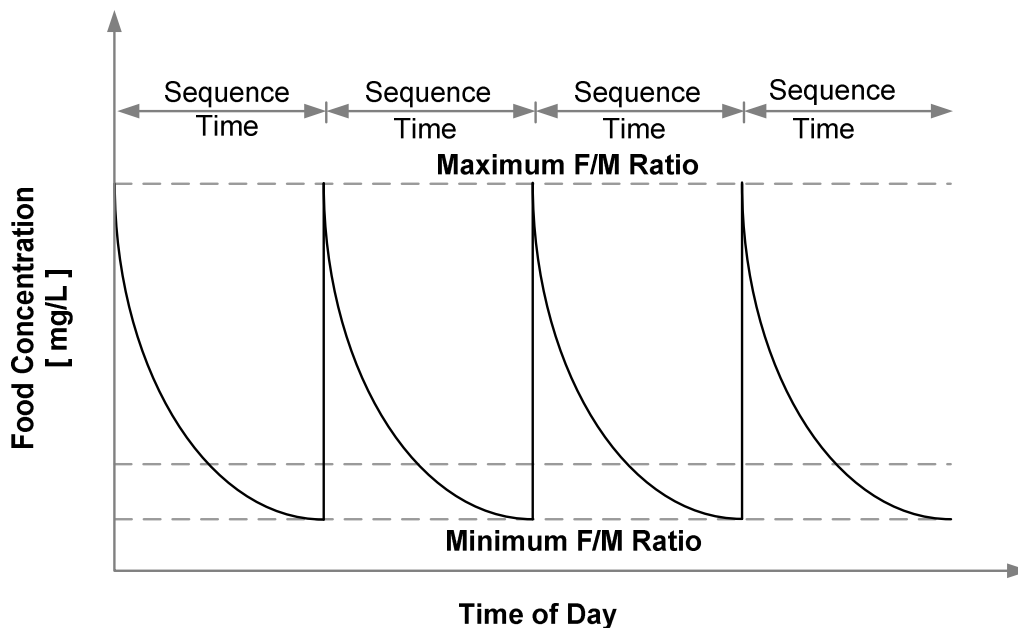


Figure 3-6 The effect of batch feeding on the food concentration and F:M ratio (adapted from ref. (22))

Thus, this batch system, the concentration of the substrate just before the reactor is fed, is lower than the concentration of the substrate at any time in a reactor that is continuously fed.

This causes the batch-fed system to achieve better flocculation and granulation of the biomass and thus, improved solids separation when compared to a continuously fed system (22). This is one of the most important characteristics of the ASBR process which distinguishes it from other conventional anaerobic processes. Just after feeding, the high F:M ratio provides an increased driving force for the metabolic activity. This results in a higher reaction rate and biogas production. As the F:M ratio decreases towards the end of the cycle (i.e. when the food concentration is at its lowest), the conditions for the biomass to flocculate and to separate are at its best (22).

c) Reactor Geometry

The reactor geometric characteristic is also an important factor that may affect performance of the bioreactor. This was first shown in a study by Sung, et al. (1992), in which four reactors with different L/D ratios (5.6, 1.83, 0.93 and 0.61 respectively) were used to treat non fat dry milk (NFDm). The authors showed that the reactor configuration is important in the performance of an ASBR system. Their results also indicate that shorter, fatter reactors were more effective in achieving higher solid cell concentrations, while the thin, taller reactors were more effective in granular sludge development and better solid retention. It was concluded that the use of a reactor with higher L/D ratios (1.83-5.6) should be preferred over reactors with lower L/D ratios (0.61-0.93). It would therefore be better, when designing an ASBR, to use a L/D ratio greater than 1.83 (21). This was confirmed by another study by Sarti *et al.* (102) in which the performance of reactors with different L/D ratios (1.5 and 3, respectively) was evaluated in which the reactor with the L/D ratio of 3 performed better than the reactor with the L/D ratio of 1.5. The main problem encountered with the reactor with the smaller L/D ratio was poor solid retentions. A summary of a number of ASBR processes used to treat different wastewaters is shown in Table 3-9. The different L/D ratios used in each application are also shown.

d) Temperature

Anaerobic bacteria perform differently at different operating temperatures. In one study, the authors evaluated the performance of a two-stage mesophilic ASBR (operated at 35°C) and a two-stage thermophilic-mesophilic (operated at 55 and 35°C, respectively) treating dairy

wastewater. In terms of biogas production rate and volatile and total solids removal, it was found that the thermophilic-mesophilic reactor setup performed better than the mesophilic-mesophilic reactor setup. The thermophilic-mesophilic reactor setup destroyed the total coliforms more effectively and was therefore recommended over the mesophilic-mesophilic reactor setup. Although the thermophilic-mesophilic reactor performed better, the increased energy requirement to operate at higher temperatures should be compared to the economic benefit of employing an ASBR system that operates at higher temperatures (18).

In initial studies on the operation of the ASBR process at temperatures lower than the conventional mesophilic temperature range of 35-37 °C, it was illustrated that the process has great potential in decreasing the operating cost, when treating low strength wastewaters. This was shown by treating low-strength synthetic dairy wastewater (non-fat dry milk) at different influent COD concentrations (1000, 800, 600 and 400 mg.L⁻¹) in four similar ASBRs, each at different temperatures (15 °C, 20 °C, 25 °C and 35 °C) and different HRTs (48, 24, 16 and 12 hours). The OLRs applied to each reactor was 0.5, 1.0, 1.5 and 2 g COD.L⁻¹.day⁻¹. At 20 °C, soluble COD removal efficiencies varied between 85 and 95%, while the SCOD achieved at 25 °C varied between 88 and 98 %. The 15°C reactor did not show any COD reduction at low HRTs of 12 and 16 hours (24).

In another study, low strength synthetic wastewater with a COD concentration of 500 mg.L⁻¹ was treated in a single ASBR by varying the operating temperature from 30 °C (for 32 days) to 35 °C (for 24 days) to 25 °C (for 24 days) and then to 15 °C (for 29 days). The system achieved 81 to 83 % soluble COD removal efficiencies at 25 and 30 °C, while the removal efficiencies at 15 and 20 °C was significantly lower at 61 to 65 %. Their results showed that by decreasing the operating temperature by 5 °C (from the reference temperature of 30 °C) did not alter the removal efficiency significantly, but when the temperature is decreased more, the removal efficiency in the reactor was significantly worse (106).

In a study treating stronger, real wastewater from a local slaughterhouse with COD concentration ranging between 7510-12590 mg.L⁻¹, four different ASBRs were operated at 30 °C (for 80 days), 25 °C (for 137 days) and 20°C (for 469 days). The total COD removal efficiency

was above 90% for all the reactors at all the operating temperatures when operating at OLRs of 2.75, 2.94 and 4.93 g COD.L⁻¹.day⁻¹. Although the COD removal efficiency was not greatly affected by the difference in operating temperatures, the methane production rate was reduced by a factor of three when treating wastewater at 20 °C (107).

Dague *et al.* (20) investigated the treatment of dilute wastewater (non-fat dry milk) at 5, 7.5, 10, 12.5, 15, 17.5, 20 and 25 °C at HRTs of 24, 16, 12, 8 and 6 hours and he concluded that the ASBR process is a wastewater treatment technology that has intrinsic characteristics to efficiently remove COD from diluted wastewater at the temperature range investigated. The COD removal efficiency at an OLR of 2.4 g COD.L⁻¹.day⁻¹ was higher than 90% at 20 and 25 °C, while at the lowest temperature of 5 °C, the COD removal efficiency was 75%.

All of the above mentioned studies indicate that operation at lower temperatures than the conventional mesophilic temperature range of 35 to 37 °C could decrease the energy requirements of the system, but may lead to the process being more sensitive and less efficient in terms of biogas production and COD removal.

e) Granulation of biomass

Granulation in anaerobic processes occurs when microorganisms adhere to each other and other organic and/or inorganic material to form rigid granules, resulting in highly active biomass. The reason why granulation is of importance in an ASBR is due to the enhanced activity and settling characteristics as a result of the higher settling velocity of the granules compared to flocculant biomass (108). The reason for the enhanced settling velocity of granules is due to the fact that it will be heavier than flocculant biomass (sludge) and will therefore have a higher settling velocity). The increased activity of granulated biomass is not as well understood as in the case of the enhanced settling characteristics. Several authors have explained that it is due to the importance of keeping a low H₂ partial pressure inside the reactor in order to prevent longer chain fatty acids that are toxic to the anaerobic bacteria to form (see section 3.1.2 on acetogenesis). When granulated biomass is used, the acetogenic and methanogenic bacteria will be in closer contact than in the case of flocculated biomass, causing intermediate products (such as H₂) to be transferred more efficiently between the different

bacteria groups (108). In an ASBR system, it is important that granulation happens as rapidly as possible. Once granulation has occurred, the performance of the reactor will have an increased efficiency in COD removal and more stable operation (22). However, granulation in ASBR systems is a long process and can take up to several months. In a study by Sung *et al.* (21), granulation was observed only after 300 days. The aim of that specific study was however not to investigate granulation and the ASBR was not operated under optimum conditions for granulation (such is low HRTs and high OLRs).

In a study by Randall and Dague (108), the time to achieve granulation of flocculant biomass in a laboratory scale ASBR was investigated. The ASBR was operated at a low HRT (1 to 2 days) in order to biomass that has not settled out well, and at high OLRs (3, 4, 5, 6, 8, 10 and 12 g COD.L⁻¹.day⁻¹) in order to produce biomass more rapidly. The addition of different attachment matrices was also investigated. Sufficient granulation was achieved after 4 months without any attachment matrices added. The addition of granular activated carbon (GAC) and powdered activated carbon (PAC) shortened the granulation time by 1.5 to 2 months and 1 month respectively (108).

Despite of the advantages of using granulated biomass, many authors have used flocculent biomass successfully in an ASBR. Flocculent biomass is more easily available from the majority of municipal wastewater treatment plants.

f) Feeding strategy

The feeding time of a typical ASBR process is usually low compared to the total cycle length results in a low feed to total cycle length ratio (F:C) (22). Angenent and Dague (99) suggested that the shorter the feeding time, the higher possibility of volatile fatty acids build-up, that will cause an unwanted drop in the pH of the system. Cheong and Hansen (109) investigated the effect of the feeding time on the efficiency of an ASBR at different OLRs (1.5 to 24 g COD.L⁻¹.day⁻¹) and HRTs (1.25, 2.5 and 5 days) treating a stock media. They compared the operation of a fed-batch reactor to a batch reactor and found that the fed-batch mode proved to be more efficient at high OLRs (109).

In another study the the F:C ratio was varied from very low (batch operation) to 0.25 and 0.5 (fed batch operation) treating diluted whey in an ASBR at OLRs between 2 and 12 g COD.L⁻¹.day⁻¹. They found sufficient COD removal at all the different operating conditions investigated. At the lower OLRs (2 to 4 g COD.L⁻¹.day⁻¹), the reactor operated at a F:C ratio of 0.25 showed better results than at the reactor operated at a F:C ratio of 0.5. No tendency to acid accumulation was observed during the cycles (110).

The feed-to-react time ratios (F:R) were varied (0.2, 0.5 and 2) when treating a synthetic substrate consisting of sucrose with a COD concentration of 7000 mg.L⁻¹ were also investigated. The OLR was varied between 2.5 and 18.5 gCOD.L⁻¹.day⁻¹. They found that at OLRs lower than 9 g COD.L⁻¹.day⁻¹, the process performance was not affected, but at OLRs higher than 9 g COD.L⁻¹.day⁻¹, the removal efficiencies decreased with as much as 25 % at low feeding-time-to-cycle-time ratios (111). This was confirmed in a study that showed improved ASBR performance at longer feed times, even though the performance at shorter feed times was sufficient (112). Rodrigues *et al.* (113) also investigated ASBR performance at different F:C ratios (0.017, 0.17, 0.33, 0.67 and 0.89) treating low-strength domestic wastewater at an OLR of 7.7 g COD.L⁻¹.day⁻¹ and found high overall performance for all the different F:C ratios, but actually found that the shorter F:C ratio (0.017) yielded the best COD removal efficiency (87 %) compared to the highest F:C ratio (0.89) that yielded an 84% COD removal efficiency (113). The same trend in the results was obtained in another study that also investigated the effect of feeding time on the performance of an ASBR treating synthetic wastewater (COD of 500 mg.L⁻¹, OLR not mentioned). The F:C ratios were varied between (0.017, 0.17, 0.33 and 0.67) and the authors found that an increase in the feeding time lead to a slight decrease in the removal efficiency inside the reactor which can be attributed to the decrease in contact time between the substrate and the immobilised biomass used (114).

When the studies mentioned above are considered, it is clear that there are no fixed way to calculate the optimum F:C ratio. It will be different for each system, but is it clear that at higher OLRs (typically above 9 g COD.L⁻¹.day⁻¹) it would be best to operate the ASBR at longer feeding times.

Various wastewater types at various OLRs have been treated in an ASBR process. Table 3-9 shows a summary of a number of applications of the ASBR process treating different wastewater types. The respective range of OLRs at which the reactors were operated together with the COD removal efficiency obtained are also given.

Previous studies on ASBR systems

Despite all the advantages mentioned earlier, one possible disadvantage associated with the ASBR process is the maximum loading rate. According to literature, the highest load fed to an ASBR was when sucrose was fed to an ASBR at an OLR of $19 \text{ g COD.L}^{-1}.\text{day}^{-1}$. This was significantly lower than the $100 \text{ g COD.L}^{-1}.\text{day}^{-1}$ which have been reported as the maximum OLR for an UASB reactor when milk powder and sucrose were fed to the reactor (112). Even though the maximum loading rate will differ for different substrates, the strategy on which the ASBR is operated may be the main reason for the low maximum achievable OLR in ASBRs compared to other anaerobic reactor configurations. A number of effluents from a variety of sources such as olive mills, slaughterhouses, distilleries and dairies have been treated successfully in ASBR systems at various OLRs (92; 98; 115; 17; 116; 102; 117; 118; 119; 82)(120). Table 3-9 shows a summary of the different studies together with the size of reactor used, the OLR at which the reactors operated, the cycle times, the operating temperature, mixing type, influent COD of wastewater as well as the COD removal efficiency. The OLRs at which these reactors have been operated ranges from $0.54 \text{ g COD.L}^{-1}.\text{day}^{-1}$ (in the case of the treatment of coking wastewater) up to $9.43 \text{ g COD.L}^{-1}.\text{day}^{-1}$.

As far it is known, no work has been done on the anaerobic treatment of milking parlour wash water generated from the CIP washing of the milking equipment. However, a few studies have investigated the treatment of wastewater from dairy factory effluents as well as synthetic dairy wastewater with an ASBR. Most of the studies focussing on the fundamental principles of the ASBR process done by Richard Dague and his fellow workers at Iowa State University have been done on non-fat dry milk (NFDM), showing that wastewater from the dairy industry (containing mostly milk), will be suitable for treatment in an ASBR process (19; 20; 21; 22; 108). Basic principles for design and operation for the purification of wastewater from the food industry

have been investigated by Ruiz *et al.* (82). Synthetic dairy wastewater consisting of diluted skimmed milk with COD concentration of 20 000 mg.L⁻¹ was treated in a laboratory scale ASBR at 35 °C. The maximum OLR at which the ASBR operations was stable was shown to be 6 g COD.L⁻¹.day⁻¹ as higher OLRs have shown to cause problems in the past with sludge wash-out and decreasing in removal efficiencies (82; 13).

3.4 Summary

From the literature study it is clear that the ASBR process have several advantages over continuous-flow anaerobic wastewater treatment processes including:

- No short-circuiting due to batch feeding of the reactor;
- No need for separate clarifier (as in the case of the anaerobic contact process)
- No need for solids or liquid recycling;
- The need for a feed distribution system at the bottom of the ASBR is not required (as in the case of the UASB or UAF systems); and
- Minimum loss of biomass occurs as discharging of effluent only takes place after settling stage is completed.

When taking these advantages into consideration, the ASBR process appears to be a suitable process to treat wastewater generated from the washing of milking equipment from milking parlours on dairy farms. Although a number of different types of wastewaters have been treated successfully with ASBR systems, the student is not aware of any work that has been done on the treatment of CIP milking parlour equipment wash water in an ASBR.

In a typical milking parlour, the concentration of detergents in the wastewater may vary from time to time due to possible accidental spillages of detergents. The effect of an increase in detergent concentration in the wastewater will therefore also be investigated. The majority of anaerobic wastewater treatment plants are operated at conventional mesophilic temperature range of 35 to 37 °C. However, from the literature review, it is evident that treatment at lower temperatures should also be considered.

Table 3-9 Various wastewaters treated in an ASBR according to literature

Type of Wastewater	Volume [L]	L/D	OLR [g COD.L ⁻¹ .day ⁻¹]	Cycle Time [hours]	Temp [°C]	Mixing	COD [mg/L]	COD removal efficiency	Reference
Raw cheese wastewater	24	9.8	0.2-3.2	24	35	Mechanical, intermittent (8min/30min), 60-80 rpm	25000 - 51000	93%	(92)
Landfill Leachate	2	1.3	0.38-9.43	24	35	Magnetic stirrer, intermittent (1min/hour)	16200 - 20000	64-85%	(98)
Olive Mill Wastewater	2	nm	5.3	72	30±2	Magnetic stirrer	4000 - 32000	83%	(115)
Brewery Wastewater	45	3.6	1-5.5	8	33	Mechanical stirrer (150 rpm)	1500 - 5000	> 90%	(17)
Slaughterhouse Wastewater	42	nm	0.49-3.29	24	30	Biogas recycling (1min/5min)	6908 - 9445	90-96%	(116)
Domestic Sewage	1200	1.5 & 3	0.6-1.2	8	29±5	Liquor recirculation (1.5m ³ /h) Mechanical mixing (30rpm)	445 - 681	71-78%	(102)
Coking Wastewater	12	4.7	0.37-0.54	24	35	Biogas recycling	400 - 1300	38 - 50.3%	(117)
Distillery Wastewater	180	31	3-4	8	35	Mixed liquor recycling	20000 - 120000	70 - 80%	(118)
Whey	5	nm	0.6-4.8	8	30±2	Mechanical stirrer (50 - 75 rpm)	500 - 4000	79 - 93%	(119)
Slaughterhouse wastewater	3.5	nm	0.6-6.1	12	35	Magnetic Stirring	3500 - 4300	86%	(82)
Concentrated dairy wastewater	3.5	nm	1.5-6.25	12	35	Magnetic Stirring	20000	90 - 97%	(82)
Yeast wastewater	0.7	nm	1.4-9.16	24	35	Magnetic Stirring	14400 - 25700	84%	(120)

The possibility of treating wastewater generated from the CIP washing of milking equipment in a milking parlour in an ASBR at 35 °C will therefore be investigated. By treating wastewater at lower temperatures, one would reduce the heating energy required. The possibility of treating milking parlour equipment wash water in an ASBR system at a lower temperature than the conventional, mesophilic temperature (35 °C) will also be investigated. Typical OLRs found in literature when dairy wastewater is treated in an ASBR at lower temperatures than the conventional mesophilic range found in literature ranges between 0.5 and 2.4 g COD.L⁻¹.day⁻¹.

3.5 Hypotheses

The following hypotheses are drawn from the literature review:

- COD removal efficiencies greater than 95 % can be achieved when treating wastewater generated from the CIP washing of milking parlour equipment in a laboratory-scale ASBR operated at 35 °C when OLRs larger than 5 g COD.L⁻¹.day⁻¹ are applied.
- COD removal efficiencies greater than 95% can be achieved at lower temperatures (between 20 and 25 °C) when OLRs up to 3 g COD.L⁻¹.day⁻¹ are applied.
- Approved CIP chemicals used at milking parlours do not affect the performance of the ASBR process significantly.

Chapter 4 - Materials and Methods

4.1 Approach

This study was carried out in two parts. In the first part of the study (Part 1), the performance of an ASBR reactor was investigated when treating synthetic wastewater mimicking wastewater generated from equipment washing in a milking parlour. This was done in order to investigate the sensitivity of the ASBR process to varying concentrations of CIP chemicals. The way in which this was done was to monitor the reactor performance at different OLRs applied to the reactor as well as by varying the detergent concentration in the synthetic wastewater fed to the reactor. The ASBR used during part 1 of the study will be referred to as “Reactor 1”.

In the second part of the study (Part 2), real wastewater generated in a milking parlour during CIP washing was fed to two identical ASBRs operated at two different temperatures at increasing OLRs. Reactor 2 was operated at 22.5 °C (which is slightly higher than the average ambient temperature of 19 °C in Cape Town). Reactor 3 was operated at 35 °C. The performance of the two reactors was monitored at different OLRs applied to the reactor.

4.2 Bioreactors setup

Figure 4-1 shows a schematic drawing of the ASBRs used in the study while Table 4-1 shows a summary of the specifications of the ASBRs. The three laboratory-scale ASBRs that were used in this study was designed by the author and then it was constructed at the workshop of the Department of Process Engineering at Stellenbosch University. The reactors consisted of cylindrical glass batch reactors (with a 5 mm thick wall) with a PVC top and bottom. The effective working volume of Reactor 1 was 5.2 litres while that of Reactors 2 and 3 was 2.6

litres. Reactor 1 had a diameter of 150 mm, a total height of 360 mm, which results in a L/D ratio of 2.4. Reactors 2 and 3 had a diameter of 120 mm, total height of 288 mm and L/D ratio of 2.4. No fixed value for the optimum L/D ratio is given in literature and the L/D ratio of 2.4 was chosen according to the recommendations made by the Sung, et al. (1992) to use a reactor with a L/D ratio greater than 1.83.

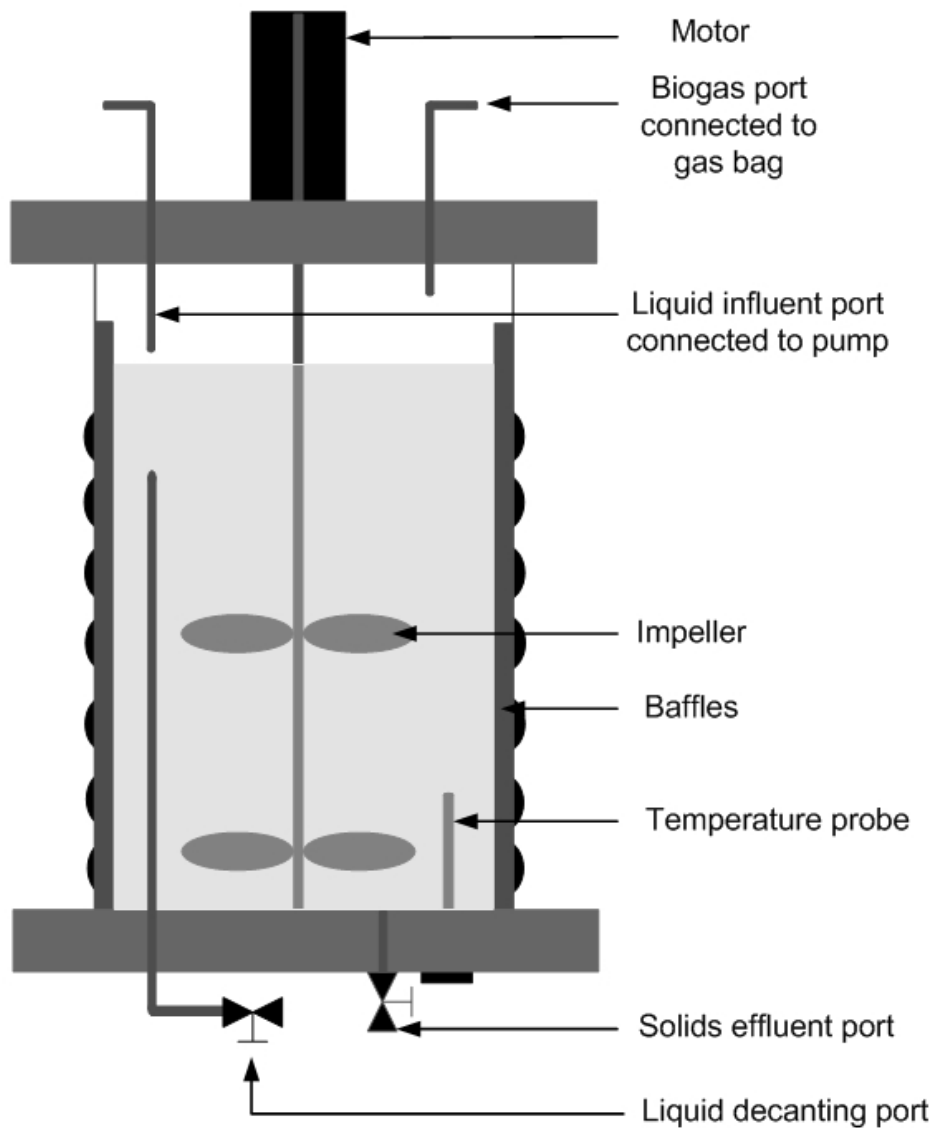


Figure 4-1 Schematic representation of the ASBRs used

A decanting port with adjustable height was situated at the bottom of each reactor, while the feeding port was situated at the top. A port through which the biogas was allowed to escape from the reactor was situated at the top of each reactor. This port was connected to a gasbag into which the biogas formed during each cycle was collected. The gasbags that were used were bags that are usually used in the winery industry and are made from aluminium foil. The bags were donated by Distell Winery. A reactor feed port was also situated at the top of each reactor through which the reactors were fed daily by means of a peristaltic pump connected to the port.

Table 4-1 Specifications of ASBRs used

Parameter	Units	Reactor 1	Reactor 2	Reactor 3
Operating temperature	°C	35	22.5	35
Total Reactor volume	L	6.40	3.30	3.30
Operating Volume	L	5.20	2.60	2.60
Reactor Diameter	mm	150	120	120
Reactor Height	mm	360	288	288
L/D		2.4	2.4	2.4
$D_{\text{reactor}}/D_{\text{impeller}}$		3	3	3
Number of impellers		2	2	2
Type of impellers		Propeller	Propeller	Propeller
Number of baffles		4	4	4
Baffle width	mm	20	15	15
Stirring speed	rpm	180	180	180

The temperature was measured by means of a temperature probe (PT 100), situated at the bottom of the reactor that was connected to a control system (built at the Department of Process Engineering at Stellenbosch University). External heating was provided by means of electrical heating tape arranged around each reactor. The heating cables were also connected

to a control system. The control system controlled the temperature of the reactors at the respective operating temperatures with a 0.5 °C accuracy.

Mechanical mixing was provided intermittently to the reactors by two propeller-type impellers at a stirring rate of approximately 180 rpm for 2 minutes every 30 minutes. The ratio of the reactor diameter and the propeller diameter were 3 for Reactors 1, 2 and 3. The impellers were driven by a 160W, 7000 rpm motor used in sewing machines. Sewing machine motors were used as these motors were not too costly and easily available. However, these motors operate at a very high speed (7000 rpm) that had to be reduced by means of a pulley system. The motor was therefore connected to a double pulley system (designed and constructed at the Department of Process Engineering at Stellenbosch University) that reduced the stirring speed from 7000 to 180 rpm (See Section B.2 in Appendix B for drawing of the pulley system). The motors were connected to an automatic electrical timer (built into the control system) that switched on for 2 minutes every 30 minutes. Four baffles were inserted inside each reactor to help with the mixing. The size of the baffles in the Reactor 1 was 20 mm and in Reactor 2 and 3, 15 mm. Table 4-1 shows a summary of the specifications of the ASBRs used.

4.3 Reactor operations

Figure 4-2 shows a schematic representation of the experimental setup. The reactors were operated on 24 hour batch cycles. Each cycle consisted of four distinct steps including feed, react, settle and decant. The reason why a 24-hour cycle time was chosen is due to the high COD concentrations in the wastewater (higher than 10 000 mg.L⁻¹). A longer cycle time is assumed to result in higher conversion of organic material to biogas. The most common cycle times used in literature was also found to be 24 hours (see Table 3-9). When operated on 24 hour batch cycles, feeding and decanting only happens once a day. Table 4-2 shows the duration of each step during one ASBR cycle operation.

During the feeding stage, the wastewater was fed to the reactors by means of a Watson Marlow 520S peristaltic pump (Reactor 1), Watson Marlow 313 (Reactor 2) and Watson Marlow

505U peristaltic pump at a constant flow rate of 130 mL.min⁻¹ (Reactor 1) and 75 mL/min (Reactors 2 and 3).

Table 4-2 Cycle time and duration of each step

Cycle step	Duration [hours]	Time commenced	Time ended
Feed	0.25	12:45	13:00
React	21.50	13:00	10:30
Settle	2.00	10:30	12:30
Decant	0.25	12:30	12:45
Total	24 hours		

Once all the wastewater was fed to the reactor, the system was purged with nitrogen gas in order to remove the excess oxygen that might have entered the system while empty gas bags were inserted in their place. The gas bags filled with biogas and nitrogen (from the purging) was removed and measured 3 times a week and on these days, the gasbags filled with biogas were replaced by gasbags filled with nitrogen before decanting. After the filling stage, the mixer was switched on and the react stage commenced. At 10:30 every morning, the reactor mixer was switched off and the sludge was allowed to settle for 2 hours until 12:30. After the settling period, a fixed amount of effluent was decanted from the reactor through the decanting port at the bottom of the reactor after which the reactor was filled again with the same volume of wastewater that was decanted from the reactor during the decanting step. The mixers were switched on at 13:00. The reactors were situated in a dark cupboard in order to prevent light from interfering with the anaerobic process. Figure 4-3 provides a photo of the three reactors used (the reactor on the left hand side is the reactor used during part 1 while the other two reactors are the reactors used during part 2 of the study). The P&ID of the system can be found in Section C.1 of Appendix C, while the standard operating procedures followed each day can be found in Section C.2 of Appendix C.

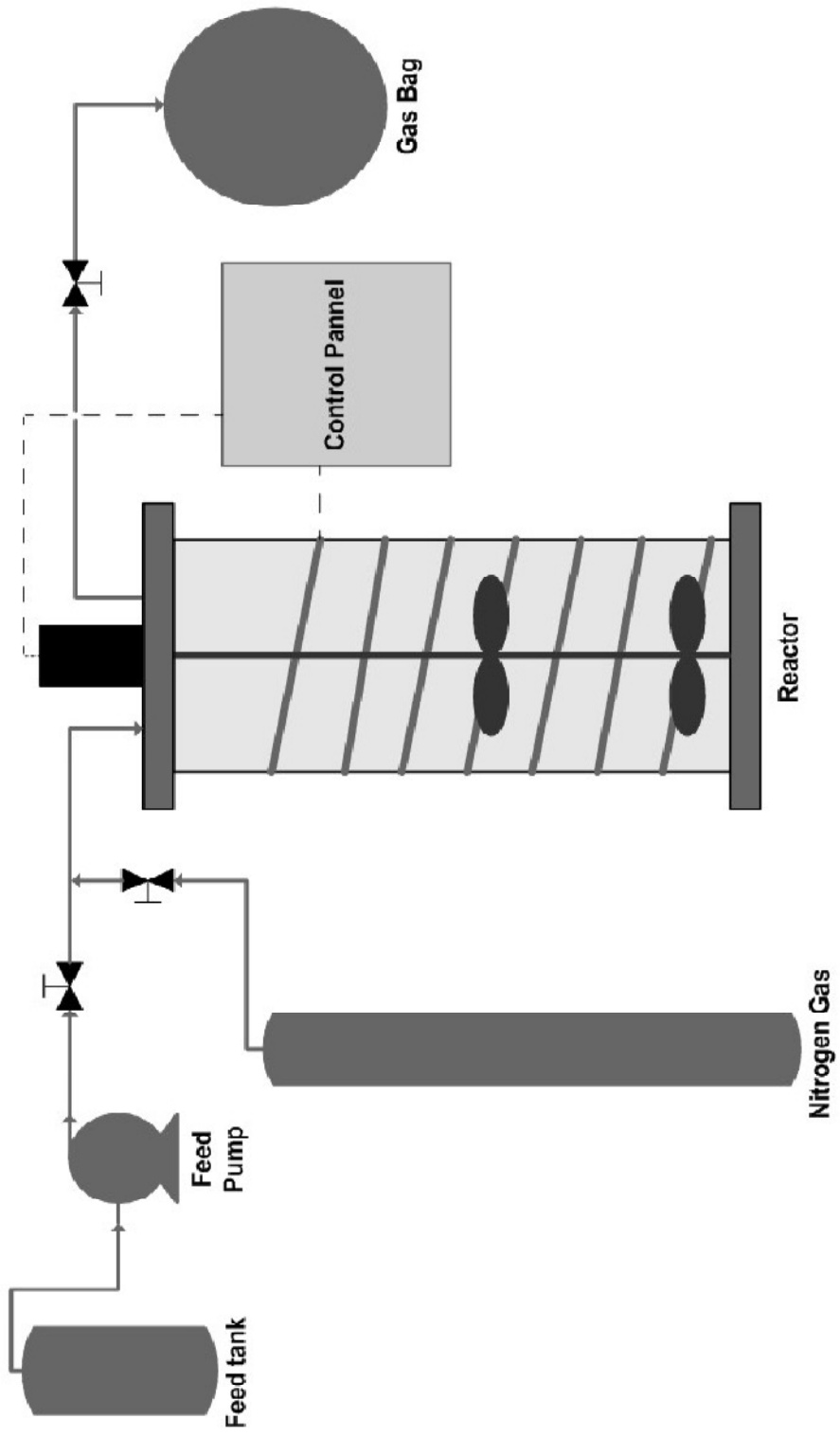


Figure 4-2 Schematic representation of reactor setup



Figure 4-3 Photo of reactor setup

4.3.1 Part 1: Treatment of synthetic milking parlour wastewater in an ASBR at increasing detergent concentrations and OLRs

a) Wastewater

The synthetic wastewater used in this part of the study consisted of milk, detergents and tap water. The synthetic wastewater composition was based on the CIP washing process of Milking Parlour 2 (see Chapter 2). The CIP washing process in the specific milking parlour consisted of 5 distinct steps:

- First rinse (120 litres, twice a day).
- Second rinse (120 litres, twice a day).

- Detergent wash (120 litres, twice a day).
- Rinse (120 litres, twice a day).
- Sanitising rinse (120 litres, twice a day) or acid rinse (120 litres, once a week).

The detergents used were supplied by Ecolab and are most commonly used in milking parlours for CIP washing processes. The three types of detergents were SuperKlenz (used during the detergent rinse), AcidEx (used during the acid rinse) and SuperSan (used during the sanitising rinse). According to the suppliers, the amount of detergents used during the washing process per litre of water is SuperKlenz (5 mL per litre used during the detergent wash step), SuperSan (2 mL per litre used during the sanitising rinse step) and AcidEx (5 mL per litre used during the acid rinse). The main ingredients of the detergents are shown in Table 4-3.

Table 4-3 Main composition of detergents used

Main hazardous Compound	Composition
Acidex	
Nitric Acid	50 %
Superklenz	
Sodium Hydroxide	13 %
Sodium Hypochlorite	3 %
Supersan	
Acetic Acid	14 %
Peroxy Acid	6 %
Hydrogen Peroxide	16 %

A component volume balance was done by calculating the total amount of milk, water and detergents that would be present in the wastewater generated during a period of one week. The composition (volume %) of the wastewater in terms of water, milk, SuperKlenz, SuperSan and AcidEx was then determined. The composition was then converted to the amount of detergents, milk and water needed to prepare 1 litre of wastewater. Table 4-4 provides a summary of the component volume balance. The table shows the amount of each component

needed to prepare 1 litre of synthetic wastewater under normal operations (see Appendix A for a more detailed outline of the component balance done).

Fresh, full cream milk from Darling Creamery was used. The COD of the synthetic wastewater varied between 12 600 and 13 400 mg.L⁻¹. The wastewater composition is presented in the next chapter together with the results and discussion of the reactor performance (see Table 5-4).

Table 4-4 Composition of synthetic wastewater

Constituent	Volume per week [litres]	Volume per litre of wastewater [mL]
Water	8627.04	932.05
Milk	616.00	66.55
Super Klenz	8.40	0.91
Super San	3.36	0.36
AcidEx	1.20	0.13
Total	9256	1000

b) Seed sludge

The reactor was seeded with 2.6 litres of anaerobic sludge obtained from a mesophilic digester operated at 30 °C at the Cape Flats Municipal Wastewater Treatment Plant in Cape Town. The TS and VS content of the sludge was 2.4 % and 76.6 % (% of TS), respectively. The pH and total alkalinity of the sludge during inoculation was 7.5 and 2367 mg.L⁻¹ CaCO₃, respectively. The reason why flocculent biomass (sludge) was used and not granulated biomass is due to the fact that sludge is more easily obtained than granules. Granules are also rather expensive and if a farmer would want to seed an ASBR on his farm, it would be much easier and cheaper to obtain sludge than it would be to obtain granules.

c) Reactor start-up and operation

Once the reactor was seeded with anaerobic sludge, 2.6 litres of tap water was added to the reactor to allow the microorganisms to acclimatize for 24 hours. The reactor was then fed with a synthetic substrate to ensure that the microorganisms have enough micro- and

macronutrients for growth during the start-up period. The synthetic substrate contained glucose, sodium lactate, acetic acid, urea, K_2HPO_4 and trace element solution according to a study done by the Department of Food Science at Stellenbosch University (51). The diluted substrate was fed to the reactor daily at an OLR of $0.5 \text{ g COD.L}^{-1}.\text{day}^{-1}$ for 10 days after which it was replaced with diluted synthetic dairy wastewater which only contained milk and water (COD of approximately 6200 mg.L^{-1}) for 21 days. Day 1 is referred to the first day on which synthetic wastewater was fed to the reactor (this was 10 days after the inoculation).

The experiment was divided into 9 phases. Table 4-5 shows a summary of the volume of detergents, milk and tap water in the synthetic wastewater fed during the 9 different phases of the reactor operational period that consisted of a total of 158 days. The volume of wastewater fed, HRT and OLR during the 9 phases are also shown.

During phase 2, the reactor was fed daily with 500 mL of a more concentrated wastewater that contained double the amount of milk than the wastewater that was fed previously, but no detergents. Thereafter, the reactor was operated at constant OLR of $1.3 \text{ gCOD.L}^{-1}.\text{day}^{-1}$ and HRT of 10.4 days. During phases 3, 4, 5 the concentrations of the detergents were gradually increased until it was double the concentration that it would be normally (according to the component volume balance). The OLR applied during phases 2 to 5 were more or less constant at $1.3 \text{ gCOD.L}^{-1}.\text{day}^{-1}$ while the HRT was constant at 10.4 days. During phase 6 to 8, the OLR was gradually increased to $5.2 \text{ g COD.L}^{-1}.\text{day}^{-1}$ by decreasing the HRT (this was done by increasing the volume of wastewater fed to the reactor to 1000, 1500 and 2000 mL).

During the last period, the concentrations of the detergents were doubled and the OLR and HRT was kept constant. The reactor was operated for a total of 158 days. Throughout the duration of the experiment, the reactor performance was monitored by measuring the biogas composition, biogas volume, COD removal efficiency, pH and alkalinity of the effluent as well as the VFA concentrations in the effluent. During the duration of the study, supplement alkalinity was added in the form of $NaHCO_3$ when the PA dropped below $1100 \text{ mg.L}^{-1} \text{ CaCO}_3$. In order to keep the operating cost as low as possible and the process as simple as possible (for

applications in milking parlours), no adjustment was made to the nutrients concentration in the influent wastewater.

4.3.2 Part 2: Treatment of real milking parlour wastewater in two ASBRs with increasing OLRs at 22.5 °C and 35 °C

a) Wastewater

The wastewater used in this study was generated from the CIP washing of milking equipment in a milking parlour. Samples were collected once every two weeks at a specific local dairy farm (farm 3) and stored in a fridge close to 4 °C during the course of the project.

Sampling and analyses

As mentioned earlier, the CIP washing process consists of 5 distinct steps in which a fixed amount of water is used during each step. The wastewater flows to the drainage system which leads the water to the wastewater dam. It was not possible to collect all the water generated during the CIP washing process and grab samples during each washing step was therefore taken and added together to create a composite sample. Two grab samples of 2 litres each were taken during each step of the one and a half hour CIP milking equipment washing process and added to a 25 litre container. After sampling, the wastewater was stored in the fridge at $\pm 4^{\circ}\text{C}$ and sent to the CSIR for analyses as soon as possible.

Wastewater analyses

In order to know the characteristics of the wastewater, analyses of the wastewater were done by the CSIR in Stellenbosch. The TCOD were also measured at the local laboratory and the average values obtained from the CSIR and at the local laboratory were taken. The wastewater were analysed for the following characteristics:

- Total COD,
- Soluble COD,
- Total Suspended Solids (TSS),
- Volatile Suspended Solids (VSS),

Table 4-5 Summary of volume of wastewater, volume of detergents, OLR, HRT used in Reactor 1

Parameter	Units	Phase								
		1	2	3	4	5	6	7	8	9
Total water	mL.L ⁻¹	967	933	932	932	932	932	932	932	932
Total milk	mL.L ⁻¹	33	67	67	67	67	67	67	67	67
SuperKlenz	mL.L ⁻¹	0	0	0.455	0.910	1.820	1.820	1.820	1.820	3.640
Super San	mL.L ⁻¹	0	0	0.180	0.360	0.720	0.720	0.720	0.720	1.420
Acid Ex	mL.L ⁻¹	0	0	0.065	0.130	0.260	0.260	0.260	0.260	0.52
Total volume fed	mL	500	500	500	500	500	1000	1500	2000	2000
HRT	Days	10.4	10.4	10.4	10.4	10.4	5.2	3.5	2.6	2.6
Average TCOD	mg.L ⁻¹	6182	12643	13716	12874	13410	13410	13410	13410	13047
OLR	gCOD.L ⁻¹ .day ⁻¹	0.6	1.2	1.3	1.2	1.3	2.6	3.9	5.2	5.0

- Total Phosphates (TP),
- Nitrates and Nitrites,
- Total Kjeldahl Nitrogen (TKN),
- Ammonia, and
- pH and Alkalinity.

The COD concentration varied between 14 900 and 28 700 mg.L⁻¹, while the pH varied between 2.5 and 10.6. Due to the great variation in the pH, it was set to approximately 7.4 by either adding 2M HCl solution or 2M KOH solution to the wastewater (depending on the initial pH of the wastewater) before feeding it to the reactor.

According to literature, PA values below 1 200 mg.L⁻¹ CaCO₃ in the reactor effluent can result in reactor instability (63). Therefore, when necessary, alkalinity was added in the form of NaHCO₃ in order to maintain the PA in the reactors above 1 100-1 200 mg.L⁻¹ CaCO₃. The rest of the wastewater characteristics are presented in Table 5-4 in Chapter 5.

b) Seed sludge

The two reactors were seeded with 1.3 litres of anaerobic digester sludge each, obtained from the mesophilic digester operated at 30 °C at the Cape Flats Municipal Wastewater Plant in Cape Town. The sludge had a TS content of 2.8% and VS content equivalent to 81.1% of the TS. The pH, partial alkalinity and total alkalinity of the sludge were 7.2, 2079 mg.L⁻¹ CaCO₃ (PA) and 2966 mg.L⁻¹ CaCO₃ (TA), respectively.

c) Reactor operations

After seeding both reactors, 1.3 litres of tap water was fed to the reactors in order to allow the microorganisms to acclimatize for 24 hours. After the stabilization period, the same synthetic substrate that was used in part 1 of the study was fed to the reactors daily for 6 days at an OLR of 0.3 gCOD.L⁻¹.day⁻¹. After this, real wastewater was fed to both reactors at an OLR of 0.7 gCOD.L⁻¹.day⁻¹ (Reactor 2) and 0.8 gCOD.L⁻¹.day⁻¹ (Reactor 3). The day on which the feeding of the real wastewater to the reactor commenced is referred to as “day 1”. The OLR applied to Reactor 3 was increased from 0.8 to 6.6 gCOD.L⁻¹.day⁻¹ throughout the total duration of the

study of 148 days. The OLR applied to Reactor 2 was increased from 0.7 to 2.5 gCOD.L⁻¹.day⁻¹ and operated for 65 days after which reactor failure occurred due to suspected overloading. The reactor was reseeded with 1.3 litres of new fresh sludge obtained from the same anaerobic digester as in the case of the first inoculation. The new sludge had a pH of 7.4 while the partial and total alkalinity was measured to be 1 764 and 2 775 mg.L⁻¹ CaCO₃, respectively. The TS content was 2.81 % and VS content was of 76.03 % of the TS. Tap water (1.3 litres) was added to the new sludge and the reactor was allowed to stabilise at 25 °C for 24 hours after which the temperature was decreased to 22.5 °C. After the new inoculation, the reactor was operated for 75 days and OLR applied to the reactor was increased more gradually than in the case of the first inoculation from 0.6 to 3.3 gCOD.L⁻¹.day⁻¹.

Throughout the duration of the experiment, the performance of both reactors was monitored by measuring the pH, alkalinity (partial and total), COD (total and soluble), biogas volume and biogas composition as well as VFA concentrations. Supplement alkalinity was added in the form of NaHCO₃ when the PA dropped below 1 100-1 200 mg.L⁻¹ CaCO₃.

4.3.3 Analytical Procedures

The parameters which were monitored include the pH and alkalinity of the effluent, the COD of the influent and effluent, the biogas composition and volume as well as the VFA concentrations.

a) pH and alkalinity

The pH and alkalinity of the effluent were measured at least every second or third day in order to monitor the stability of the reactor. This was done by means of an auto titrator (Radiometer TitraMaster 85). 40 mL of sample was titrated with a 0.1N HCl standard solution. This method first measures the pH of the sample after which the partial alkalinity (PA) and total alkalinity (TA) is measured. The PA was measured by titrating the sample to a pH of 5.75, while the TA was measured by titrating the sample to a pH of 4.3. The values were expressed in terms of mg/l CaCO₃ (see Section C.3.2 in Appendix C for a full description of the operating procedure for measuring pH and alkalinity and how the intermediate alkalinity and IA/PA ratios were calculated).

b) COD

The COD of the effluent from the reactor was measured three times a week. The COD was measured calorimetrically using a NOVA 60 Spectroquant® spectrophotometer (Merck) according to standard methods (59). The total COD (TCOD) of the samples were measured as well as the soluble COD (SCOD) of the samples. In this case, the SCOD of the samples were defined as the COD of a sample which were centrifuged at 10 000 rpm for 10 minutes in a Beckman CoulterTJ-25 Centrifuge in order to get rid of the solids in the sample (see Section C.3.1 in Appendix C for a full description of the procedure followed to measure the COD concentrations as well as how the TCOD and SCOD removal efficiencies and COD removal rate was calculated).

c) Biogas composition and volume

The biogas produced from the reactor was collected in a gas tight gasbag. The gas bags were aluminium foil bags used in the winery industry and were obtained from Distell Winery. The bags were modified in such a way that it can be attached to the biogas ports leading from the reactors. The daily volume and composition of the biogas produced during a cycle was measured three times a week. The composition of the biogas was measured at the Department of Food Science by means of a Varian 3000 Gas Chromatograph containing a thermal conductivity detector and a 2.0 m x 3.0 m inner diameter column which is packed with Hayesep Q (Supelco, Bellefonte, PA), with an 80/100 mesh. Helium was used as the carrier gas at a flow rate of 30 mL/min with an oven temperature of 55 °C (51). Four millimeters of biogas was drawn from the gasbags by means of a syringe and injected into the GC.

The volume of biogas was measured by withdrawing 60 mL of biogas from the bag at a time with a 60 mL gas-tight syringe (see Sections C.3.5 and C.3.6 in Appendix C for a description of the detailed operating procedures followed).

d) VFA concentrations

Short chain VFA (acetic, propionic, iso-butyric, butyric, iso-valeric and valeric) were measured by means of Varian 3700 Gas Chromatograph (GC) containing a 30 m Nukol Column (Supelco, Inc., Bellefonte, PA) using Nitrogen as carrier gas. The column is a fused silica capillary column

which has a diameter of 0.53 mm and a film thickness of 0.50 μm containing a flame ionisation detector. The injection temperature of the column was 150 $^{\circ}\text{C}$ while the detector temperature was 300 $^{\circ}\text{C}$. The initial column temperature was kept at 105 $^{\circ}\text{C}$ for 2 minutes after which it was increase to 190 $^{\circ}\text{C}$ (increase temperature rate of 10 $^{\circ}\text{C}\cdot\text{min}^{-1}$). The temperature was then held at 190 $^{\circ}\text{C}$ for 10 minutes. The flow rate of the carrier gas was set to 6.1 $\text{mL}\cdot\text{min}^{-1}$. Samples were diluted with formic acid and kept at -18 $^{\circ}\text{C}$ until analysed (see Section C.3.3 in Appendix C for full description of the method followed). The analyses were done in duplicate or triplicate. 2 μl of Hexanol were added to the sample after which 1 μl was injected to the GC. The concentrations of the short chain fatty acids were then determined by the integration method with the Borwin Version 1.2 integration software (JMBS Developments, Le Fontanil, France). The short chain VFA's that were measured were acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, valeric acid.

e) TS and VS

The total solids and volatile solids concentration of the sludge was determined according to Standard Method for Examination of Water and Wastewater (59). The % of total solids was determined by adding a specific weight of sludge in a crucible and weighing the crucible before (A) and after the sludge is added (B). The sample is then dried overnight at 105 $^{\circ}\text{C}$ in a convection oven after which it is cooled down and weighed again (C). In order to determine the % volatile solids, the sample is ignited in a muffle furnace at 550 $^{\circ}\text{C}$ for 3 hours. It is then allowed to cool down after which it is weighed (D). The %VS can then be expressed as % of the TS (see Section C.3.4 of Appendix C for a full description of the operating procedures followed and the equations used to calculate that % TS and % VS).

4.4 Experimental challenges

Throughout the duration of the study, some challenges occurred. One of these was the sludge settling. The sludge inside the reactors was observed to rise to the top of the reactor during the settling step of each cycle, forming a sludge blanket phase at the top and a clear, liquid phase at the bottom of the reactor. This was possibly due to gas bubbles being entrapped between the sludge particles, causing the sludge rise to the top of the reactor with the bubbles. All three

reactors had an effluent port with an adjustable height and the wastewater could therefore been drawn from the reactor without biomass washout. Although this is not how conventional ASBRs are run, the separation of the two phases was sufficient and the reactor design made it possible to draw the wastewater from the bottom. Figure 4-4 shows a schematic representation of how the different phases inside the reactor looked at the end of the settling phase when compared to conventional ASBR's. Despite this challenge, the reactor still operated satisfactory. Granulation of biomass was not investigated in this study, but, at the end of the study, when the sludge was disposed from the three reactors, it was evident that granulation was occurring in all three reactors. Further work on granulation in these specific ASBRs used will be useful.

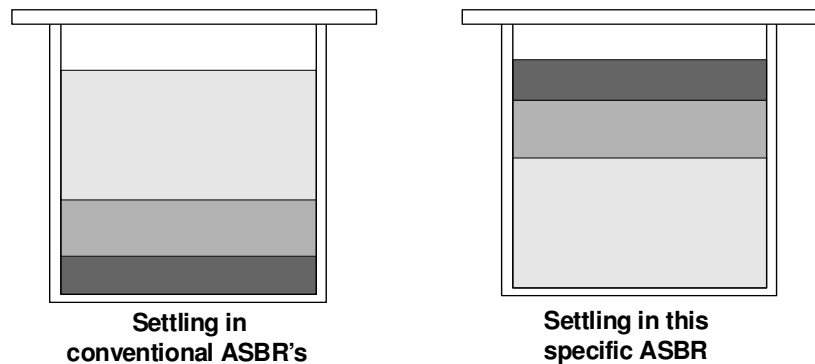


Figure 4-4 Picture showing difference in settling in the ASBR compared conventional ASBRs

4.5 Summary

This chapter describes the materials and methods used during the experimental work performed during this research study. In Chapter 5, the results obtained from the test work described in this chapter will be discussed in detail.

Chapter 5 - Results and discussions

5.1 Approach

When the feasibility of an anaerobic digestion process is investigated, the assessment must include the reactor performance in terms of methane yield, COD removal efficiency and operating OLR as these results play an important role in the economical viability of an anaerobic reactor (14). The performance of the ASBRs will therefore be discussed in terms of the above mentioned criteria. Throughout the discussion, Reactor 1 is referred to the reactor used during part 1 of the study, while Reactors 2 and 3 are the reactors used during part 2 of the study, operated at 22.5 °C (Reactor 2) and 35 °C (Reactor 3), respectively.

5.2 Part 1: Treatment of synthetic milking parlour wastewater in an ASBR at increasing detergent concentrations and OLRs

This part of the study was divided into 9 phases in which either the concentration of detergents in the wastewater or the OLR applied to the reactor was varied. See Table 4-5 for the different OLRs, HRTs and detergent concentrations of the wastewater fed during each phase.

5.2.1 Substrate

The average composition of the wastewater fed to the reactor throughout the study is shown in Table 5-1. The wastewater fed during phase 1 (day 1-21) is a 1:1 dilution of the wastewater fed during phase 2. The wastewater samples were analysed at the analytical laboratories of the CSIR for pH, alkalinity (total), filtered and unfiltered COD, TSS, VSS, TKN, nitrate + nitrite, ammonia and total phosphorus.

A summary of the main results are shown in Table 5-2. The values obtained at each OLR were taken after stable operations (pseudo steady state) have been observed (i.e. the TCOD removal efficiency of three consecutive samples had a standard deviation of less than 5 %).

Table 5-1 Composition of synthetic dairy wastewater used during part 1 of the study

Parameter	Units	Phase				
		2	3	4	5-8	9
Unfiltered COD	mg.L ⁻¹	12 644	13 716	12 874	13 410	13 047
Filtered COD	mg.L ⁻¹	10 920	11 456	11 073	10 996	10 977
pH (at 20 °C)		7.3	8.1	9.0	10.0	10.5
Alkalinity	mg.L ⁻¹ CaCO ₃	203	257	303	407	590
TSS	mg.L ⁻¹	416	430	436	498	353
VSS	mg.L ⁻¹	180	74	162	86	331
TKN	mg.L ⁻¹ N	60	43	47	65	328
Nitrate + Nitrite	mg.L ⁻¹ N	0.05	4.18	10.3	22.3	42
Ammonia	mg.L ⁻¹ N	3.0	4.8	4.1	5.9	4.5
Total Phosphorus	mg.L ⁻¹ P	24.9	27.4	27.9	30.6	43

5.2.2 COD removal

The average TCOD and SCOD removal efficiencies as well as the TCOD removal rate (in terms of g COD removed per day) at the different OLRs are shown in Figure 5-1. The results for the TCOD and SCOD removal efficiency as well as the amount of TCOD and SCOD removed during the duration of the study can be seen in Figure D-1 in Appendix D.

The minimum TCOD and SCOD removal efficiencies achieved were 89.5 % and 94.5 %, respectively at an OLR of 0.6 g COD.L⁻¹.day⁻¹ and HRT of 10.4 days. The maximum removal efficiencies achieved were 97.9 % (TCOD) and 98.2 % (SCOD) and were achieved at an OLR of 2.6 g COD.L⁻¹.day⁻¹ and HRT of 5.2 days. The fact that the removal efficiencies were higher than 89 % throughout the study at all the different OLRs applied, showed that the ASBR operated effectively in terms of TCOD and SCOD removal efficiencies. In both the TCOD and SCOD, an increase in the OLR from 0.6 to 1.3 g COD.L⁻¹.day⁻¹ showed a significant increase in the removal

Table 5-2 Summary of results obtained from treatment of synthetic milking parlour wastewater in an ASBR at 35 °C (Reactor 1)

Parameter	Units	Phase									
		1	2	3	4	5	2-5	6	7	8	9
COD of substrate	mg.L ⁻¹	6182	12644	13716	12874	13410	13410	13410	13410	13410	13047
pH of substrate		7.2	7.3	8.1	9.0	10.0	10.0	10.0	10.0	10.0	10.5
Alkalinity of substrate	mg.L ⁻¹ CaCO ₃	115	203	257	303	407	407	407	407	407	590
HRT	days	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	2.6
OLR	g COD.L ⁻¹ .day ⁻¹	0.6	1.2	1.3	1.2	1.3	1.3	1.3	2.6	3.9	5
pH effluent		7.29	7.27	7.44	7.52	7.36	7.4	7.37	7.51	7.48	7.55
TCOD removal efficiency	%	89.5	97.1	98.1	97.8	97.9	97.7	97.9	97.1	95.0	93.7
SCOD removal efficiency	%	94.5	97.6	98.1	98.0	97.9	97.9	98.2	97.9	96.7	96.0
COD removal rate	g COD _{removed} .day ⁻¹	0.53	1.18	1.29	1.21	1.25	1.24	2.52	3.76	4.90	4.70
Volume biogas	L.day ⁻¹	0.63	2.04	2.11	2.14	3.11	2.35	4.94	8.62	12.09	12.23
Volume methane	L.day ⁻¹	0.46	1.38	1.41	1.41	1.53	1.45	3.11	5.34	7.47	7.58
% Methane in biogas	%	73.4	67.7	66.9	65.8	65.6	66.5	63.2	61.8	61.8	62.0
Methane yield	L _{CH₄} (g COD _{removed}) ⁻¹	0.168	0.224	0.210	0.223	0.234	0.223	0.237	0.273	0.293	0.310
Methane productivity	L _{CH₄} .(L _{reactor} .day) ⁻¹	0.089	0.265	0.272	0.270	0.295	0.276	0.599	1.027	1.436	1.458

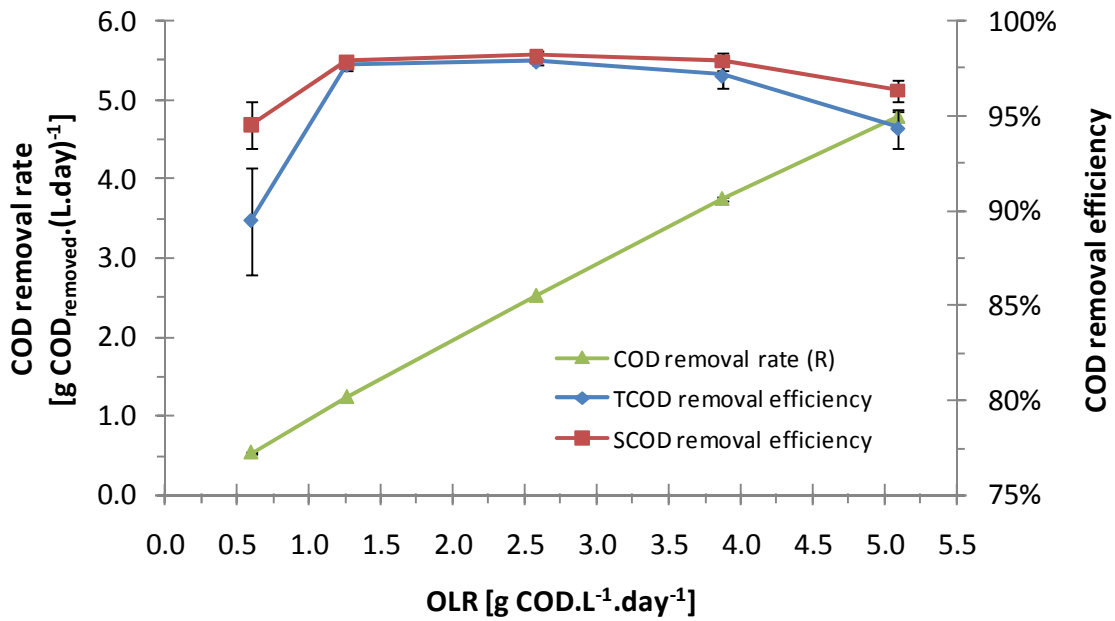


Figure 5-1 COD removal efficiencies and removal rate achieved at different OLRs during anaerobic treatment of synthetic milking parlour wastewater in an ASBR

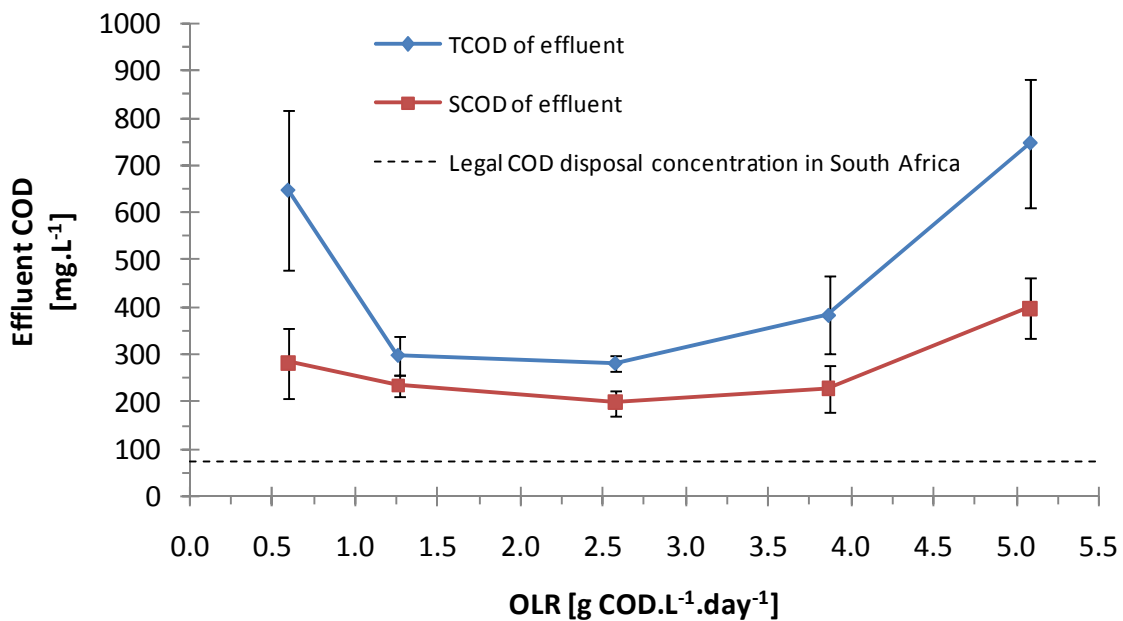


Figure 5-2 COD effluent concentrations achieved at different OLRs during the treatment of synthetic milking parlour wastewater in an ASBR stating at 0.6 g COD.L⁻¹.day⁻¹

efficiencies. When the OLR applied to the reactor was increased further to 2.6 and 3.9 g COD.L⁻¹.day⁻¹ (i.e. the HRT was decreased to 5.2 and 3.5 days), the removal efficiencies stayed more or less constant. Further increase in the OLR to 5.1 g COD.L⁻¹.day⁻¹ (and decrease in the HRT to 2.6 days) caused the TCOD removal efficiency to decrease to 94.3 % which is a decrease of nearly 3 % from the 97.1 % achieved at an OLR of 3.9 g COD.L⁻¹.day⁻¹ (and HRT of 3.5 days). The SCOD removal efficiency also decreased from 97.9 % to 96.4 % with an increase in the OLR from 3.9 to 5.1 g COD.L⁻¹.day⁻¹, but not as significant as the TCOD.

The COD removal rate for the ASBR system is also shown in Figure 5-1. From Figure 5-1 it can be seen that the removal rate increases linearly with an increase in the OLR. This shows that despite the slight decrease in the removal efficiencies at the highest OLR of 5.1 g COD.L⁻¹.day⁻¹, the reactor has not yet reached its maximum operating limit and could most probably have handled higher OLRs. According to Strydom *et al.* (12), the removal rate will usually increase with an increase in the OLR up until the reactor has reached its maximum operating limit. Once this limit is reached, the removal rate will reach a plateau after which it will start to decrease.

Figure 5-2 shows the average effluent TCOD and SCOD concentrations achieved during the study at the different OLRs. From the figure it can be seen that the average TCOD concentration of the effluent from the reactor throughout the duration of the experiment ranged between 747 and 283 mg.L⁻¹, while, the average SCOD in the effluent ranged between 198 and 400 mg.L⁻¹. Despite the high TCOD and SCOD removal efficiencies achieved by the reactor, both the TCOD and SCOD concentrations of the effluent is still higher than the South African legal COD concentration of 75 mg.L⁻¹ at which industrial effluent may be disposed into a natural water body (see Figure 5-2) (121). The SCOD concentration in the effluent is lower than the TCOD concentration due to the removal of solids by means of centrifugation. A final polishing step (such as membrane filtration) is therefore necessary if one wants to reuse the water specifically for equipment washing or if the water is going to be disposed into a fresh water source. The COD concentration in the effluent from the reactor is however, much lower than the legal limit of 5 000 mg.L⁻¹ when irrigating up to 50 m³ wastewater per day and can therefore be used for irrigational purposes (8).

During phases 2 to 5, the concentrations of the detergents were increased during each phase while the HRT (and OLR) were kept constant. From Table 5-2 it is evident that the COD removal efficiency did not decrease significantly when the detergent concentrations in the wastewater were increased at an OLR of $1.3 \text{ g COD.L}^{-1}.\text{day}^{-1}$ indicating that at this specific OLR, the concentration of the detergents is still low enough that it will not cause inhibition to the anaerobic bacteria. Under normal conditions in a milking parlour, the concentrations of the detergents will be similar to that fed during phase 4 of the experiment. The ASBR reactor showed very good performance in terms of TCOD (97.8 %) and SCOD (98.0 %) removal efficiencies during phase 4. The detergent concentrations in the wastewater fed during phase 5 was doubled from phase 4 and the reactor still showed very good performance in terms of TCOD (97.9 %) and SCOD (98.0 %) removal efficiencies (see Table 5-2 for results). However, at the higher OLR (5.0 to $5.2 \text{ g COD.L}^{-1}.\text{day}^{-1}$) and shorter HRT (2.6 days), when the detergent concentration was further increased to double the concentration fed during phases 5 to 8, the removal efficiencies decreased slightly from 95.0 % to 93.7 % (TCOD) and from 96.7 to 96.0 % (SCOD). Despite of the slight decrease in the removal efficiencies, both the TCOD and SCOD removal efficiencies achieved were still very good. This shows that the microorganisms adapted well to the increased detergent concentrations in the wastewater and the reactor could still possibly be operated at higher OLRs and short HRTs with good removal efficiencies. This also shows that if, for some reason the wastewater generated from a milking parlour contains four times more detergents than usual, the microorganisms will still be able to achieve high removal efficiencies.

Table 5-3 shows a comparison of the results obtained in this study with other similar studies. In terms of COD removal efficiencies, the reactor shows good performance when compared to other ASBR system treating similar wastewaters. In a study by Upendrakumar *et al.* (92), TCOD and SCOD removal efficiencies of 93 % and 98 % was achieved in an ASBR treating wastewater from a cheese factory with influent average COD concentration in the influent of $38\,000 \text{ mg.L}^{-1}$. The average OLR applied was $1.8 \text{ g COD.L}^{-1}.\text{day}^{-1}$. Ruiz *et al.* (82) obtained 90 to 97 % TCOD removal efficiency treating synthetic dairy wastewater (consisting of milk and water) with an

Table 5-3 Summary of other similar anaerobic wastewater treatment technologies treating dairy effluent

Wastewater	Reactor configuration	Temperature [°C]	COD removal (%)	OLR [g COD.L ⁻¹ .day ⁻¹]	Methane yield [L _{CH₄} .(g COD _{removed}) ⁻¹]	Methane productivity [L _{CH₄} .(g COD _{removed}) ⁻¹]	Reference
Cheese factory wastewater	Single phase ASBR	35	93	1.8	0.17	0.33	(92)
Cheese factory wastewater	Two-phase ASBR	35	98	2	0.20	0.45	(92)
Synthetic dairy wastewater	ASBR	35	90-97	1.5-6.26			(82)
Cheese whey	ASBR	50	1.6-12.8		0.263-0.122	0.4-1.1	(97)
Cheese whey	ASBR	30	> 90	0.6-4.8		0.18	(119)
Non-fat dry milk	ASBR	35, 25, 20, 15	86-99 (35 °) 77-96 (25°C) 70-95 (20 °C)	0.2-2			(24)
Synthetic dairy factor effluent	Anaerobic Hybrid reactor	35	90-98	2.5-6.1	0.206-0.354	0.49-2.2	(12)
Synthetic milking parlour wastewater	ASBR	35	89-98	0.6-5.1	0.168-0.310	0.10-1.46	This study
Real milking parlour wastewater	ASBR	35	91-98	0.8-6.6	0.163-0.331	0.18-1.56	This study

average COD concentration of 20 000 mg.L⁻¹ in an ASBR at an OLR between 1.5 and 6.25 g COD.L⁻¹.day⁻¹.

The performance of the ASBR in terms of COD removal efficiencies also shows good performance when compared to other anaerobic wastewater treatment technologies treating similar wastewater. In a study by Strydom *et al.* (12) a synthetic milk factory effluent consisting of yoghurt, cottage cheese and water was treated in a laboratory scale anaerobic hybrid reactor. Removal efficiencies between 90 and 97 % were achieved when operating at an OLR between 2.5 and 6.1 g COD.L⁻¹.day⁻¹ while effluent COD concentrations ranged between 287 and 810 mg.L⁻¹.

The reactor also shows superior performance when compared to the performance of the aerobic treatment systems used in the study by Christopherson *et al.* (11) in which 41 to 75 % COD removal efficiencies were achieved when treating milking parlour equipment wash water from 5 different milking parlours.

5.2.3 Biogas production, methane yield and methane productivity

The average biogas composition as well as the average volume biogas and methane produced at the different OLRs during the experiment are shown in Figure 5-3 while the average methane yield and the average methane productivity are shown in Figure 5-4. The daily volume of biogas produced as well as the biogas content can be found in Figure D-2 in Appendix D.

From Figure 5-3, it can be seen that, throughout the experiment, the methane content in the biogas generally decreased from 73.5 % at an OLR of 0.6 g COD.L⁻¹.day⁻¹ to 61.9 % at an OLR of 5.1 g COD.L⁻¹.day⁻¹. According to Droste (50), the normal methane and carbon dioxide composition in biogas from industrial wastewater plants is in the range of 60 to 70 % and 30 to 40 %, respectively. However, this is not a fixed rule and the biogas content will depend on a number of conditions such as OLR applied and characteristics of the wastewater, HRT and many more. The decrease in the methane content in the biogas as the OLR was increased was also observed by Strydom *et al.* (12) during the treatment of synthetic dairy factory effluent in an anaerobic hybrid reactor. The methane content at an OLR of 6.1 g COD.L⁻¹.day⁻¹(which was the

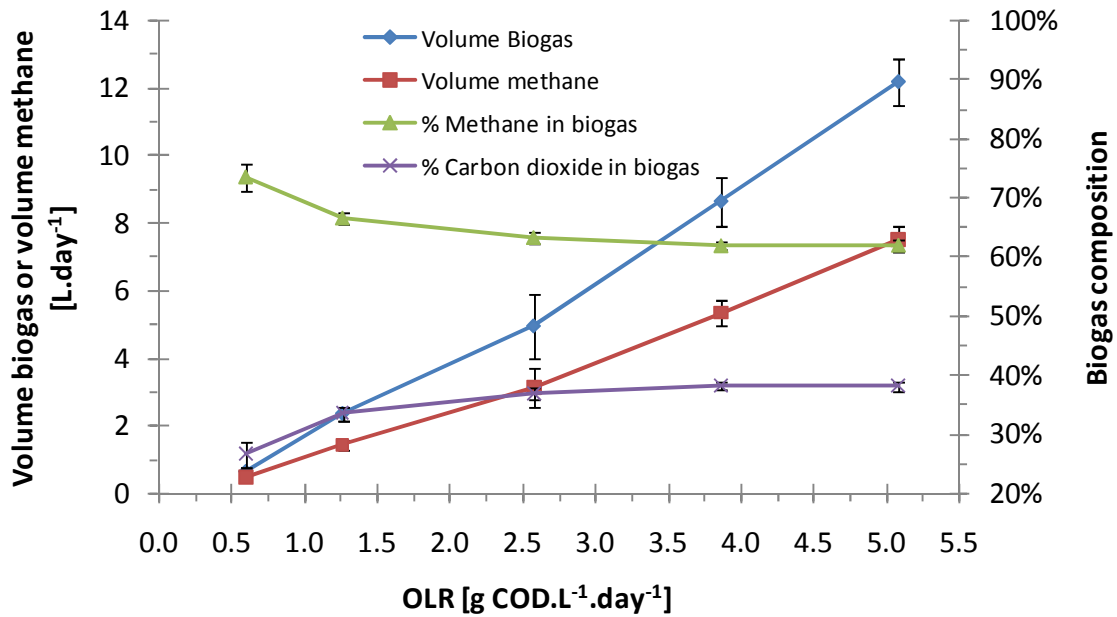


Figure 5-3 Biogas composition and volume biogas and methane produced at different OLRs during the treatment of synthetic milking parlour wastewater in an ASBR

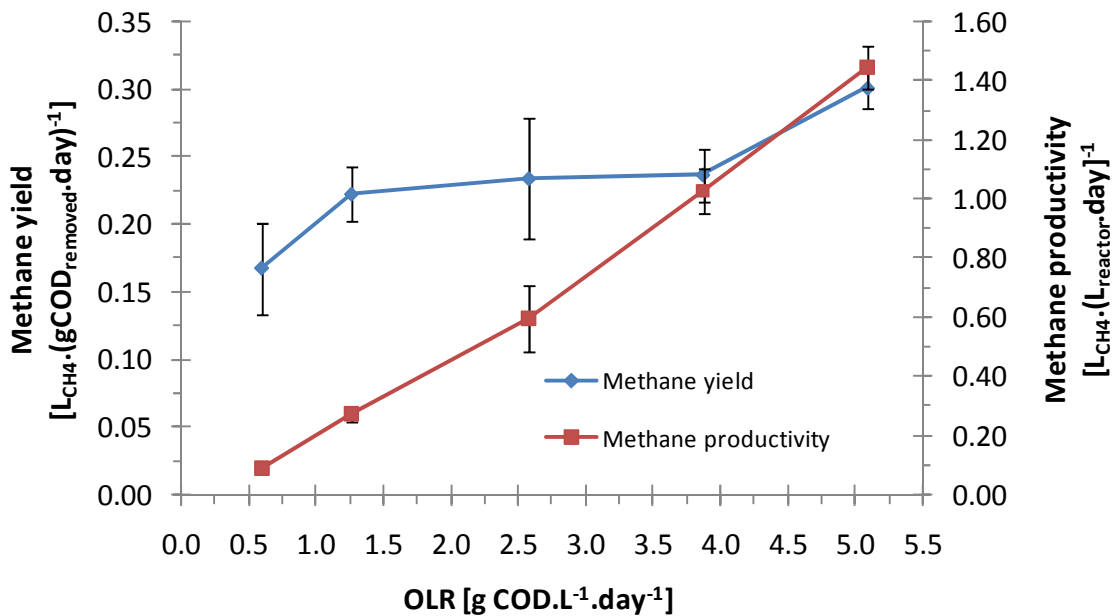


Figure 5-4 Methane yield, methane and methane productivity achieved at different OLR's during the treatment of synthetic milking parlour wastewater in an ASBR

highest OLR applied to the anaerobic reactor hybrid reactor used in the study by Strydom *et al.* (12)), was 56 %, which is almost 4 % lower than that achieved in this specific study. It is also evident from Figure 5-3 that the volume biogas and hence, the volume methane produced generally increased with an increase as the OLR was increased. This indicates that the microorganisms were adapted to the higher OLRs and the reactor could possibly have been operated at even higher OLRs if time allowed it.

According to literature, the methane yield is a function of the quantity of organic material removed (122). This is a result of the activity of the methanogenic bacteria inside the reactor. According to literature, for a specific carbon source being digested under anaerobic conditions, the methane yield will, ideally, be constant if the system is at true steady state (14). A decrease in the methane yield can usually be ascribed to the methanogenic bacteria being physiologically stressed due to possible organic overloads. The methane yield is therefore a good indicator of the condition of the methanogenic bacteria activity inside the reactor (122). Throughout the whole experiment, the methane yield and the methane productivity increased with an increase in the OLR applied to the reactor (see Figure 5-4). The maximum methane yield achieved, was $0.30 \text{ L}_{\text{CH}_4} \cdot (\text{g COD}_{\text{removed}})^{-1}$. This was achieved at the maximum OLR applied to the system ($5.1 \text{ g COD} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$). Although the methane yield increased throughout the duration of the experiment, it never reached the maximum theoretical yield of $0.40 \text{ L}_{\text{CH}_4} \cdot (\text{g COD}_{\text{removed}})^{-1}$ that can be achieved when glucose is used as carbon source at 35 °C and 1 atm. The fact that the methane yield was not constant during this specific study shows that, although the COD removal efficiencies were more or less constant at each OLR applied, the system most probably have not achieved true steady state conditions.

The volumetric methane productivity is the volume of methane produced per volume of reactor. By expressing the methane volume in such a way, one normalises it and it can be compared to the methane volume generated in other reactors with different volumes. The methane productivity showed a nearly linear increase with an increase in the OLR, indicating the microbial biomass adapted well to the operating conditions. The maximum methane productivity was observed to be $1.46 \text{ L}_{\text{CH}_4} \cdot (\text{L}_{\text{reactor}} \cdot \text{day})^{-1}$ and was achieved at an OLR of

5.1 g COD.L⁻¹.day⁻¹. Although the TCOD removal efficiency decreased slightly when the OLR was increased to 5.1 g COD.L⁻¹.day⁻¹, the methane productivity and methane yield increased. This shows that the increase in the OLR was too high to obtain better removal efficiencies, but enough to cause an increase in the methane productivity and methane yield.

When comparing the methane yield and methane productivity achieved in this specific ASBR with other ASBRs treating dairy wastewater, it shows good performance. Upendrakumar *et al.* (92) achieved a methane yield of 0.17 L_{CH₄}.(g COD_{removed})⁻¹ and methane productivity of 0.33 L_{CH₄}.(L_{reactor}.day)⁻¹ while treating cheese wastewater in an a single phase ASBR at a loading rate of 1.8 g COD.L⁻¹.day⁻¹ and HRT of 22 days. The performance of the ASBR treating synthetic milking parlour wastewater also shows superior behaviour when compared to the two-stage ASBR used in the study by Upendrakumar *et al.* (92) in which a a methane yield of 0.20 L_{CH₄}.(g COD_{removed})⁻¹ and methane productivity of 0.45 L_{CH₄}.(L_{reactor}.day)⁻¹ at an OLR of 2 g COD.L⁻¹.day⁻¹ was achieved. In another study in which dairy wastewater (whey) was treated in an ASBR, the methane yield showed a decrease from from 0.263 to 0.122 L_{CH₄}.(g COD_{removed})⁻¹ as the OLR was increased gradually from 1.6 to 12.8 g COD.L⁻¹.day⁻¹ while the methane productivity increased from 0.4 to 1.1 L_{CH₄}.(L_{reactor}.day)⁻¹ as the OLR was increased (97). In a study by Strydom *et al.* (12) synthetic dairy factory wastewater was treated in an anaerobic hybrid reactor at 35 °C. The methane yield obtained ranged from 0.206 to 0.354 L_{CH₄}.(g COD_{removed})⁻¹ at an OLRs between 2.53 and 6.11 g COD.L⁻¹.day⁻¹ (HRT of 4.1 to 1.7 days).

The inconsistency in the methane yield at different OLRs for a specific anaerobic system has also been recorded in literature. Parawira (14) found that the methane yield increased with an increase in the OLR while treating potato waste leachate in an UASB and anaerobic packed-bed reactor. Strydom *et al.* (12) also found that the methane yield was not constant during the treatment of synthetic dairy factory effluent. The reason for the inconsistency in the methane yield observed during this study as well as those in literature, is most probably due to the fact that the microorganisms were still being established in the anaerobic reactors and used more of the carbon in the substrate to produce biomass than it would under true steady state

conditions and, hence, reducing the proportion of substrate that can be converted to methane. Another possible explanation may be that some of the organic matter may have accumulated in the reactor, which may result in an over-estimation of the COD removal efficiency even if the OLR was increased. This may result in an under estimation of the methane yield (see Eq. 3.7).

One possible reason why the methane yield achieved in this study (and in most of the studies mentioned in literature treating dairy wastewater) is lower than the theoretical methane yield can most probably be due to the high concentration of fats in the dairy wastewater. Literature has shown that the presence of fat and long chain fatty acids (as a result of lipolysis of milk fat) in milk have caused inhibition to the anaerobic process by reducing the methanogenic activity and the ATP levels in the cells (causing the physiological activity of the cell to decrease) (123). The low concentration of nutrients in the wastewater fed to the reactor might be the reason why the methane yield achieved was relatively low compared to the theoretical methane yield. The optimum C:N:P ratio for anaerobic reactors is approximately 1000:10:1, which corresponds to a COD:N:P ratio of 350:5:1 (48). Throughout the experiment, the COD:N:P ratios of the wastewater had sufficient nitrogen, but lacked phosphorous. In order to keep the operating cost as low as possible, the reactor was operated without nutrient control.

Thus, a possible way to improve the activity of the methanogenic bacteria (and hence, the methane yield), a suitable treatment method to reduce the fat concentrations in the wastewater should be installed prior to anaerobic treatment (such as a fat trap). In order to provide sufficient nutrients to the anaerobic bacteria, Phosphorus can be added to the wastewater in the form of K_2HPO_4 . Another possibility can be to co-treat the wastewater with other, more phosphorus-rich wastewater, but this will have other implications as combining the wastewater with another wastewater stream will change the composition of the wastewater.

As in the case of the COD removal efficiencies, an increase in the detergent concentration at an OLR of $1.3 \text{ g COD.L}^{-1}.\text{day}^{-1}$ did not result in a significant difference in the biogas composition, methane yield or volume methane produced per day. This confirms the statement made earlier that the microorganisms adapted well to the increase in the detergent concentrations.

5.2.4 Alkalinity, pH and VFA concentrations

The pH and TA of the synthetic wastewater fed to the reactor increased as the detergents concentrations were increased and ranged from 7.3 to 10.5 and 203 to 590 mg.L⁻¹ CaCO₃, respectively. The pH, PA and TA (and consequently, the IA) was measured at least 2-3 times a week. Throughout the duration of the experiment, the pH of the reactor effluent ranged between 7.1 and 7.8. The results for the PA, TA, IA and pH throughout the duration of the study can be found in Figure D-3 in Appendix D.

For the first 17 days, the reactor was operated without any addition of supplement alkalinity in order to see whether the reactor would be able to operate without the addition of alkalinity. During this time, the PA of the effluent dropped from 1848 mg.L⁻¹ CaCO₃ (on day 1) to 673.8 mg.L⁻¹ CaCO₃ (on day 17), showing that the alkalinity in the synthetic wastewater was not sufficient for the reactor to operate without addition of alkalinity. Although PA values below 1200 mg.L⁻¹ have previously shown to result in reactor instability (63), the reactor performance in this study was still acceptable with pH values ranging between 7.1 and 7.3 (average 7.6±0.2). According to literature, IA/PA ratios below 0.3 indicate stable operations, while IA/PA ratios above 0.3 are a sign of possible disturbances inside the anaerobic reactor (62; 48). The average IA/PA ratio during this time was still acceptable at 0.15. However, it was expected that reactor failure would have been unavoidable if suppliant alkalinity was not added. Subsequently, 2 g.L⁻¹ of alkalinity in the form of NaHCO₃ was therefore added to the reactor feed every time the partial alkalinity in the effluent approached 1 200 mg.L⁻¹ CaCO₃.

The general trend of the alkalinity shows a sharp increase once supplement alkalinity has been added to the reactor. For the first few days just after the addition of additional alkalinity, the alkalinity in the effluent decreases more rapidly than the other days. It was interesting to note that during phase 4 of the experiment, the PA and TA did not decrease below 1200 mg.L⁻¹ CaCO₃. The TA of the wastewater fed during this time was 409 mg.L⁻¹ CaCO₃. This is approximately 100 mg.L⁻¹ CaCO₃ higher than the alkalinity of the wastewater fed during phase 4. The average IA/PA ratio throughout the duration of the experiment was 0.14±0.04, indicating

stable operations. Figure 5-5 shows the pH of the effluent throughout the duration of the study as well as the VFA concentrations of the effluent from day 29 onwards.

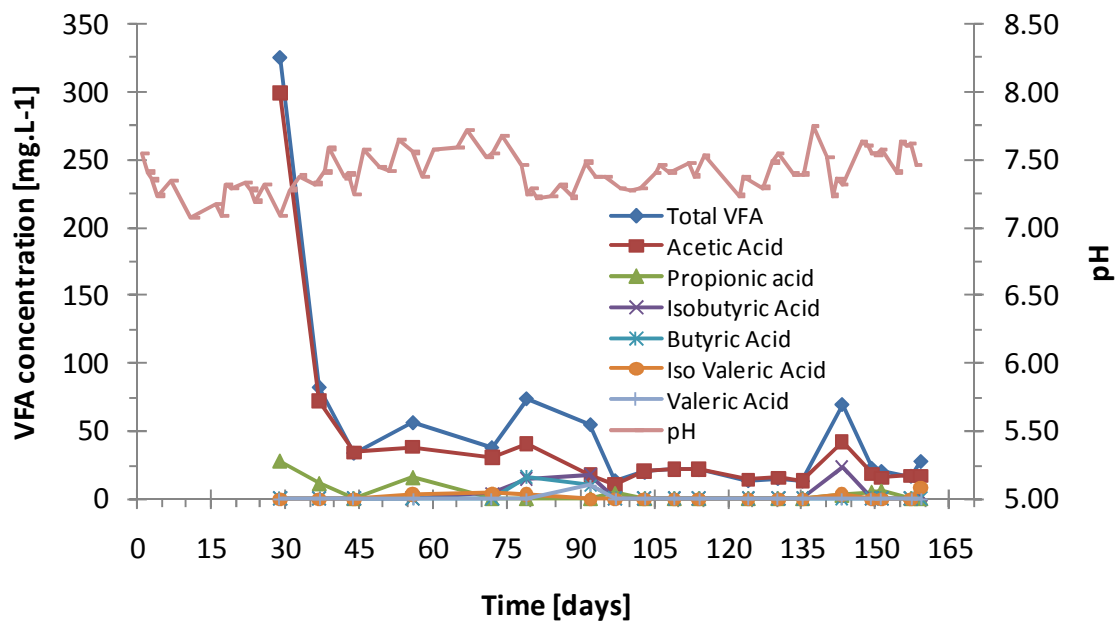


Figure 5-5 VFA concentrations and pH of effluent from ASBR treating synthetic milking parlour wastewater

According to literature, no absolute VFA concentration in the effluent can be defined as the “normal” concentration (64). The reason for this is that each anaerobic system will have its own unique “normal” VFA concentration which is dependent on the operating conditions in the reactor as well as the composition of the substrate being digested (i.e. the wastewater). When considering the concentrations of the VFA at different times during the study, it can be seen that the acetic acid was the main VFA contributing to the TVFA concentrations in the effluent. On day 29 the TVFA concentration (especially the acetic acid concentration) was significantly higher than for the rest of the study. The high VFA concentrations in the effluent on day 29 were accompanied with low PA measurements (986 mg.L⁻¹ CaCO₃) and a slightly higher IA/PA ratio of 0.21, which confirm the higher VFA concentrations.

The TVFA concentration of the effluent varied between 326.3 on day 29 and 13.29 mg.L⁻¹ day 135. On days 79, 92 and 143, the VFA concentrations were notably higher than during the rest of the experiment. This can be explained by the fact that the PA of the effluent on these days were close to 1200 mg.L⁻¹ CaCO₃ indicating that the buffering capacity inside the reactor is which will lead to higher TVFA concentrations in the reactor. If supplement alkalinity have not been added, the TVFA concentration would most probably have increased which could have lead to possible reactor failure. However, although the TVFA concentrations were higher than “normal” during these periods, the reactor performance was still acceptable indicating that the microorganisms have adapted well to the operating conditions.

5.2.5 Summary

The results above show that an increase in the detergent concentration in the wastewater (generated from the CIP washing of milking equipment in milking parlours) up to ½, 1, 2, and 4 times the normal concentration did not affect the performance of the reactor in terms of COD removal efficiencies, biogas production (methane yield and methane productivity) or in terms of pH of the effluent. It also shows that synthetic wastewater which has similar characteristics than the wastewater generated from the washing of milking equipment in milking parlours is suitable for treatment in an anaerobic sequencing batch reactor at 35 °C at OLR between 0.6 and 5.2 g COD.L⁻¹.day⁻¹. Significant COD reduction (89 to 98 %) was achieved, reducing the COD concentration of the effluent to as low as 266 mg.L⁻¹. Despite the good performance of the ASBR, supplement alkalinity was necessary to keep the partial alkalinity above 1 200 mg.L⁻¹ CaCO₃.

5.3 Part 2 - Treatment of real milking parlour wastewater in two ASBRs at increasing OLRs at 22.5 °C and 35 °C

During Part 2 of this study, real wastewater generated from the CIP washing of milking equipment was treated in two ASBRs at 22.5 °C and 35 °C at increasing OLRs. The performance of the reactors in terms of TCOD and SCOD removal efficiencies, COD removal rate, biogas

production, methane yield and methane productivity (as in the case of Part 1), will be presented and discussed.

During the first 66 days of operation, Reactors 2 and 3 were operated (as far as possible) at the same OLRs. The OLRs were also increased at the same times in both reactors. On the 68th day of operation, the reactor operated at 22.5 °C (Reactor 2) failed when the OLR was increased to 2.9 g COD.L⁻¹.day⁻¹ due to suspected overload. The temperature of the reactor was increased to 25 °C and supplement nutrients in the form of urea (500 mg.L⁻¹) and K₂HPO₄ (500 mg.L⁻¹) were added to the reactor in order to allow the bacteria to acclimatise, but after two days, the biogas production was still zero. The colour of the sludge turned grey and a very strong odour arose from the reactor and it was suspected that the methanogenic bacteria were not alive anymore. The reactor content was then removed and the reactor was washed and seeded with new sludge obtained from the same anaerobic digester at the Cape Flats Municipality Wastewater Treatment Plant. The OLR applied to Reactor 2 was once again increased but not as rapidly as in the previous case in order to establish whether the OLR applied to Reactor 2 in the first case was increased too rapidly. The result for the first and second inoculation of Reactor 2 will first be presented and discussed. This will be followed by the results and discussion of the performance of Reactor 3.

5.3.1 Substrate

The characteristics of the wastewater fed to Reactor 2 and 3 are shown in Table 5-4. The pH of the wastewater ranged between 2.5 and 10.6, depending of an acid rinse was employed during the specific washing cycle. In order to obtain a much more accurate representation of the wastewater generated per day, it would be best to collect all the wastewater generated from the CIP washing in one day (i.e. from all three washing cycles) into a vessel. A sample from the total, combined wastewater should then be taken. However, during this study, it was impractical and grab samples were therefore collected. This contributed to the variation in the wastewater characteristics observed in Table 5-4. It does, however, indicate how conditions may change on a full-scale installation.

Table 5-4 Wastewater characteristics used in part 2 of the study

Parameter	Units	Range	Average
Unfiltered COD	mg.L ⁻¹	14 902-28 726	21 511
Filtered COD	mg.L ⁻¹	5 333-12 773	9 198.2
pH (at 20 °C)		2.5-10.6	8.2
Alkalinity	mg.L ⁻¹ CaCO ₃	0-832	522.2
TSS	mg.L ⁻¹	3 324-5 956	4 537.7
VSS	mg.L ⁻¹	3 322-5 824	4 451.3
TKN	mg.L ⁻¹ N	34-741	351.2
Nitrate + Nitrite	mg.L ⁻¹ N	0.6-168	31.6
Ammonia	mg.L ⁻¹ N	1.2-24.2	9.8
Total Phosphorus	mg.L ⁻¹ P	11.4-69	39.9

5.3.2 Reactor 2 (22.5 °C)

A summary of the results obtained in Reactors 2 during the first inoculation shown in Table 5-5 while that obtained during the second inoculation are shown in Table 5-6. The values obtained at each OLR were taken after stable operations (pseudo-steady state) have been observed (i.e. the TCOD removal efficiency of 3 consecutive samples had a standard deviation of less than 5 %).

a) COD removal

Figure 5-6 and Figure 5-7 shows the TCOD and SCOD removal efficiencies as well as the COD removal rate at different OLRs of the first and second inoculation for Reactor 2, respectively. The results for the TCOD and SCOD removal efficiency as well as the amount of TCOD and SCOD removed during the first and second inoculation of Reactor 2 can be seen in Figure D-4 and Figure D-7 in Appendix D. From Figure 5-6 it can be seen that, during the first inoculation, the TCOD and SCOD removal efficiencies were above 90 % for all the OLRs applied except when the OLR was increased to 2.9 g COD.L⁻¹.day⁻¹, indicating that the reactor operated effectively in terms of COD removal efficiencies at OLRs lower or equal to 2.5 g COD.L⁻¹.day⁻¹. The TCOD

Table 5-5 Summary of results from the treatment of real milking parlour wastewater in an ASBR at 22.5 °C (first inoculation)

Parameter	Units	OLR [g COD.L ⁻¹ .day ⁻¹]				
		0.7	1.2	2.0	2.5	2.9
COD of substrate	mg.L ⁻¹	15119	23190	15485	15945	14130
pH of substrate		7.4	7.4	7.4	7.4	7.4
HRT	days	21.3	19.3	8.0	6.5	4.9
pH effluent		7.31	7.47	7.31	7.14	6.82
TCOD removal efficiency	%	95.9	97.6	92.0	90.6	85.9
SCOD removal efficiency	%	96.9	98.7	91.1	94.6	81.5
COD removal rate	g COD _{removed} .day ⁻¹	0.63	1.18	1.83	2.25	1.24
Volume biogas	L	0.225	0.211	0.503	0.943	0
Volume methane	L	0.180	0.180	0.406	0.715	0
% Methane in biogas	%	80.2	85.1	81.2	75.9	0
Methane yield	L _{CH₄} .(g COD _{removed}) ⁻¹	0.106	0.059	0.085	0.122	0
Methane productivity	L _{CH₄} .(L _{reactor} .day) ⁻¹	0.069	0.069	0.156	0.275	0

removal efficiency increased from 96.0 to 97.6 % as the OLR was increased from 0.7 to 1.2 g COD.L⁻¹.day⁻¹, but after further increase to 2.0 and 2.5 g COD.L⁻¹.day⁻¹, the TCOD removal efficiency decreased to 92.1 and 90.6 %, respectively. The final increase of the OLR to 2.9 g COD.L⁻¹.day⁻¹ resulted in a further decrease in the TCOD removal efficiency to 85.6 %. The SCOD removal efficiency shows a similar trend except for the increase at an increase in the OLR to 2.5 g COD.L⁻¹.day⁻¹.

After the OLR was increased to 2.9 g COD.L⁻¹.day⁻¹, reactor failure occurred and the biogas production decreased to zero. A possible reason why the COD removal efficiency was still relatively high (85.6 %) when an OLR of 2.9 g COD.L⁻¹.day⁻¹ was applied, despite the fact that no biogas has been produced is due to the relatively long HRT of approximately 7 days that was applied during this time. This means that wastewater fed to the reactor 7 days earlier may still be present in the reactor, causing the COD removal efficiency to still appear satisfactory, while very little or no digestion of organic matter has actually taken place in the reactor.

Table 5-6 Summary of results from the second inoculation of the treatment of real milking parlour wastewater in an ASBR at 22.5 °C

Parameter	Units	OLR [g COD.L ⁻¹ .day ⁻¹]					
		0.6	0.8	1.0	1.5	2.7	3.3
COD of substrate	mg.L ⁻¹	16 357	20 069	16 774	15 163	22 988	26 733
pH of substrate		7.4	7.4	7.4	7.4	7.4	7.4
HRT	days	28.5	26.0	17.3	10.3	8.7	8.1
pH effluent		7.3	7.4	7.6	7.32	7.5	7.5
TCOD removal efficiency	%	88.6	94.5	96.3	96.2	96.5	96.3
SCOD removal efficiency	%	92.6	95.7	98.1	96.6	96.3	95.1
COD removal rate	g COD _{removed} .day ⁻¹	0.51	0.73	0.93	1.41	2.56	3.17
Volume biogas	L	nd	0.231	0.263	0.693	2.151	2.449
Volume methane	L	nd	0.198	0.214	0.499	1.368	1.632
% Methane in biogas	%	nd	86.4	83.2	71.4	65.2	66.6
Methane yield	L _{CH₄} .(g COD _{removed}) ⁻¹	nd	0.105	0.089	0.136	0.210	0.198
Methane productivity	L _{CH₄} .(L _{reactor} .day) ⁻¹	nd	0.076	0.082	0.192	0.526	0.628

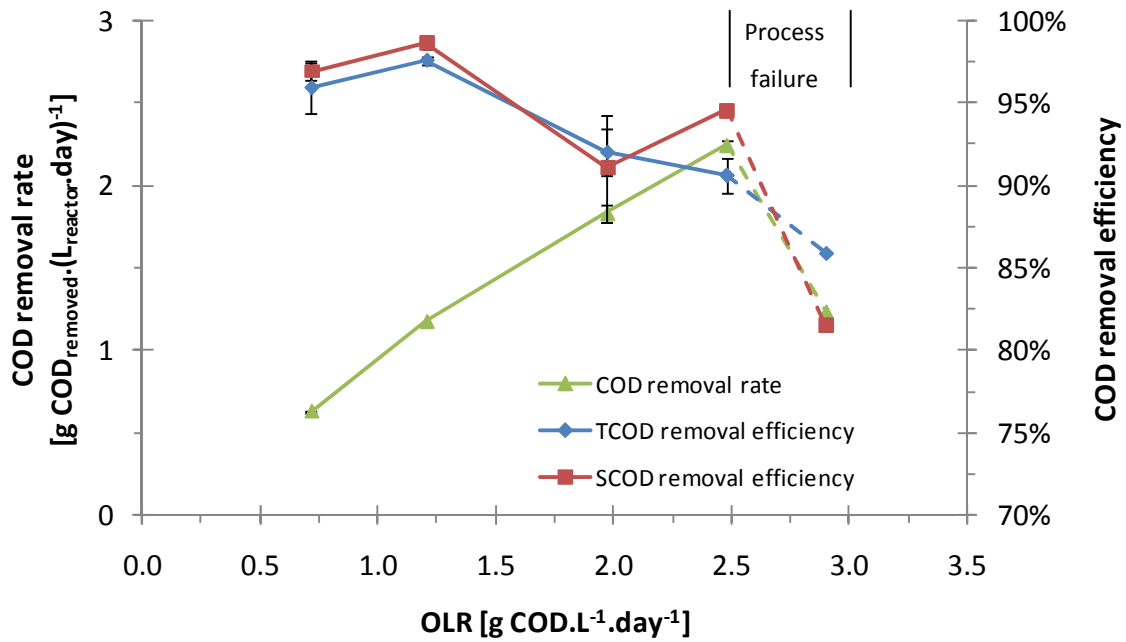


Figure 5-6 COD removal efficiencies and removal rate achieved at different OLRs during anaerobic treatment of real milking parlour wastewater in an ASBR operate at 22.5 °C (first inoculation)

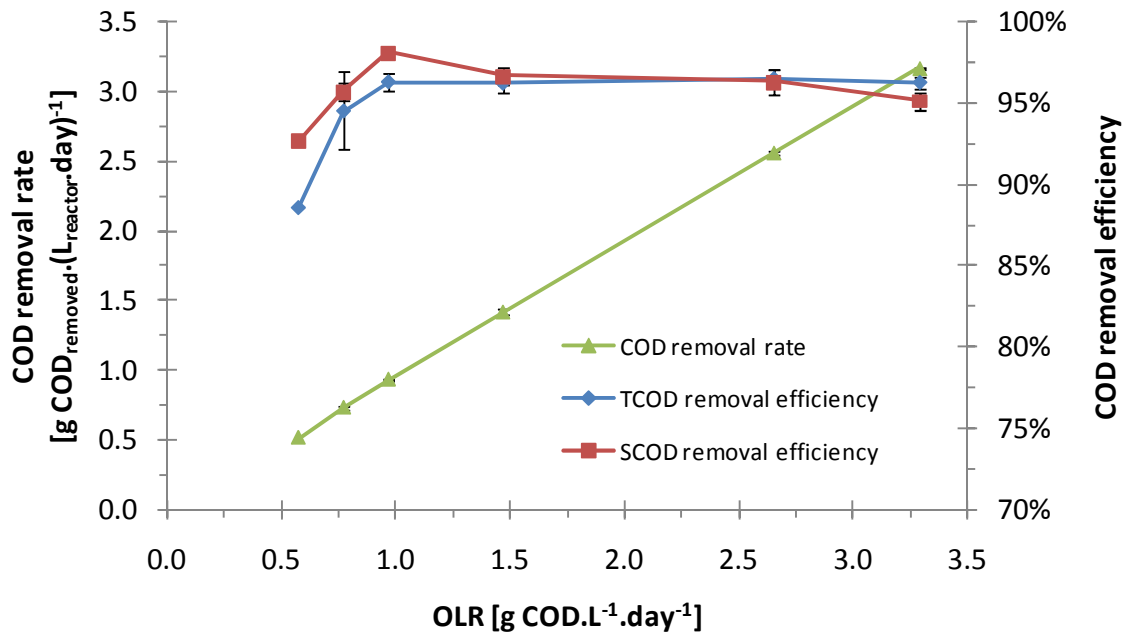


Figure 5-7 COD removal efficiencies and removal rate achieved at different OLRs during anaerobic treatment of real milking parlour wastewater in an ASBR operate at 22.5 °C (second inoculation)

The COD removal rate during the first inoculation (see Figure 5-6) showed an almost linear increase as the OLR was increased up until an OLR of 2.5 g COD.L⁻¹.day⁻¹ after which it decreased significantly when an OLR of 2.9 g COD.L⁻¹.day⁻¹ was applied. According to Strydom *et al.* (12), the COD removal rate will usually increase with an increase in the OLR after which it will reach a plateau when the maximum OLR at which the reactor can operate is approached. Once the maximum operating OLR is reached, the removal rate will decrease. The linear increase in the removal rate indicates that the reactor was still operating below its maximum operating limit up until an OLR of 2.5 g COD.L⁻¹.day⁻¹, but the sharp decrease as the OLR was increased to 2.9 g COD.L⁻¹.day⁻¹ is a result of reactor overload, indicating that the reactor was being operated above the maximum operating capacity of this specific microbial community in Reactor 2 under the specific anaerobic conditions.

According to the results shown in Figure 5-7, during the second inoculation, Reactor 2 also showed very good performance in terms of COD removal efficiencies. The maximum TCOD removal efficiency achieved was 96.5 % at an OLR 2.7 g COD.L⁻¹.day⁻¹ while the maximum SCOD removal efficiency achieved was 98.1 % at an OLR of 1.0 g COD.L⁻¹.day⁻¹. The general trend observed for both the TCOD and SCOD removal efficiencies shows an increase in the removal efficiencies as the OLR is increased up to 1.0 g COD.L⁻¹.day⁻¹ after which it reached a plateau. The TCOD removal efficiency at the highest OLR applied (3.3 g COD.L⁻¹.day⁻¹) was 96.3 %, while the SCOD removal efficiency was 95.1 %. This indicates that the reactor performed very well in terms of organic matter removal, and had, unlike during the first inoculation, not reached its maximum operational limit when the experiment was stopped. The COD removal rate in Figure 5-7 shows a linear increase with an increase in the OLR. This also indicates that the OLR applied to the reactor could have been increased even more, as the reactor has not yet reach its maximum operational limit.

The OLRs applied to Reactor 2 during the two different inocula were not the same, and a direct comparison between the performance of the reactor during the two inocula can therefore not be made, but the overall performance will be compared. The reason why the reactor was not loaded in the same manner during the first and second inoculation was to determine whether it

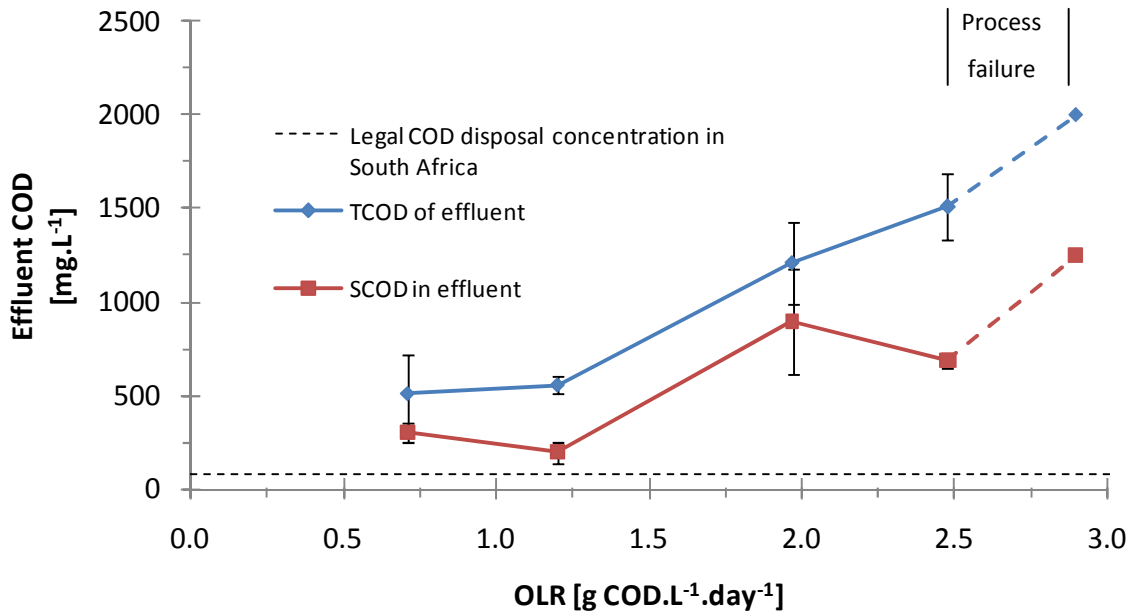


Figure 5-8 COD effluent concentrations achieved at different OLRs during the treatment of real milking parlour wastewater in an ASBR operated at 22.5 °C (first inoculation)

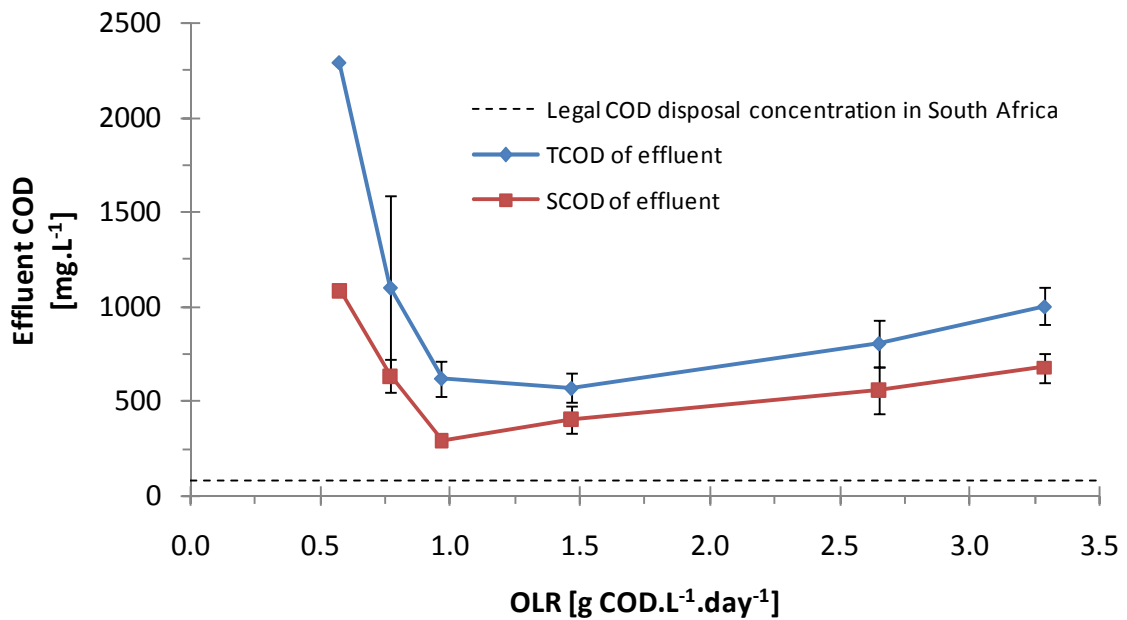


Figure 5-9 COD effluent concentrations achieved at different OLRs during the treatment of real milking parlour wastewater in an ASBR operated at 22.5 °C (second inoculation)

was possible to obtain a better reactor performance if the reactor was not loaded as rapidly as during the first inoculation. There was no significant difference in the maximum TCOD removal efficiency achieved during the first or second inoculation. However, it is clear that Reactor 2 operated more steadily during the second inoculation than during the first inoculation, as reactor failure occurred at an OLR of 2.9 g COD.L⁻¹.day⁻¹ during the first inoculation, but during the second inoculation, the reactor was still operating steadily at an OLR of 3.3 g COD.L⁻¹.day⁻¹, without showing signs that it had reached its maximum operating limit.

In term of COD removal efficiencies, the reactor performance during the first inoculation (before failure) and second inoculation compares well to previous studies done on similar substrates in ASBRs at lower temperatures than the conventional mesophilic range of 35 to 37°C (see Table 5-7). Ndon and Dague (24) achieved 70 to 95 % COD removal efficiencies when treating low-strength synthetic dairy wastewater (COD concentration ranging between 400 and 1 000 mg.L⁻¹) in an ASBR at various OLRs (0.5 to 2 g COD.L⁻¹.day⁻¹) at 20 °C and 77 to 96 % when treating the same wastewater at the same OLRs at 25 °C. However, the authors did not state anything about occurrence of reactor failure which suggests that the reactors were operating steadily at the OLRs applied. Dague *et al.* (20) also achieved high COD removal efficiencies (greater than 90 %) at 20 and 25 °C when OLRs up to 2.4 g COD.L⁻¹.day⁻¹ were applied.

Figure 5-8 and Figure 5-9 show the effluent COD concentration obtained at the different OLRs during the first inoculation and second inoculation of Reactor 2, respectively. According to Figure 5-8, the effluent COD concentrations at the different OLRs during the first inoculation generally increased as the OLR was increased, with a minimum effluent TCOD concentration of 513 mg.L⁻¹ achieved at an OLR of 0.7 g COD.L⁻¹.day⁻¹ and minimum SCOD concentration of 195 at an OLR of 1.2 g COD.L⁻¹.day⁻¹. From Figure 5-9 it is evident that the reactor performance during the second inoculation was an improvement from that during the first inoculation as the increase of the effluent concentration at the different OLRs was not as prominent as during the first inoculation. As in Part 1 of this study, the COD removal efficiencies were very good but, the effluent concentration was still too high (greater than 75 mg.L⁻¹) to dispose the water into a fresh water source or to reuse for the same equipment washing purposes. A final polishing step

Table 5-7 Summary of other similar anaerobic wastewater treatment technologies treating dairy effluent

Wastewater	Reactor configuration	Temperature [°C]	COD removal (%)	OLR [g COD.L ⁻¹ .day ⁻¹]	Methane yield [L _{CH₄} .(g COD _{removed}) ⁻¹]	Methane productivity [L _{CH₄} .(g COD _{removed}) ⁻¹]	Reference
Non-fat dry milk	ASBR	35, 25, 20, 15	86-99 (35 °C) 77-96 (25 °C) 70-95 (20 °C)	0.5-2			(24)
		5, 7.5, 10, 12.5, 15, 17.5, 20, 25	> 90 % (25 °C) > 90 (20 °C) 62-75 % (5 °C)	0.6-2.4			(20)
Real milking parlour wastewater	ASBR	22.5	85-97	0.7-2.9	0.06-0.122	0.07-0.28	This study
Real milking parlour wastewater	ASBR	22.5	88-96	0.8-3.3	0.15-0.21	0.08-0.63	This study

will therefore be required if water is to be reused for the same purpose. Further investigation into a suitable, inexpensive polishing step will be necessary. However, the COD concentration in the reactor effluent, was, as in the case of Reactor 1, lower than $5\,000\text{ mg.L}^{-1}$, which is the legal COD concentration for water used for irrigational purposes.

b) Biogas production, methane yield and methane productivity

The average biogas composition as well as the average volume biogas and volume methane produced at the different OLRs during the first inoculation and second inoculation of Reactor 2, are shown in Figure 5-10 and Figure 5-11, respectively. The daily biogas production and biogas composition throughout the duration of both the first and second inoculations of Reactor 2 can be seen in Figure D-5 and Figure D-8 respectively (in Appendix D).

According to Figure 5-10, the methane content in the biogas during the first inoculation ranged between 85 % (at OLR of $1.2\text{ g COD.L}^{-1}.\text{day}^{-1}$) and 75 % (at an OLR of $2.5\text{ g COD.L}^{-1}.\text{day}^{-1}$). There were no significant difference in the volume of biogas (and hence volume methane produced) when the two lowest OLRs of 0.7 and $1.2\text{ g COD.L}^{-1}.\text{day}^{-1}$ were applied. As the OLR was increased further, the volume of biogas and volume of methane produced in the reactor also increased up until $2.5\text{ g COD.L}^{-1}.\text{day}^{-1}$. However, when the OLR was increased further to $2.9\text{ g COD.L}^{-1}.\text{day}^{-1}$, the biogas production drop drastically to zero which indicates reactor failure.

From Figure 5-11 it can be seen that, as in the case when the synthetic milking parlour wastewater was treated during Part 1 of this study, the methane content in the biogas generally decreased with an increase in the OLR. The methane content in the biogas ranged between 86.4 and 66.7 %. The daily biogas production and daily methane production increased as the OLR was increased. The maximum volume of biogas and methane produced was 2.45 and 1.63 litres which were achieved at an OLR of $3.3\text{ g COD.L}^{-1}.\text{day}^{-1}$.

Figure 5-12 illustrates the efficiency of the ASBR in terms of methane yield and methane productivity at different OLRs during the first inoculation of Reactor 2, while Figure 5-13 shows methane yield and methane productivity of Reactor 2 during the second inoculation. According

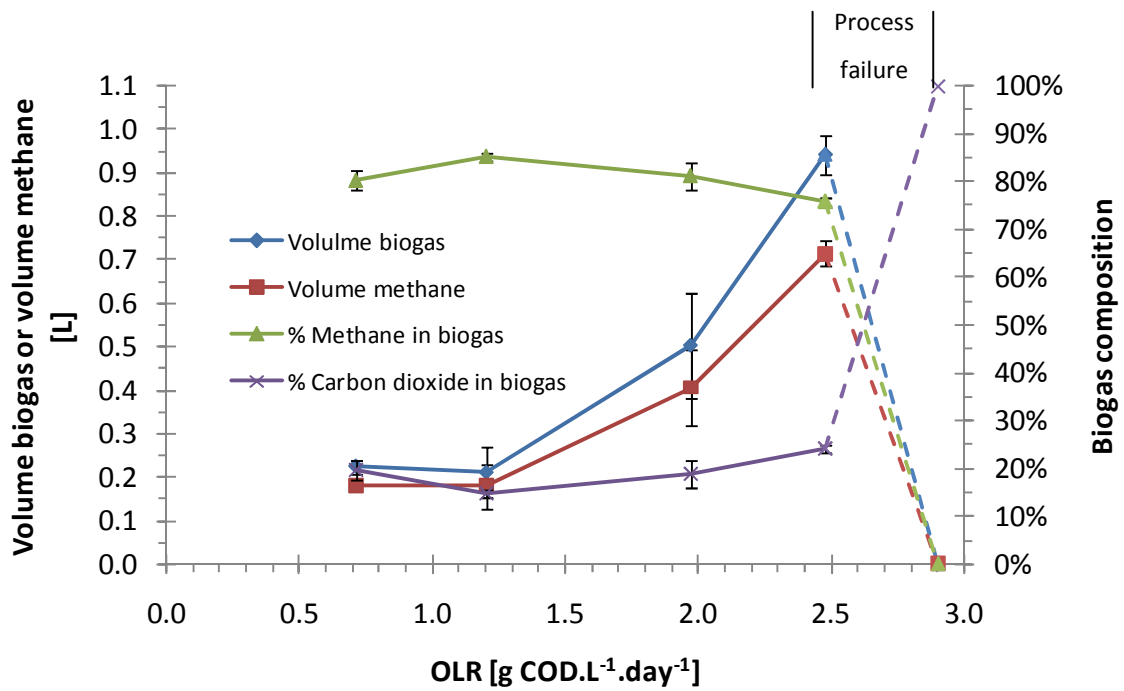


Figure 5-10 Biogas composition and volume biogas and methane produced at different OLRs during the treatment of real milking parlour wastewater in an ASBR at 22.5 °C (first inoculation)

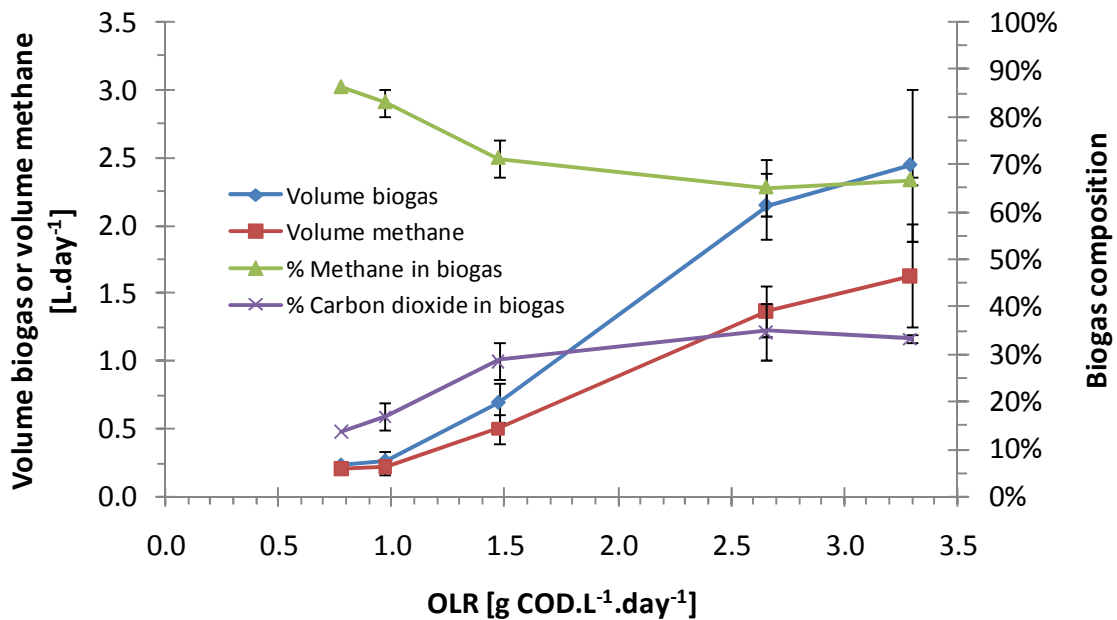


Figure 5-11 Biogas composition and volume biogas and methane produced at different OLRs during the treatment of real milking parlour wastewater in an ASBR at 22.5 °C (second inoculation)

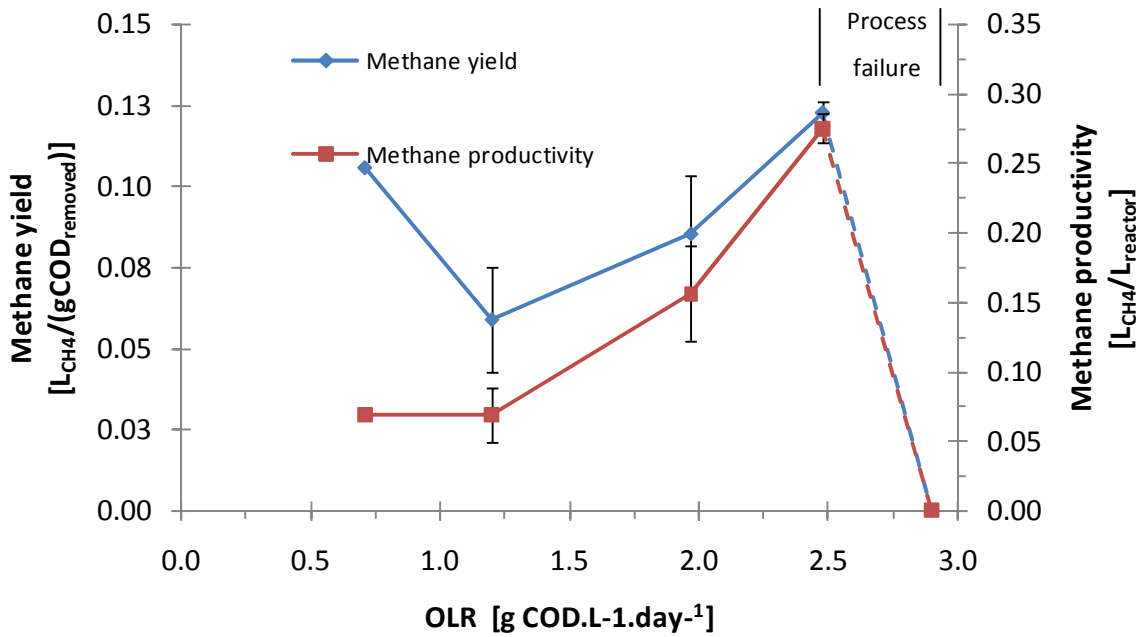


Figure 5-12 Methane yield and methane productivity achieved at different OLRs during the treatment of real milking parlour wastewater in an ASBR operated at 22.5 °C (first inoculation)

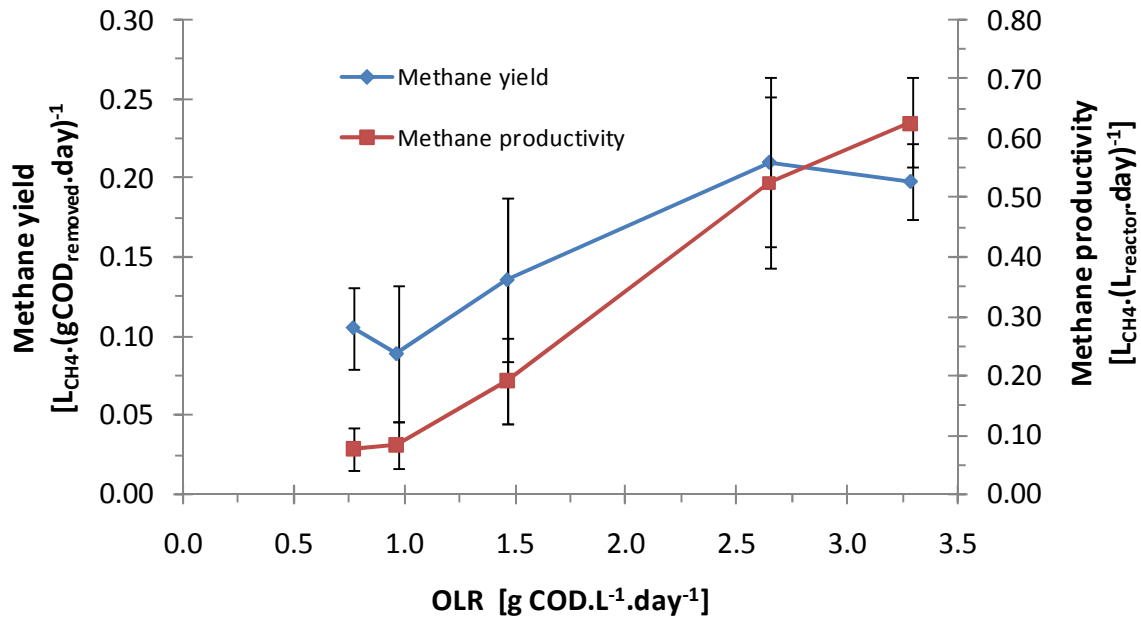


Figure 5-13 Methane yield and methane productivity achieved at different OLRs during the treatment of real milking parlour wastewater in an ASBR operated at 22.5 °C (second inoculation)

to Figure 5-12 it can be seen that, during the first inoculation, the methane yield decreased when the OLR was increased from 0.7 to 1.2 g COD.L⁻¹.day⁻¹ after which it showed a slight upwards trend up until an OLR of 2.5 g COD.L⁻¹.day⁻¹ was applied during the first inoculation. Once the OLR was further increased to 2.9 g COD.L⁻¹.day⁻¹, the methane yield and methane productivity dropped to zero indicating that the methanogenic bacteria were not producing any methane which resulted in reactor failure. The maximum methane yield achieved in the reactor was 0.122 L_{CH₄}.(g COD_{removed}.day)⁻¹.

During the second inoculation, the methane yield decreased slightly when the OLR was increased from 0.8 to 1.0 g COD.L⁻¹.day⁻¹ after which it generally increased and reaches a plateau at an OLR of 3.3 g COD.L⁻¹.day⁻¹ (see Figure 5-13). The minimum methane yield achieved in the reactor was 0.09 L_{CH₄}.(g COD_{removed}.day)⁻¹ (at an OLR of 1.0 g COD.L⁻¹.day⁻¹), while the maximum methane yield achieved was 0.21 L_{CH₄}.(g COD_{removed}.day)⁻¹ at an OLR of 2.7 g COD.L⁻¹.day⁻¹. This is almost double that of the maximum methane yield achieved during the first inoculation, but, it was still lower than the theoretical methane yield of 0.379 L_{CH₄}.(g COD_{removed}.day)⁻¹ which can be achieved during anaerobic digestion at 22.5 °C. As in the case during the treatment of synthetic milking parlour wastewater during part 1 of this study, the methane yield was not constant throughout the duration of either the first or the second inoculation. This indicates that true steady state was most probably not achieved under the anaerobic conditions in the reactor. This can, once again, be ascribed to the fact that the microorganisms were most probably still adapting to the environment and used a high portion the substrate for biomass production, which decreases the amount of substrate that the microorganisms can use for methane formation resulting in lower methane yields. Accumulation of organic matter due to batch operation may also contribute to underestimation of the methane yield.

The fat content (FOG) in the influent and effluent was not monitored in this study. However, the FOG concentration of one of the wastewater samples was measured and was approximately 5000 mg.L⁻¹. This is very high and is most probably due to the presence of milk fat and detergents in the wastewater. A study by Perle *et al.* (123) showed that milk fat in the

substrate can cause inhibition to the anaerobic process. The high fat content in the wastewater and the lower temperature at which the anaerobic digestion took place, are therefore most probably the reasons why the methane yields during both inoculation of Reactor 2 were significantly lower than the theoretical methane yield. Another possible reason for the low methane yield achieved, is the deficiency of phosphorus in the wastewater. The COD:N:P ratio was in the range of 350:5-10:0.6-0.9 which is lower than the ratio of 350:5:1 suggested in literature for efficient anaerobic digestion (48). Although the wastewater was short in nutrients and contained high fat concentrations, the microorganisms were still able to digest sufficient amounts to achieve high COD removal efficiencies during both the first and second inoculation of Reactor 2. It is therefore expected that, by reducing the fat content in the wastewater prior to feeding and adding phosphorus, would result in higher methane yields and more stable reactor operations.

The methane productivity during the first inoculation increased as the OLR was increased up until $2.5 \text{ g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$, but decreased sharply when the OLR was increased to $2.9 \text{ g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$. The maximum productivity achieved at an OLR of $2.5 \text{ g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ was $0.28 \text{ L}_{\text{CH}_4}\cdot(\text{L}_{\text{reactor}}\cdot\text{day})^{-1}$. The methane productivity during the second inoculation shows an almost linear increase from $0.035 \text{ L}_{\text{CH}_4}\cdot(\text{L}_{\text{reactor}}\cdot\text{day})^{-1}$ at an OLR of $0.8 \text{ g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ to $0.63 \text{ L}_{\text{CH}_4}\cdot(\text{L}_{\text{reactor}}\cdot\text{day})^{-1}$ at an OLR of $3.3 \text{ g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$. The increase in the methane productivity coincides with an increase in the COD removal rate at all the OLRs which confirm the statement made earlier that the microorganisms were adapting well to the increase in the OLRs and the reactor could possibly have been operated at even higher OLRs if more time were allowed for the study. The methane productivity at an OLR of $2.0 \text{ g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ was $0.16 \text{ L}_{\text{CH}_4}\cdot(\text{L}_{\text{reactor}}\cdot\text{day})^{-1}$, while that achieved during the second inoculation at a lower OLR of $1.5 \text{ g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ was $0.19 \text{ L}_{\text{CH}_4}\cdot(\text{L}_{\text{reactor}}\cdot\text{day})^{-1}$. It is therefore clear that Reactor 2 performed much better during the second inoculation as the methane productivity at a higher OLR during the first inoculation was lower than the methane productivity at a lower OLR during the second inoculation.

c) Alkalinity, pH and VFA concentrations

First inoculation

During the first inoculation, the pH and TA of the wastewater ranged between 0 and 832 mg.L⁻¹ CaCO₃. Because of the great variation in the pH of the wastewater, the pH was adjusted to 7.4 before being fed to the reactor. The pH, PA and TA of the effluent was measured at least 3 times a week. The pH of the effluent throughout the study ranged between 6.8 and 8.2. The results for the PA, TA, IA and pH throughout the duration of this study can be seen in Figure D-6 in Appendix D.

Generally, the PA and TA of the effluent did not decrease as rapidly as in the case when the synthetic wastewater was treated during part 1 of the study. On day 28, the PA dropped to a value of 891 mg.L⁻¹ CaCO₃. This was accompanied with an increase in the IA/PA ratio from 0.20 to 0.24. An IA/PA ratio of 0.3 has shown in the past to indicate process instability in anaerobic reactors (62; 48). Although the IA/PA ratio was not higher than 0.3 yet, the increase in the IA/PA ratio, together with the low PA measurement was an indication of the possibility of reactor instability that might have occurred in the near future. Supplement alkalinity in the form of 5 g NaHCO₃ was therefore added to the reactor. On day 41, the PA dropped below 1 100 mg.L⁻¹ CaCO₃ and 5 g NaHCO₃ was added once again. The IA/PA ratio during this time was 0.47, indicating possibility of process instability. The addition of NaHCO₃ caused the IA/PA ratio to drop to 0.28 (on day 43) and 0.2 (on day 46).

The concentrations of the VFAs at different stages of the study (from day 18 onwards) are shown in Figure 5-14. Once again, acetic acid was the main VFA contributing to the TVFA concentration. Despite the addition of alkalinity and the decrease in the IA/PA ratio, the TVFA concentration measured on day 45 was higher than usual (625 mg.L⁻¹). However, the reactor operation in terms of methane production did not show a decrease during this time, which indicates that the system quickly recovered. The reactor failure after the increase of the OLR to 2.9 g COD.L⁻¹.day⁻¹ was accompanied with an increase in the VFA concentrations (especially acetic acid and iso-butyric acid) and a sudden decrease in the pH to 6.8. The PA also decreased significantly from 2 133 mg.L⁻¹ CaCO₃ on day 64 to 1 032 mg.L⁻¹ CaCO₃ on day 67. The IA/PA

ratio on day 67 was 0.54, which is notably higher than the average IA/PA ratio of 0.25 obtained throughout the study. This confirms the finding from Almeida *et al.* (62) which stated that IA/PA ratios higher than 0.3 may result in process instability or even process failure.

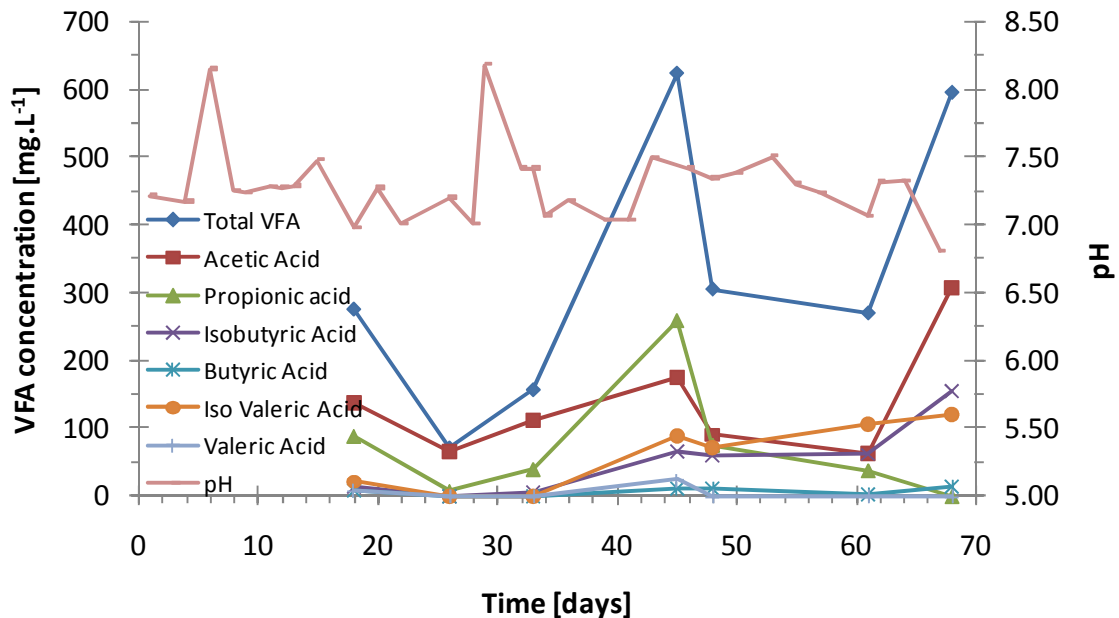


Figure 5-14 VFA concentrations and pH of effluent from ASBR treating real milking parlour wastewater at 22.5 °C (first inoculation)

The increase in the VFA concentrations during reactor overload indicates that disintegration, hydrolysis and acidogenesis happened rapidly, while acetogenesis from the longer chained VFAs and methanogenesis were probably the rate limiting step.

The results suggests that the microorganisms in anaerobic sludge are very sensitive when operating at lower temperatures, especially during start-up and care should be taken that the reactor is not loaded too high too rapidly. The results confirms the statement made in literature that the start-up of anaerobic reactors at lower temperatures is one of the key limits for the application of the process and care should be taken in keeping the concentration of the VFAs low in the reactor (124). The pH dropped to 6.8 when the reactor failed during the first inoculation, confirms the statement made earlier that VFAs concentrations in the effluent

(especially acetate and propionate) and pH of the effluent are good indicators of anaerobic reactor performance (125).

Second inoculation

As in the case of the first inoculation of Reactor 2, the pH of the wastewater fed to the reactor was set to 7.4 each day before feeding. The pH of the effluent from the reactor ranged from 7.2 to 7.8. Figure D-9 in Appendix D shows the PA, TA, IA and pH of the effluent throughout the duration of the study. Figure 5-15 shows the VFA concentrations and the pH of the effluent throughout the duration of the study.

As in the case of the first inoculation, additional alkalinity in the form of $2 \text{ g.L}^{-1} \text{ NaHCO}_3$ was added to the wastewater fed to the reactor when the PA measured in the effluent were close to or below $1200 \text{ mg.L}^{-1} \text{ CaCO}_3$. The PA and TA decreased throughout the duration of the study, but not as rapid as during the first inoculation (see Figure D-6 and Figure D-9 in appendix D). Additional alkalinity only had to be added three times during the study. The first time that alkalinity was added to the reactor was on day 2 after the PA dropped to $942 \text{ mg.L}^{-1} \text{ CaCO}_3$. The other two times that addition alkalinity was added was on day 45 and day 62.

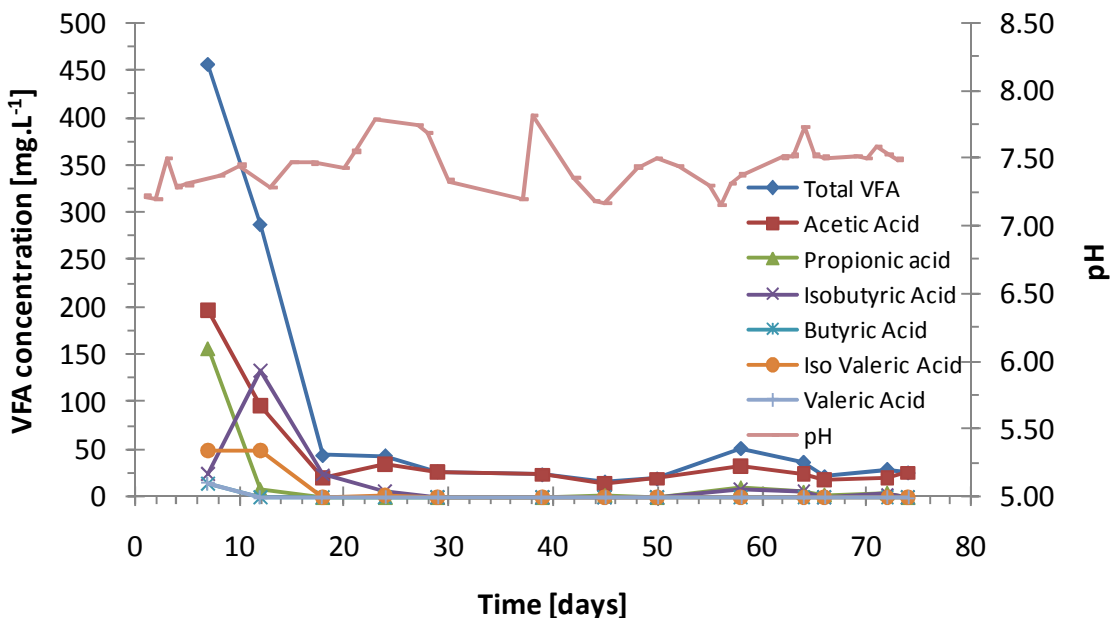


Figure 5-15 VFA concentrations and pH of effluent from ASBR treating real milking parlour wastewater at 22.5 °C (second inoculation)

The average IA/PA ratio throughout the study was 0.15 ± 0.07 . The maximum IA/PA ratio was 0.33 which was during the first 2 days of operation. After additional alkalinity was added on day 2, the IA/PA ratio decreased to 0.26. From there onwards, the IA/PA ratio stayed below 0.3. This indicates that the reactor operation was stable as IA/PA ratios above 0.3 have shown to indicate reactor instability in the past (62). The average IA/PA ratio during the period of the study when the reactor was operated at the highest OLR of $3.3 \text{ g COD.L}^{-1}.\text{day}^{-1}$, was 0.09, which is significantly lower than 0.3 indicating that the reactor operation was still stable.

The TVFA concentration on day 7 and 12 (during the first 2 weeks of operation) was 457 and 288 mg.L^{-1} , respectively. This is very high in comparison with the average TVFA concentration of 31.4 mg.L^{-1} measured during the rest of the reactor operation period (days 18 to 74). Although the PA dropped to a value below $1200 \text{ mg.L}^{-1} \text{ CaCO}_3$ on day 45, no VFA accumulation was detected as the TVFA concentration measured in the effluent on day 45 was 16.8 mg.L^{-1} which is almost half of the average TVFA concentration measured between days 18 and 74. As in the case of the previous study, acetic acid was the main VFAs contributing to the TVFA concentration in the effluent from the reactor.

5.3.3 Reactor 3 (35 °C)

A summary of the main results achieved in Reactor 3 are shown in Table 5-8. The values obtained at each OLR (between 0.8 and $6.6 \text{ g COD.L}^{-1}.\text{day}^{-1}$) and HRT (20.2-3.7 days) were taken after stable operations have been observed (i.e. the TCOD removal efficiency of 3 consecutive samples had a standard deviation of less than 5%).

a) COD removal

The average TCOD and SCOD removal efficiencies as well as the TCOD removal rate (in terms of g COD removed day) at the different OLRs are shown in Figure 5-16. The results for the TCOD and SCOD removal efficiency as well as the amount of TCOD and SCOD removed in Reactor 3 throughout the duration of the study can be seen in Figure D-10 in Appendix D.

From Table 5-8 and Figure 5-7 it is clear that Reactor 3 showed very good performance in terms of TCOD and SCOD removal efficiencies throughout the during of study. The TCOD removal

Table 5-8 Summary of results from the treatment of real milking parlour wastewater in an ASBR at 35 °C

Parameter	Units	OLR [$\text{g COD}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$]										
		0.8	1.2	2.0	2.5	2.9	3.5	3.2	4.0	4.0	6.6	
COD of substrate	$\text{mg}\cdot\text{L}^{-1}$	15119	23190	15820	16110	14130	18377	16774	15163	24171		
pH of substrate		7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
HRT	days	20.2	19.3	8.1	6.5	4.9	5.3	5.2	3.8	3.7		
pH effluent		7.62	7.92	7.69	7.52	7.40	7.50	7.34	7.29	7.54		
TCOD removal efficiency	%	91.9	94.8	97.8	98.3	98.4	98.5	98.1	97.3	96.5		
SCOD removal efficiency	%	96.9	98.1	97.9	98.2	97.1	98.3	98.4	97.7	97.2		
COD removal rate	$\text{g COD}_{\text{removed}}\cdot\text{day}^{-1}$	0.60	1.14	1.93	2.44	2.83	3.38	3.16	3.86	6.35		
Volume biogas	L	0.584	0.613	1.348	2.138	3.080	4.368	2.379	4.180	6.557		
Volume methane	L	0.46	0.47	1.01	1.48	2.04	2.91	1.73	2.79	4.05		
% Methane in biogas	%	78.5	77.0	74.9	69.1	66.1	66.7	72.6	67.1	61.7		
Methane yield	$\text{L}_{\text{CH}_4}\cdot(\text{g COD}_{\text{removed}})^{-1}$	0.293	0.163	0.201	0.233	0.276	0.331	0.210	0.278	0.244		
Methane productivity	$\text{L}_{\text{CH}_4}\cdot(\text{L}_{\text{reactor}}\cdot\text{day})^{-1}$	0.176	0.182	0.388	0.568	0.783	1.120	0.664	1.072	1.557		

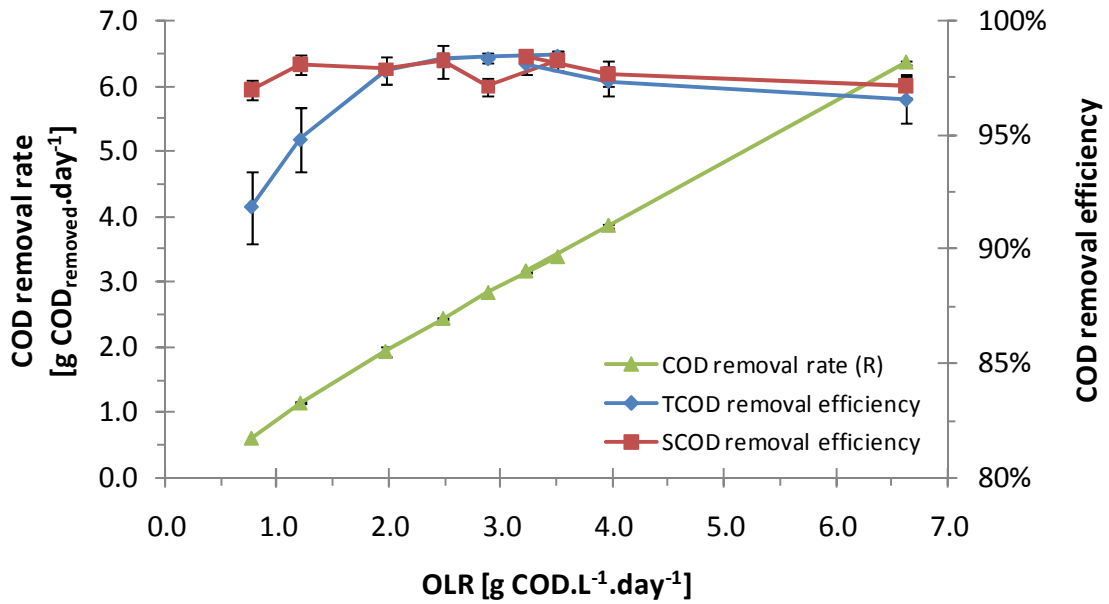


Figure 5-16 COD removal efficiencies and removal rate achieved at different OLRs during anaerobic treatment of real milking parlour wastewater in an ASBR operate at 35 °C (Reactor 3)

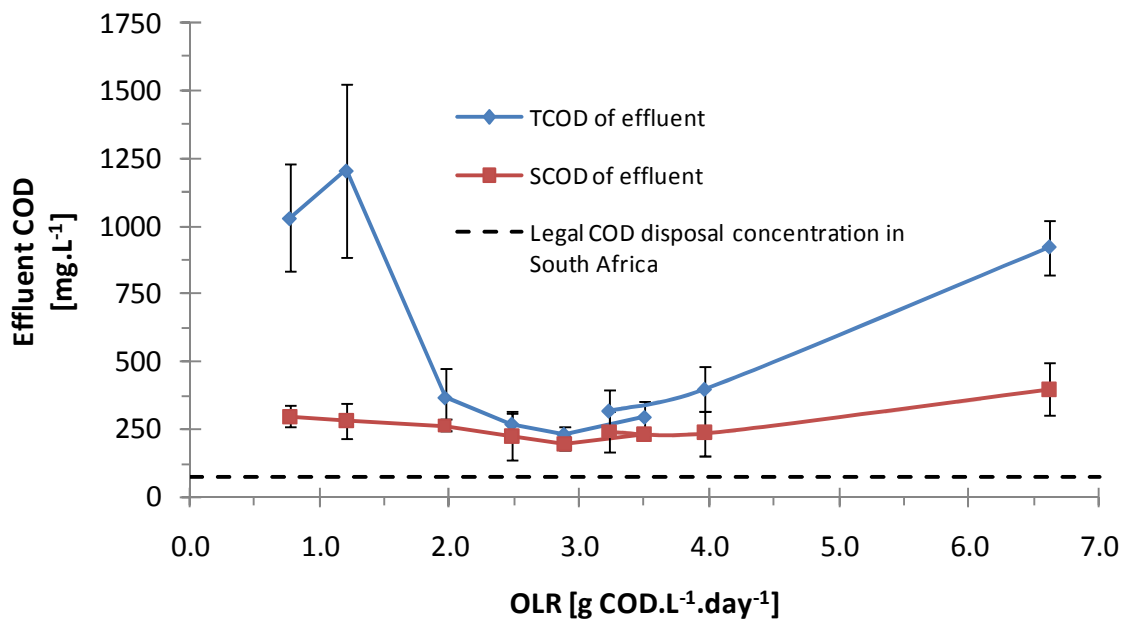


Figure 5-17 COD effluent concentrations achieved at different OLRs during the treatment of real milking parlour wastewater in an ASBR operated at 35 °C (Reactor3)

efficiency at all of the OLRs applied to the reactor did not drop below 91 %, while the SCOD removal efficiency at all of the OLRs applied did not drop below 97 %. The maximum TCOD removal efficiency achieved during the study was 98.5 % and was achieved at an OLR of 3.5 g COD.L⁻¹.day⁻¹ and HRT of 5.3 days. On day 94 (at an OLR of 3.5 g COD.L⁻¹.day⁻¹), a mechanical failure in Reactor 3 occurred, causing approximately 300 ml of sludge to be wasted from the reactor. The OLR applied to the reactor was then decreased to 3.2 g COD.L⁻¹.day⁻¹ in order to prevent possible disruption and overload of the system. The OLR to the reactor was later increased to 4.0 g COD.L⁻¹.day⁻¹ and after 15 days it was increased further with 2.6 g COD.L⁻¹.day⁻¹ to 6.6 g COD.L⁻¹.day⁻¹ in order to see whether the reactor is able to withstand such a high and sudden increase in the OLR. The maximum SCOD removal efficiency was 98.4 % and was achieved when the OLR was decreased to 3.2 g COD.L⁻¹.day⁻¹ (HRT of 5.2 days). There was no significant decrease in the TCOD and SCOD removal efficiencies during the time that the OLR was decreased to 3.2 g COD.L⁻¹.day⁻¹ to prevent reactor failure. Both the TCOD and SCOD removal efficiencies were still more or less constant at 98 %. This indicates that the loss of biomass from the reactor did not alter the ability of the biomass to achieve high removal efficiencies in the reactor. The increase in the OLR from 4.0 g COD.L⁻¹.day⁻¹ to 6.6 g COD.L⁻¹.day⁻¹ did not cause a significant change in either the TCOD or SCOD removal efficiencies which was 96.5 % (TCOD removal efficiency) and 97.2 % (SCOD removal efficiency), respectively.

The COD removal rate as a function of OLR is also shown in Figure 5-16. The COD removal rate increases linearly with an increase in the OLR. When the OLR was decreased from 3.5 to 3.2 g COD.L⁻¹.day⁻¹ after the mechanical failure, the removal rate shows a decrease, but with further increase in the OLR to 4.0 and 6.6 g COD.L⁻¹.day⁻¹, the removal rate increased linearly once again. The slope of the removal rate as a function of OLR during the increase in the OLR up to 3.5 g COD.L⁻¹.day⁻¹ and the increase from 3.2 to 6.6 g COD.L⁻¹.day⁻¹ after the mechanical failure, were more or less constant.

The linear increase in the removal rate as the OLR was increased throughout the study indicates that, although the COD removal efficiency decreased slightly when the OLR was increased to

6.6 g COD.L⁻¹.day⁻¹, the reactor's maximum operational limit had not been reached and the OLR could therefore have been increased even more. When the removal efficiency reaches a plateau, it is an indication that the reactor has reached its maximum operational limit and once this happens, the removal efficiency will decrease with a further increase in the OLR.

Figure 5-17 shows the COD effluent concentrations achieved at the different OLRs during the study. It can be seen that the minimum TCOD and SCOD concentration in the effluent was 233 and 197 mg.L⁻¹ respectively and was achieved when an OLR of 3.5 g COD.L⁻¹.day⁻¹ was applied to the reactor. Although the TCOD and SCOD removal efficiencies were still relatively high when the OLR was increased to 6.6 g COD.L⁻¹.day⁻¹, the TCOD and SCOD concentrations in the effluent showed an increase to 924 and 400 mg.L⁻¹ respectively. The increase in the effluent COD concentration can be ascribed to the high COD concentration in the wastewater fed during that specific period. The COD removal efficiency stayed more or less constant, but the influent COD concentration fed during the time when an OLR of 6.6 g COD.L⁻¹.day⁻¹ was applied was almost 10 000 mg.L⁻¹ higher than when an OLR of 4.0 g COD.L⁻¹.day⁻¹ was applied (see Table 5-8).

Once again, despite of the high COD removal efficiencies achieved at all of the different OLRs applied, the TCOD and SCOD concentrations measured in the effluent was still higher than the South African legal COD concentration on 75 mg.L⁻¹ at which wastewater can be disposed into a fresh water source. It was however, still lower than the legal COD concentration of 5 000 mg.L⁻¹ for irrigation. As in the case when synthetic milking parlour wastewater was treated in an ASBR during the first part of the study, a further polishing step will be necessary before the water can be disposed into a river or reused for equipment washing. However, no polishing step will be necessary if the water is used for irrigation or for other purposes in the milking parlour such as floor flushing.

When comparing the performance in terms of COD removal efficiencies with other anaerobic treatment methods treating dairy wastewater, the ASBRs shows very good performance which indicates that the ASBR process would in fact be suitable for the treatment of the wastewater generated from washing of the equipment from milking parlours (see Table 5-3). All of the studies shown in Table 5-3 that operated at 35 °C, achieved COD removal efficiencies higher

than 85 % treating a wide variety of wastewater from the dairy industry including cheese whey, synthetic dairy wastewater and non-fat dry milk at OLR ranging from 0.2 to 6.26 g COD.L⁻¹.day⁻¹ (92; 24; 12; 82; 97; 119). The performance of Reactor 3 in terms of COD removal efficiencies in this study showed superior performance when compared to the performance of 5 aerobic wastewater treatment technologies implemented at 5 different milking parlours to treat wastewater from the equipment of milking parlour during a study by Chistopherson *et al.* (11). The removal efficiencies achieved by Chistopherson *et al.* (11) ranged between 41 and 75 % which is significantly lower than the removal efficiencies achieved in the ASBRs in this study. This shows that the anaerobic wastewater treatment would be much more effective in the treatment of wastewater from equipment washing in milking parlours.

It must be noted that the aim of this study was not to investigate the effect of temperature on the performance of an ASBR treating milking parlour wastewater, but to look at the feasibility of treating milking parlour wastewater at the two different temperatures. If the effect of temperature was to be investigated, more reactors at more temperatures will have to be used in the study. However, some general comparisons can be made from results from the first inoculation of Reactor 2 and the first 68 days of operation of Reactor 3, as the two reactors were operated at same OLR for the same period of time.

Table 5-9 shows a comparison of the performance of Reactor 3 and the first inoculation of Reactor 2. No significant difference in terms of maximum COD removal efficiencies achieved can be observed. However, significantly lower minimum COD removal efficiencies were achieved in Reactor 2 than in Reactor 3 due to the reactor failure that occurred at an OLR of 2.9 g COD.L⁻¹.day⁻¹ in Reactor 2. A significant difference in the minimum and maximum COD concentrations of the effluent from the two reactors is observed. The minimum and maximum COD concentration of the effluent from Reactor 2 is almost double that from Reactor 3, indicating that although high removal efficiencies were achieved in both reactors, Reactor 3 was performing better than Reactor 2 in terms of providing an effluent with lower COD concentrations.

b) Biogas production, methane yield and methane productivity

Figure 5-18 shows the daily biogas and methane production as well as the methane and carbon dioxide content in the biogas at the different OLRs applied. The methane yield and methane productivity as a function of OLR are shown in Figure 5-19. The daily biogas production and biogas content at the different OLRs applied throughout the duration of the reactor operation can be seen in Figure D-11 in Appendix D.

Table 5-9 Comparison of performance of the first inoculation of Reactor 2 (operated at 22.5 °C) and Reactor 3 (operated at 35 °C) treating real milking parlour wastewater

Parameter	Units	Reactor 2 (1 st inoculation)	Reactor 3
TCOD removal	%	85-98	92-98
SCOD removal efficiency	%	81-99	97-98
TCOD concentration of effluent	mg.L ⁻¹	512-1 998	296-1 030
SCOD concentration of effluent	mg.L ⁻¹	195-1 250	197-298
Methane yield	L _{CH₄} ·(g COD _{removed}) ⁻¹	0.06-0.122	0.16-0.28
Methane productivity	[L _{CH₄} ·(L _{reactor}) ⁻¹]	0.07-0.28	0.18-0.78
Methane content in biogas	%	76-80	66-78
Biogas production	L _{CH₄} ·day ⁻¹	0.21-0.94	0.6-3.1
OLR range	g COD.L ⁻¹ ·day ⁻¹	0.7-2.9	0.8-2.9
OLR during reactor failure	g COD.L ⁻¹ ·day ⁻¹	2.9	Did not fail
Maximum TVFA concentration	mg.L ⁻¹	625	183
Operating temperature	°C	22.5	35

From Figure 5-18 it can be seen that generally, the methane content in the biogas decreased from 78.6 % to 66.7 % as the OLR was increased from 0.8 to 3.5 g COD.L⁻¹·day⁻¹. When the OLR was decreased to 3.2 g COD.L⁻¹·day⁻¹ after the mechanical failure in the reactor, the methane content in the biogas increased to 72.6 %, but as the OLR was increased further, the methane content in the biogas decreased to 61.7 %. The decrease in the methane content in the biogas with an increase in the OLR was also observed earlier in the study when synthetic milking

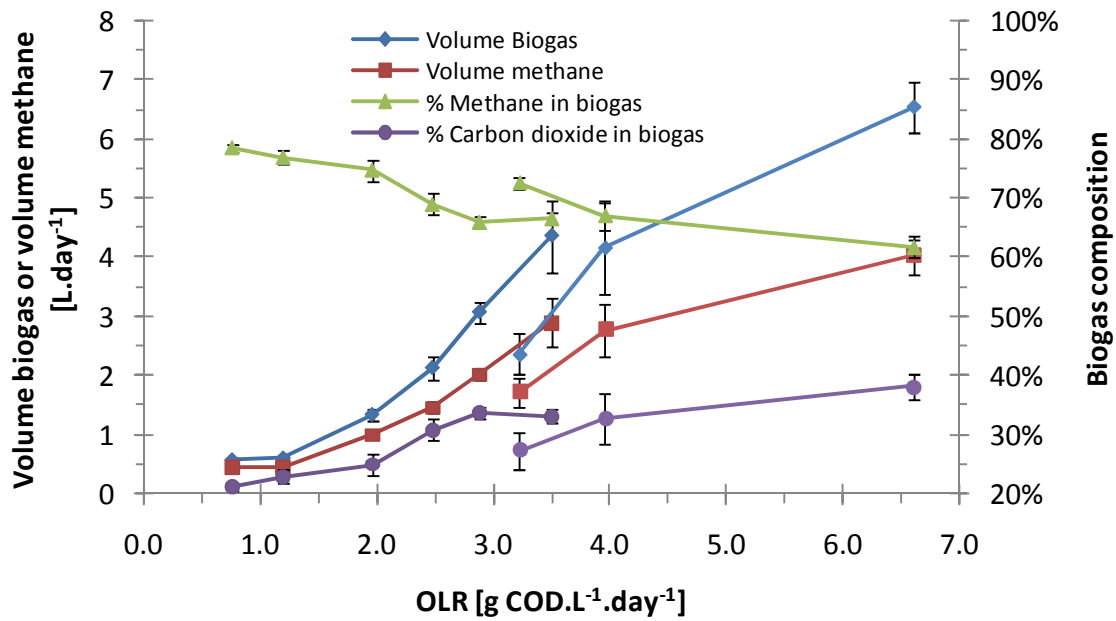


Figure 5-18 Biogas composition and volume biogas and methane produced at different OLRs during the treatment of real milking parlour wastewater in an ASBR at 35 °C (Reactor 3)

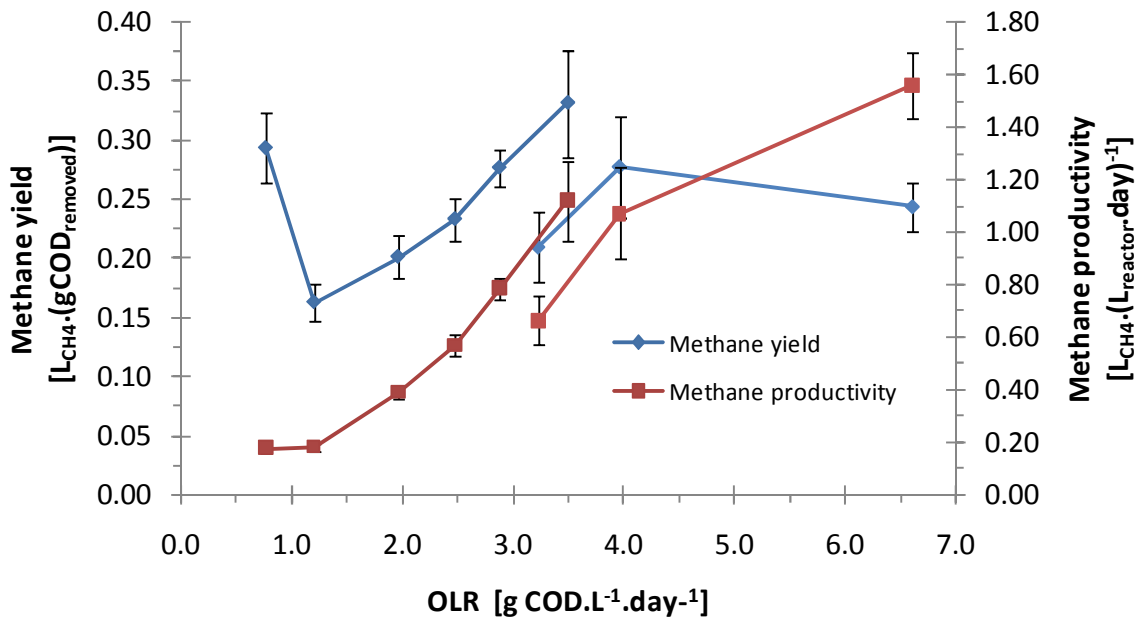


Figure 5-19 Methane yield and methane productivity achieved at different OLRs during the treatment of real milking parlour wastewater in an ASBR operated at 35 °C (Reactor 3)

parlour wastewater was treated in a similar ASBR at 35 °C (see Figure 5-3), which yielded a methane content in the biogas of 61.9% at an OLR of 5.1 g COD.L⁻¹.day⁻¹.

Despite the decrease of the methane content in the biogas with an increase in the OLR, the volume methane produced increased with an increase in the OLR. This indicates that the increase in the daily biogas production with an increase in OLR was high enough to compensate for the decrease in the methane content of the biogas. The daily volume of biogas and methane produced decreased when the OLR was decreased from 3.5 to 3.2 g COD.L⁻¹.day⁻¹ after mechanical failure in the reactor (and hence, loss of sludge from the reactor). After the OLR was increased further to 4.0 g COD.L⁻¹.day⁻¹, the volume methane produced and volume biogas produced daily increased, but it was still lower than the volumes produced at an OLR of 3.5 g COD.L⁻¹.day⁻¹ (before the loss of sludge from the reactor). This shows that the microorganisms was adapting well the new operating conditions, but the loss of some of the microorganisms from the reactor (approximately 300 mL) resulted in less methanogenic bacteria being present the reactor and hence, lower methane production.

From Figure 5-19 it can be seen that during the early stages of the study, when the OLR was increased from 0.8 to 1.2 g COD.L⁻¹.day⁻¹, the methane yield decreased drastically from 0.29 to 0.16 L_{CH₄}.(g COD_{removed}.day)⁻¹. After further increase in the OLR, the methane yield reached its maximum value of 0.33 L_{CH₄}.(g COD_{removed}.day)⁻¹ at an OLR of 3.5 g COD.L⁻¹.day⁻¹, which is only slightly less than the theoretical methane yield of 0.40 L_{CH₄}.(g COD_{removed}.day)⁻¹ that can be achieved during anaerobic digestion when glucose is used as carbon source. The methane yield decreased after some sludge was wasted from the reactor after the mechanical failure that occurred on day 94 of the reactor operation. The decrease in the methane yield to 0.21 L_{CH₄}.(g COD_{removed}.day)⁻¹ during this time indicates the possibility of a slight disruption in the reactor due the decrease in the volume of microorganisms present in the reactor. The methane yield increased again, but did not reach the maximum value of 0.33 L_{CH₄}.(g COD_{removed}.day)⁻¹ achieved earlier at an OLR of 3.5 g COD.L⁻¹.day⁻¹. As in the case the methane yields achieved in Reactors 1 and 2, the methane yield was not constant during the

study indicating once again, that, although a pseudo-steady state was reached (due to the constant COD removal efficiencies), true steady state was most probably not reached in Reactor 3 during this study. The range of methane yields achieved in this study of 0.16 to 0.33 $L_{CH_4} \cdot (g \text{ COD}_{\text{removed}} \cdot \text{day})^{-1}$ is higher than that achieved during the study by Upendrakumar *et al.* (92) when treating cheese wastewater in a single- and two-phase ASBR. The methane yields achieved in the single-phase and two-phase system was 0.17 and 0.20 $L_{CH_4} \cdot (g \text{ COD}_{\text{removed}} \cdot \text{day})^{-1}$ respectively (see Table 5-3).

The maximum methane productivity that was achieved was 1.6 $L_{CH_4} \cdot (L_{\text{reactor}} \cdot \text{day})^{-1}$ when an OLR of 6.6 $g \text{ COD} \cdot L^{-1} \cdot \text{day}^{-1}$ was applied to the reactor. The decrease in the methane productivity due to the decrease in OLR from 3.5 to 3.2 $g \text{ COD} \cdot L^{-1} \cdot \text{day}^{-1}$ and the sludge being lost from the reactor, is evident from Figure 5-19. It is also evident from the increase in the methane productivity from the OLR which increased from 3.2 to 6.6 $g \text{ COD} \cdot L^{-1} \cdot \text{day}^{-1}$ that, although the loss of sludge from the reactor resulted in a slight decrease in the methane productivity, the microorganisms soon recovered.

The results in terms of methane yield and methane productivity obtained during the treatment of real milking parlour wastewater at 35°C in Reactor 3 compares well with the results obtained in Part 1 of this study when synthetic milking parlour wastewater was treated in a similar ASBR at 35°C (see Table 5-2). A higher methane yield was obtained during the treatment of the real wastewater at 35 °C (Reactor 3) than that achieved during the treatment of synthetic wastewater at 35 °C (Reactor 1) which suggests that the microorganisms in Reactor 3 adapted better to the changing in environmental conditions than those in Reactor 1. The volumetric methane productivity achieved during the treatment of the synthetic wastewater at an OLR of 3.9 $g \text{ COD} \cdot L^{-1} \cdot \text{day}^{-1}$ in Reactor 1 was 1.03 $L_{CH_4} \cdot (L_{\text{reactor}} \text{ day})^{-1}$ while that achieved at an OLR of 4.0 $g \text{ COD} \cdot L^{-1} \cdot \text{day}^{-1}$ in Reactor 3 was 1.07 $L_{CH_4} \cdot (L_{\text{reactor}} \text{ day})^{-1}$. This shows that the reactors produced almost the same volume of methane per reactor volume per day at the respective OLRs. It also compares very well with results obtained in other studies treating similar wastewater in ASBRs and other anaerobic treatment methods (see Table 5-3).

When comparing the performance of Reactor 3 with that of Reactor 2 during the first 68 days of operation (when Reactors 2 and 3 were operated under the same OLRs), the volume of methane produced, the methane yield and methane produced in Reactor 3 were in all cases almost double than that produced in Reactor 2 (see Table 5-9). This suggests that, biomass activity is higher when operating at higher temperatures, closer to the optimum temperature for methanogenic bacteria (35 to 37 °C). Biogas formation was observed 4 days after inoculation of operation in Reactor 3, but only after 22 days after Reactor 2 was inoculated, while it was observed 4 days after inoculation in Reactor 3. The sludge used in both reactors was taken from a mesophilic anaerobic digester operated at 30 °C. This indicates that the microorganisms acclimatised better to the increase in operating temperature to 35 °C than to a decrease in the temperature to 22.5 °C. This suggests that during the treatment at a lower temperature, most of the carbon in the substrate was used by the microorganisms for growth and to establish themselves in the reactor and very little carbon were used to produce methane. Hence, the high COD removal efficiencies and low methane production in the reactor.

The methane content in the biogas was generally lower in Reactor 3 than in Reactor 2 at all of the OLRs applied. This higher methane content in the biogas at lower temperatures were also observed when slaughterhouse wastewater was treated in an ASBR at 20, 25 and 30 °C in a study by Masse and Masse (116) as well as when swine manure was treated in an ASBR at 10 and 20 °C (126).

Figure 5-20 shows the methane production as a function of COD removal rate during at OLRs up to 2.5 g COD.L⁻¹.day⁻¹ achieved in Reactor 3 and during the first inoculation of Reactor 2. The results from Figure 5-20 also shows that Reactor 3 performed better in converting COD to methane than Reactor 2. The non-linearity of the graph indicates that the microorganisms were continuously adapting to the increase in the OLR to the reactors.

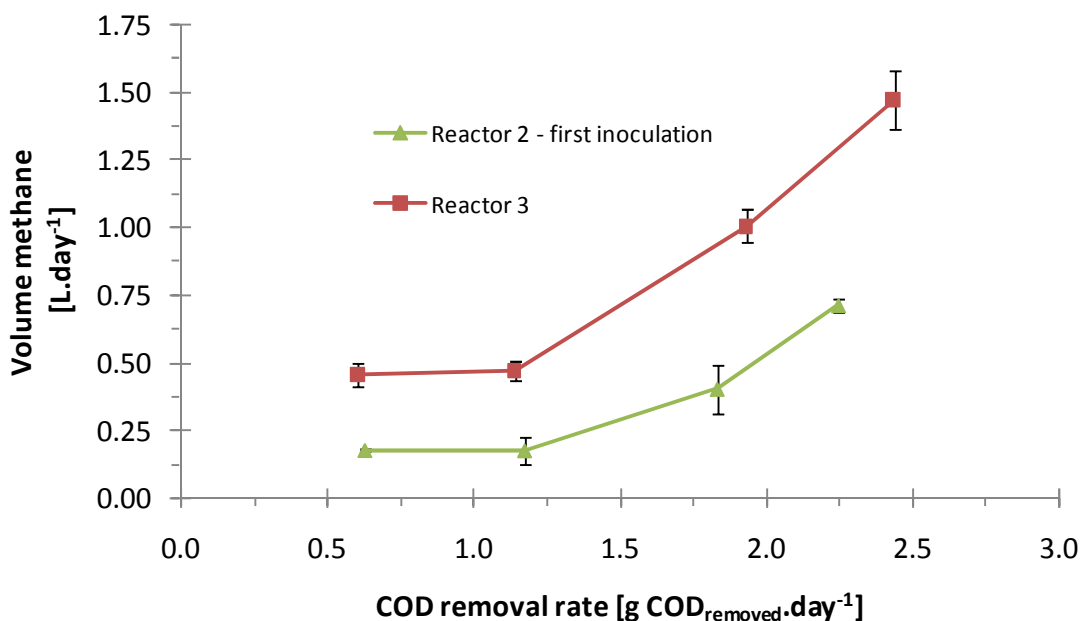


Figure 5-20 Methane production as a function of COD removal rate during the first inoculation of Reactor 2 (22.5 °C) and Reactor 3 (35 °C) treating real milking parlour wastewater

c) Alkalinity, pH and VFA concentrations

The pH of the effluent from the reactor ranged from 7.5 to 8.4. Figure D-12 in Appendix D shows the PA, TA, IA and pH of the effluent throughout the duration of the study. The alkalinity of the wastewater fed to the reactor is shown in Table 5-4. As in the case of Reactor 2, additional alkalinity was added to the reactor in the form of 5 g NaHCO₃ if the PA in the effluent was measured to be below or close to 1 200 mg.L⁻¹ CaCO₃ as PA values below 1 200 mg.L⁻¹ CaCO₃ have shown in the past to result in reactor instability (63).

Once additional alkalinity was added to the reactor, the PA in the effluent from the reactor showed a sharp increase after which it gradually decreased. The decrease in the PA of the effluent was also seen during the synthetic milking parlour wastewater treatment in an ASBR (Reactor 1) during part 1 of the study. The decrease was however not as sharp as in the case of reactor 1. The IA/PA ratio observed throughout the duration of time that the reactor was operated ranged between 0.07 and 0.21 with an average of 0.12±0.03. This indicates that the reactor operations were stable throughout the study as the IA/PA ratio was never above 0.30.

The VFA concentrations were measured once a week from day 18 up until the end of the study and can be seen in Figure 5-21. The pH of the effluent throughout the duration of the study can also be seen in Figure 5-21. Generally, the VFA concentrations in the effluent were relatively low (12.6 to 50.0 mg.L⁻¹) except on day 18 when it was 95.7 mg.L⁻¹ and on day 68 when it was 183 mg.L⁻¹. The PA, pH and IA/PA ratio of the effluent from the reactor on day 18 was 1 664 mg.L⁻¹ CaCO₃, 7.3 and 0.16 respectively. A mechanical failure to the pulley system occurred on day 67 and the reactor content could therefore not be mixed for the whole day. This was most probably the reason for the high VFA concentrations in the reactor effluent. The PA, pH and IA/PA ratio, however, were measured to be 1 783 mg.L⁻¹ CaCO₃, 7.4 and 0.11 respectively on day 68. This shows that, although the VFA concentrations on day 18 and 68 were relatively high in comparison with the VFA concentrations during the rest of the time that the reactor was operated, it was still low enough not to cause reactor instability.

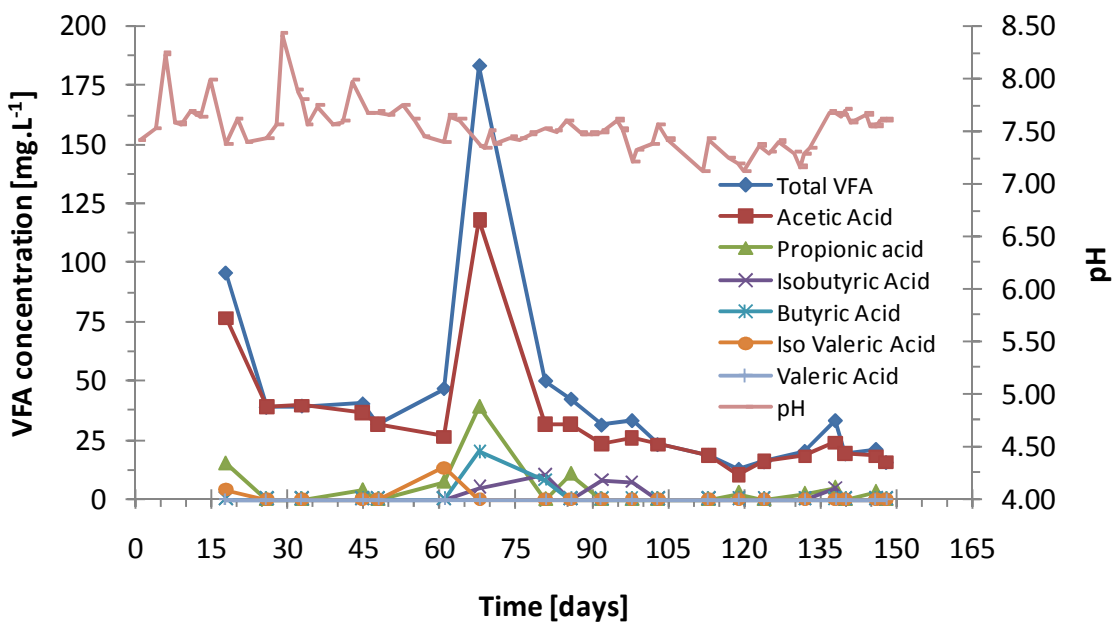


Figure 5-21 VFA concentrations and pH of effluent from ASBR treating real milking parlour wastewater at 35 °C (Reactor 3)

5.3.4 Summary

The results from the performance of Reactors 2 and 3 show that, wastewater generated from the washing of milking equipment in milking parlours is suitable for treatment in an anaerobic sequencing batch reactor at 35 °C (OLRs between 0.7 and 6.6 g COD.L⁻¹.day⁻¹ and HRTs between 20.2 and 3.7 days) as well as at 22.5 °C (OLRs OLRs between 0.8 and 3.3 g COD.L⁻¹.day⁻¹ and HRTs between 26 and 8.1 days). Significant COD reduction (85 % to 98 %) was achieved in both reactors. However, the reactor operated at 22.5 °C failed due to suspected overload and it is therefore evident that anaerobic bacteria are more sensitive at lower temperatures. Despite the good performance of the ASBR, supplement alkalinity was necessary to keep the partial alkalinity above 1 200 mg.L⁻¹ CaCO₃.

By applying these results, the conceptual sizing of an ASBR, as would be used at a typical milking parlour, are provided in Appendix E.

Chapter 6 - Conclusions and Recommendations

6.1 Conclusions

By assessing the water consumption and effluent generation at five South African milking parlours, the following can be verified:

- The total daily volume of wastewater generated in these milking parlours differs significantly between 15 and 51 L.cow⁻¹.day⁻¹.
- The volumes of wastewater generated from the CIP washing of equipment in these milking parlours are rather similar, ranging between 4.9 and 6.4 L.cow⁻¹.day⁻¹.

Based on the experimental assessment of ASBR technology for treatment of CIP effluent from milking parlours, the hypotheses (as stated at the end of Chapter 3) are proven to be true and the following conclusions can be drawn:

- Varying detergent concentrations, up to four times the typical concentrations used at milking parlours, appear to have no significant effect on the performance of the ASBR process in terms of COD removal efficiencies, methane yields or methane productivity. The ASBR that was used to treat synthetic milking parlour wastewater at varying detergent concentrations (up to two, three and four times the normal detergent concentrations) and increased OLRs (between 0.6 and 5.2 g COD.L⁻¹.day⁻¹) removed between 89 and 98 % of the TCOD at 35 °C.
- The ASBR process is suitable to treat milking parlour equipment wash water at 22.5 °C (without nutrient control) with OLRs up to at least 3.3 g COD.L⁻¹.day⁻¹. However, at an operating temperature of 22.5 °C the ASBR process appears to be more susceptible to

failure (compared to operation at 35 °C) when the OLR is increased too quickly. This is to be expected, since methanogenic bacteria acclimatise easier and operate more effectively at temperatures closer to conventional mesophilic temperatures. The ASBR that was used to treat real milking parlour wastewater at 22.5 °C achieved TCOD removal efficiencies between 86 and 98 %, but failed during an OLR of 2.9 g COD.L⁻¹.day⁻¹. In a second attempt, while applying a slower increase in OLR, TCOD removal efficiencies between 89 and 97 % were achieved up to an OLR of 3.3 g COD.L⁻¹.day⁻¹ without signs of reactor failure.

- The ASBR process is suitable to treat milking parlour equipment wash water at 35 °C (without nutrient control) with OLRs up to at least 6.6 g COD.L⁻¹.day⁻¹. When treating real milking parlour wastewater at mesophilic temperatures, COD removal efficiencies between 92 and 98 % were achieved at OLRs ranging from 0.8 to 6.6 g COD.L⁻¹.day⁻¹.
- At the maximum levels of OLR, the COD concentration in the effluent from the ASBRs was always below 1 000 mg.L⁻¹. Although this is notably higher than 75 mg.L⁻¹ (the legal limit for safe disposal into a fresh water body), it is more than acceptable for up to 50 m³.day⁻¹ irrigation.
- When treating milking parlour equipment wash water in an ASBR at both 22.5 and 35 °C, supplement alkalinity is required to keep the PA above 1 200 mg.L⁻¹ CaCO₃.
- A maximum yield of 0.33 L_{CH₄}.(g COD_{removed})⁻¹ was achieved at 35 °C, which is notably lower than the maximum yields of 0.40 L_{CH₄}.(g COD_{removed})⁻¹ that can theoretically be achieved with glucose as carbon source. Various reasons can be stated, including the fact that wastewater from milking parlour equipment washing does not contain sufficient phosphorus for optimum operation and that these batch processes only operate at pseudo steady state.

6.2 Recommendations

- A more comprehensive and dedicated study is required to accurately determine the water usage, wastewater generation and composition of wastewater generated from different milking parlours. This may include the installation of water meters and more accurate sampling methods.
- It should be determined whether the ASBR process can operate effectively for long periods of time (longer than 1 year) at the OLRs tested in this study, as well as at higher OLRs at both 22.5 and 35 °C.
- The impact of nutrient concentrations (e.g. P and N) should be investigated to optimise performance. However, one would prefer to keep the process as simple as possible for dairy farm operations, therefore minimising operator interactions related to nutrient control, etc.
- More work on the fundamental principles of the operation of ASBRs (such as granulation and mixing) should be done to help understand the settling mechanism in the ASBR process.
- The possibility of recovering heat for temperature control from the biogas produced in the ASBR should be investigated in order to minimise the heating requirements for the process.
- The effect of milk fats on the activity of the methanogenic bacteria should be investigated in order to possibly achieve higher methane yields and methane productivities when treating dairy effluent in anaerobic digesters.

When considering the ASBR process as a full-scale operation at a milking parlour, the following is recommended:

- Install an equalisation tank prior to feeding the wastewater to the ASBR in order to neutralise the pH of wastewater and to add supplement alkalinity and nutrients. Supplement alkalinity can be added in the form of NaHCO_3 while supplement phosphorus can be added in the form of K_2HPO_4 in the equalisation tank.

- Fat traps or other possible methods for fat removal (such as dissolved air flotation, air flotation, enzymatic hydrolysis or gravity traps) should be installed in milking parlours prior to treating it in an ASBR in order to reduce the FOG concentration in the influent wastewater.
- If the effluent from the ASBR is to be reused for equipment washing or to be disposed into a nearby water body, a final polishing step is such as membrane filtration may be required.

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Appendix A Milking parlour wastewater

A.1 Estimating daily milking parlour wastewater volumes

A-1 Worksheet to estimate the daily volume of wastewater generated in a milking parlour (adapted from Payer, et al. (2000))

Category	Description
Milking system cleaning	
A	Sink volume x number of cleaning cycles per milking x number of milkings per day
Bulk Tank cleaning	
B	Bulk tank size x 5% x number of cleanings per week ÷ 7 days/week (<i>automatic washing</i>)
	Bulk tank size x 3% x number of cleanings per week ÷ 7 days/week (<i>manual washing</i>)
Udder cleaning and Stimulation	
C	0.95 litres per cow x number of cows x number of milkings per day (<i>when using single-service towels and buckets</i>)
	Average volume used per cow x number of cows x number of milkings per day (<i>when using spraying or automatic prep stalls</i>)
Milk Pre-cooling	
D	Average daily milk production x water-to-milk-flow ratio x fraction of water discharged to drain
Water softening	
E	Wastewater per regeneration cycle x number of cycles per week ÷ 7 days per week
Milking parlour and holding area washdown	
F	Sum of water used at each hose = flow rate (litres/min) x average time used (min/day)
Other wastewater Sources	
G	Hand and boot washing, manual equipment cleaning, milk discharge etc
Total Wastewater generated	
Total	A + B + C + D + E + F + G

A.2 Wastewater components balance

The volume balance for the synthetic dairy wastewater was done on the CIP washing system of MP 2. It was done based on the following assumptions:

- Approximately 44 litres of milk stays behind in the pipeline system which are flushed from the system by the first rinse
- The CIP washing consist of 5 different steps which all uses 120 litres
- An acid rinse is done twice a week
- Milking (and hence, equipment washing) takes place twice a day

The component volume balance was done on taking one week as the basis. The total volume of each component in the wastewater (water, milk and detergents) in a total wastewater sample of 1 week was calculated after which the fraction of each in the wastewater was calculated. The volume of each component per litre of wastewater was then calculated. The volume estimated was taken as the volume of detergents under “normal” operations.

Table A-3 shows the calculations used to determine the composition of the milking parlour synthetic wastewater, while Table A-2 shows the composition of the wastewater under “normal” conditions.

Table A-2 Summary of calculations of component volume balance to determine the composition of the synthetic milking parlour wastewater fed to Reactor 1

Constituent	Times per day	Times per week	Concentration used [ml.L ⁻¹]	Total volume per rinse [litres]	Amount of water per week [litres]	Fraction of total water [%]	Total volume of detergents used per week [litres]
Milk	2	14	n/a	44	616	6.66%	n/a
1 st Rinse	2	14	n/a	120	1680	18.15%	n/a
2 nd Rinse	2	14	n/a	120	1680	18.15%	n/a
Detergent washing	2	14	5	120	1680	18.15%	8.4
Rinse	2	14	n/a	120	1680	18.15%	n/a
Sanitising agent	2	14	2	120	1680	18.15%	3.36
Acid rinse	2	2	5	120	240	2.59%	1.2
Total				764	9256	100	12.96

Table A-3 Composition of synthetic milking parlour wastewater under "normal" conditions

Constituent	Total volume wastewater per week [Litres]	Fraction of total water	Volume per litre [ml.L ⁻¹]
Water	8627.04	0.93	932.05
Milk	616.00	0.067	66.55
Super Klenz	8.40	0.00091	0.91
Super San	3.36	0.00036	0.36
AcidEx	1.20	0.00012	0.13
Total	9256	1	1000

Appendix B Reactor drawings

B.1 Detailed design drawings of Reactors

B.1.1 Main dimensions of Reactor 1 (Reactor 2 and 3)

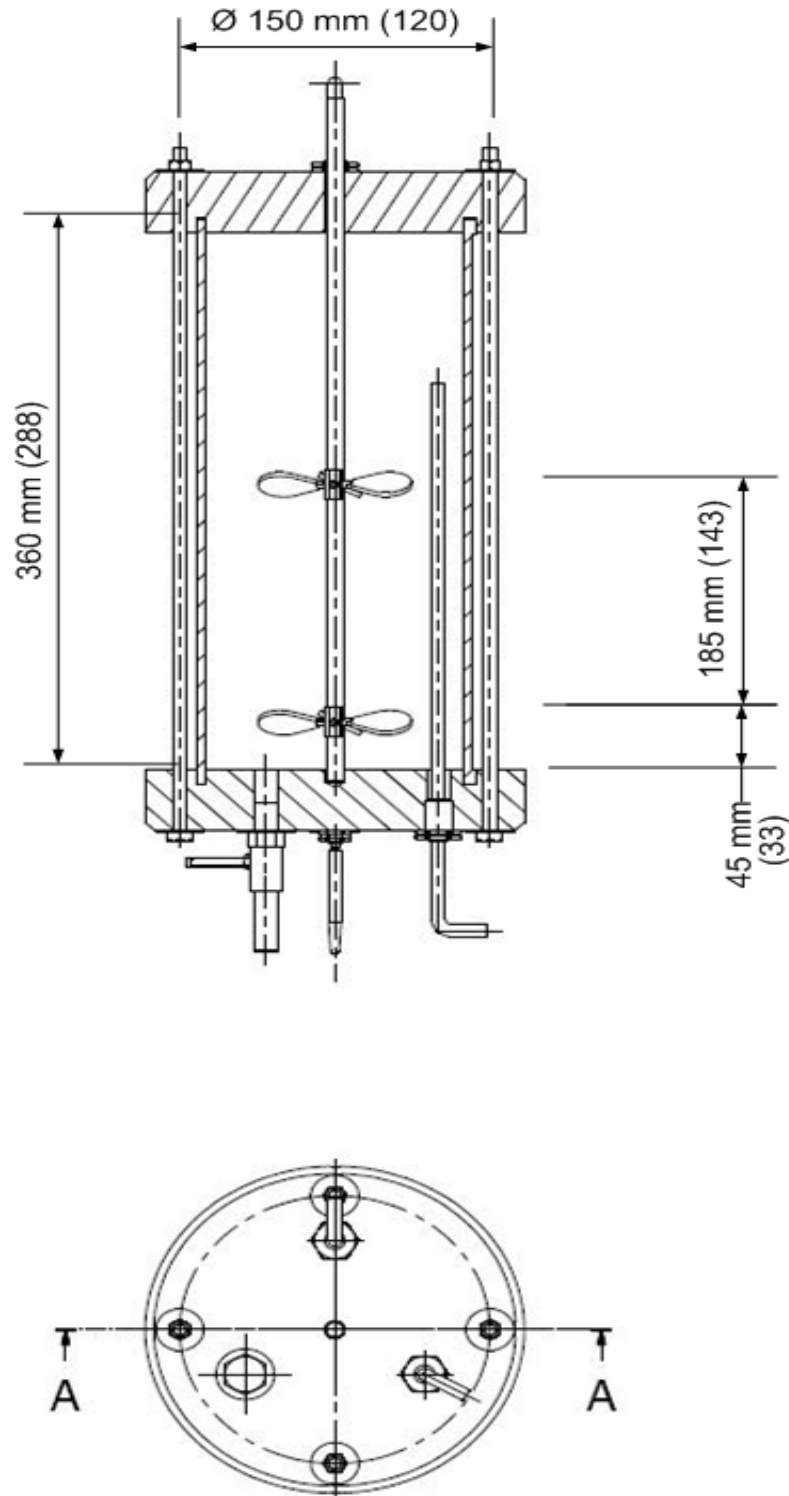


Figure B-1 Design drawing of ASBRs used in this study

B.1.2 3-D view of reactor

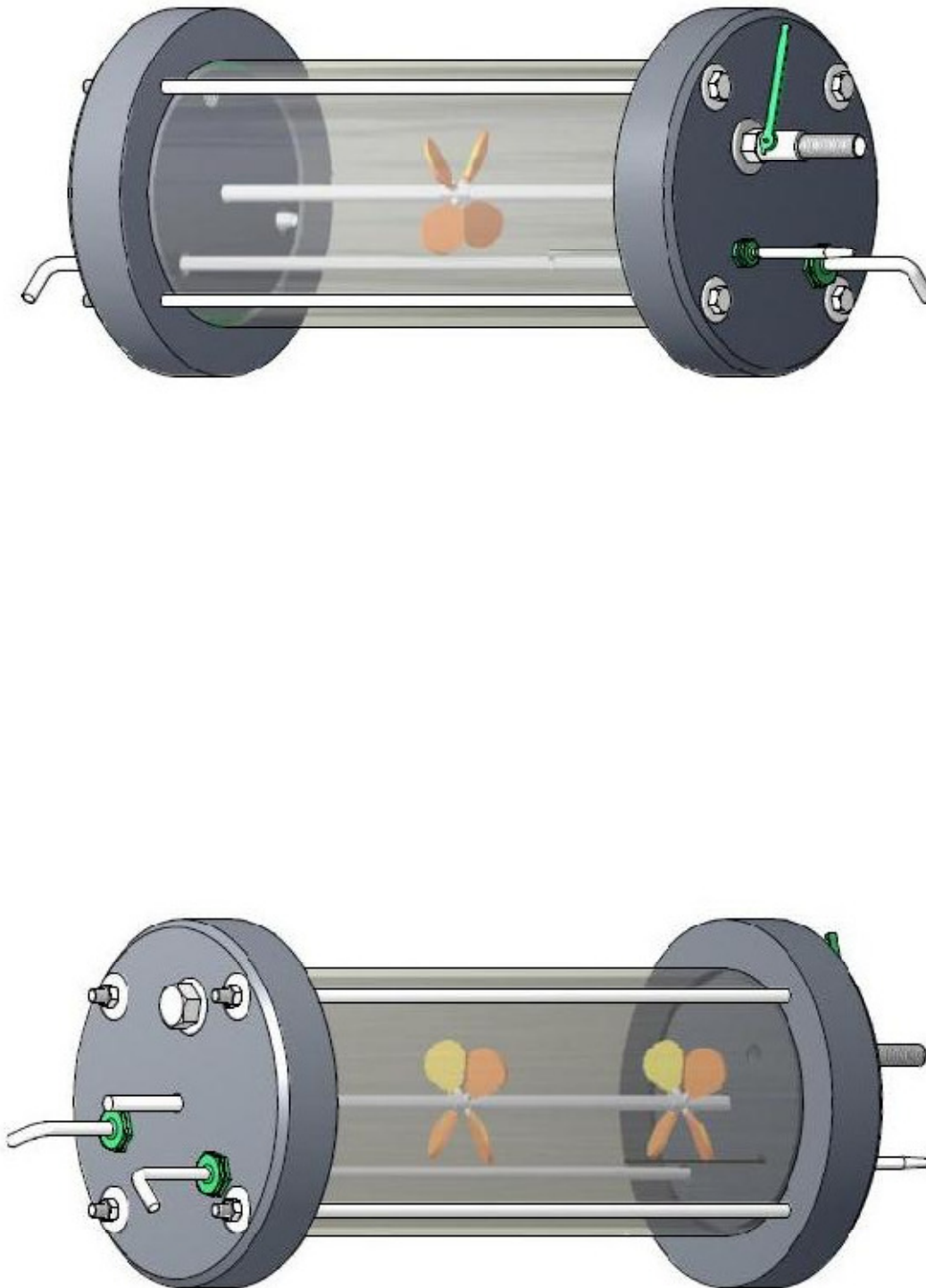


Figure B-2 3-Drawings of ASBRs used in this study

B.2 Pulley system

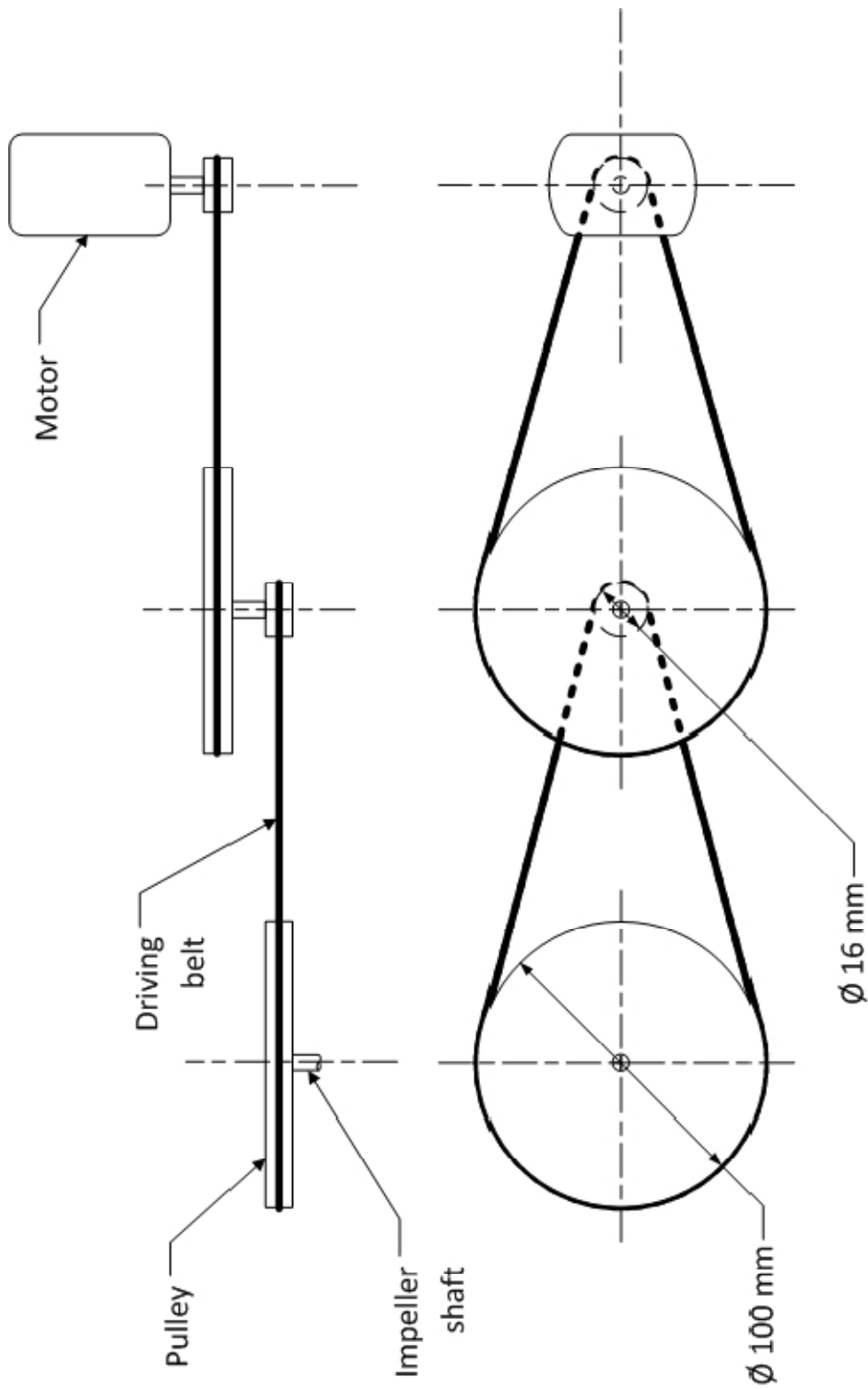
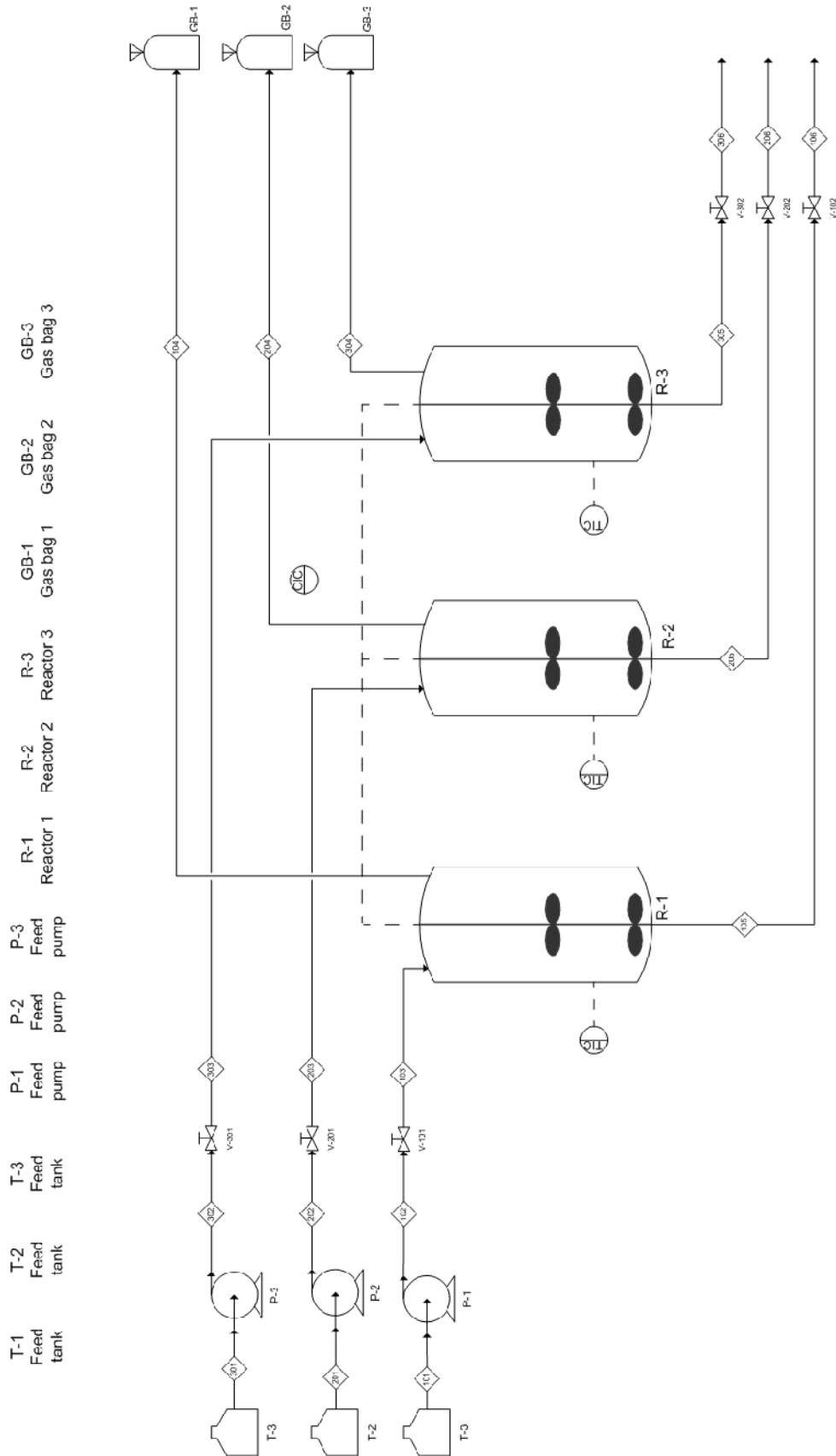


Figure B-3 Drawing of pulley system used in this study

Appendix C Experimental procedures

C.1 P&ID



TIC: Temperature indicator controller
 CIC: Timer indicator controller

Figure C-1 P&ID of experimental setup

C.2 Standard Operating Procedures

- At 10:30, the mixers of all three reactors were switched off by turning the black switches on the panel marked “Reactor 1”, “Reactor 2” and “Reactor 3” to the “off” positions
- The sludge (biomass) was then allowed to settle for 2 hours
- While waiting for the sludge to settle, the required volumes of wastewater (depending on the organic loading rate) to be added to Reactors 2 and 3 was measured in a measuring cylinder and then add the wastewater to the feeding bottles above Reactors 2 and 3
- The required volumes of milk, water and soap (depending on the operating conditions) was measured and added to a 1L bottle and mixed well and the required amount of this synthetic wastewater was added to the feeding bottle above Reactor 1
- The feeding bottles were then placed next to their respective pumps and it was ensured that the screw caps of all 3 bottles were tightly screwed on
- The gasbags were then connected to the vacuum pump (one by one) in order to remove all the air inside after which it was tightly closed with a clamp
- Three of the gasbags were then filled approximately halfway with nitrogen gas by connecting the bag to the Nitrogen bottle after which the bags were tightly closed with a clamp
- At 12:25, the valves connecting the gasbags to the reactors were closed and the gasbags from each reactor were removed and replaced with the gasbags filled with Nitrogen
- The gasbags that were removed were closed properly with a clamp to prevent any of the biogas formed to escape from the bags
- In order to decant the specific volumes from each reactor, the valves at the bottom of the reactors were opened and the effluent was decanted into a measuring cylinder in order to decant the correct volume from each reactor
- The decanted effluent from each reactor was transferred to a sample bottles
- The valves connecting the feeding bottles to the reactors were opened and the pumps used to feed each reactor were switched on

- Once all the wastewater from each of the feeding bottles were fed to its respective reactor, the pump was switched off
- The valve connected to the nitrogen bottle was then opened and each of the reactors were purged with nitrogen in order to get rid of any oxygen which may have entered the system while feeding the reactors
- Once the purging was done the feeding port valves to the reactors were closed and the gasbags (filled with nitrogen gas) were removed and replaced with empty gasbags
- Once the gasbags were connected, the valves leading to the gasbags were opened after which the reactor stirrer switches were switched on again by turning the black switches on the control panel marked “Reactor 1”, “Reactor 2” and “Reactor 3” to the “on” position

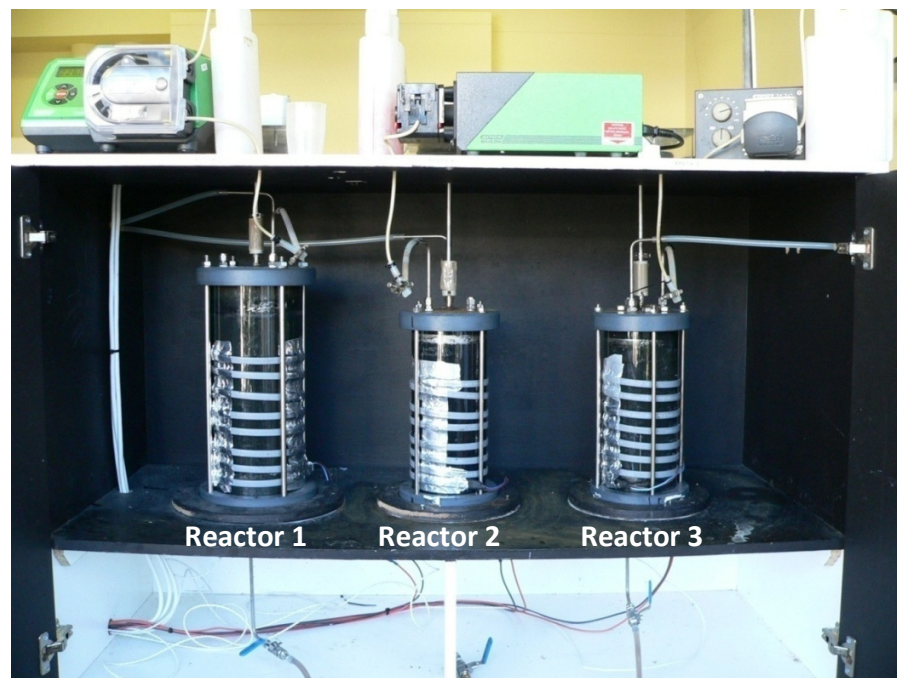


Figure C-2 Picture of experimental setup



Figure C-3 Picture of control panel and gas bags

C.3 Analytical Procedures and data analyses

C.3.1 COD

a) Measuring COD

The following procedure was followed to measure the COD of the wastewater and reactor effluent:

- According to the concentration range of the sample (see table xx), pipette the required amount of Solution A and Solution B into an empty, clean test cell and mix vigorously with the vortex mixer
- Add the required amount of sample according to the COD concentration range from Table C-1
- If it is expected that the COD concentration higher than the specified range, the sample should be diluted with distilled water
- Close the test cells tightly and mix vigorously again with the vortex mixer

- Place the test cells into the preheated thermo reactor (Thermoreaktor TR300 from Merck) at 148 °C for two hours
- Once the two hours reacting time is over, switch off the thermo reactor, remove the test cells from the thermo reactor and place it on a tube rack to cool down to room temperature for 15 minutes
- Once the cells have cooled down, switch on the Spectroquant® NOVA 60 (from Merck) spectrophotometer by opening the lid
- Place the cell into the opening of the spectrophotometer and select the appropriate method for the specific COD concentration range and measure the COD concentration
- Note the measure value
- If the sample was diluted, take into account the dilution ratio and calculate the true COD concentration

Table C-1 Volume of Solution A and B used in COD analyses

Parameter	Units	Measuring range [mg.L ⁻¹ COD]		
		10-150	100-1500	500-10000
Solution A	mL	0.3	0.3	2.20
Solution B	mL	2.85	2.30	1.80
Sample	mL	3.0	3.0	1.0
Method	mL	014	023	024

b) Calculating COD removal efficiencies and COD removal rate

The TCOD removal efficiency (ε_{TCOD}) was calculated as follow:

$$\varepsilon_{TCOD} = \frac{TCOD_{inf} - TCOD_{eff}}{TCOD_{inf}} \times 100$$

The SCOD removal efficiency was determined as follow:

$$\varepsilon_{SCOD} = \frac{SCOD_{inf} - SCOD_{eff}}{SCOD_{inf}} \times 100$$

The TCOD removal rate was calculated as follow:

$$R = \frac{TCOD_{inf} - TSCOD_{eff}}{V_{reactor}} \times Q_F$$

C.3.2 pH and Alkalinity - Auto Titrator

a) Measuring pH, PA and TA

The following operating procedures were followed when measuring pH and alkalinity with the Radiometer Titramaster 85 auto titrator:

- Switch on the auto titrator
- If electrode calibration is required, choose “calibrate electrode” and calibrate first with a pH 10 buffer solution and then with a pH 4 buffer solution
- When electrode calibration is not required, go directly to “select method” and select “pH and alkalinity” method
- Pour 40 mL of sample into the beaker and insert the stirring magnet into the beaker
- Rinse the electrode with distilled water
- Place the beaker underneath the electrodes and measure the pH
- If pH measurement is satisfactory, press “continue” to continue with alkalinity measurements
- Once the alkalinity is measured, the titrator indicates the pH, PA and TA on the screen. The PA and TA are expressed in mg/l CaCO₃
- Write down the results before continuing with the next sample
- Rinse the electrode with distilled water and pat dry very lightly with a tissue towel
- If no more measurement is going to be done, place the electrode tip in the concentrated KCl solution and switch off the auto titrator
- The electrode was cleaned once a month by dipping it in a cleaning solution for 3 minutes after which it was rinsed with distilled water



Figure C-4 Picture of autotitrator used in pH and alkalinity measurements

b) Calculating IA and IA/PA

The intermediate alkalinity is simply the difference in the PA and the TA and was calculated as follow:

$$IA = TA - PA$$

The IA/PA ratio was calculated as follow:

$$IA:PA = \frac{IA}{PA}$$

C.3.3 VFAs

Volatile fatty acids (VFA) were determined by means of Gas Chromatography (GC) with a Nukol Column. The setup for the Nukol Column of the GC is shown in Table C-2.

a) Preparation of standard

The standard fatty acids which were used are acetic acid, propionic acid, butyric acid, iso-butyrac acid, valeric acid and iso-valeric acid while the internal standard which was used is n-hexanol. In order to prepare the standard solution the following procedures were followed:

- Pipette 1 mL of each fatty acid into a 1000 mL volumetric flask
- Add 0.5 mL hexanol to the fatty acids

Table C-2 Column specifications for VFA concentration measurements

Setting	Units	Value
H ₂	mL.min ⁻¹	35
Air	mL.min ⁻¹	340
N ₂	mL.min ⁻¹	6.1
Split ratio		10:1
Split flow	mL.min ⁻¹	61
Total flow	mL.min ⁻¹	69.0
Make-up	mL.min ⁻¹	30
Initial temperature	° C	105 (105 -120)
Hold	Min	2
Rate	°C.min ⁻¹	6
Injector temperature	° C	135
FID temperature	° C	300

- Dilute the solution with 35 % formic acid in the raio 1:3 (250 mL formic acid solution + 750 mL standard solution).
- Calculate the concentration of the respective fatty acid in the standard solution as follow:

$$\text{Concentration } \left[\frac{mg}{L} \right] = 1ml_{acid} \times \rho_{acid} \times \text{purity}_{acid} \div 1000ml$$

- The standard was then injected and set as standard

b) Sample preparation

The following procedures were followed to prepare the sample:

- In order to get rid of all solid materials in the samples, centrifuge the sample at 10 000 rpm for 10 minutes at 10 °C with a Buckmann Centrifuge
- Dilute the sample with a 35% formic acid solution in the ratio 1:3 (1mL of sample + 3 mL of formic acid solution)
- Keep the sample at -18 °C until analysed

c) Sample injection

The following procedures were followed to inject the sample:

- Take sample from the freezer and allow to defrost
- Switch on the GC
- Once the sample is defrosted, add 2µl of Hexanol
- Allow the column to stabilise to the conditions specified by the manufacturers (see
- In order to condition the column for the acid medium of the samples, one or two runs were done by only injecting 35% formic acid solution
- It is important to ensure that the initial temperature of the program is between 100 and 110 °C and the injector temperature should not exceed the initial temperature by more than 20 to 30 °C
- Once the column is stable, draw 1µl sample from the prepared sample and inject it to the column

d) Integration

Integration was done by means of Borwin Version 1.2 integration software (JMBS Developments, Le Fontanil, France).

C.3.4 TS and VS

The TS and VS of the sludge before each inoculation was measured in the following manner:

- Dry empty crucibles in a muffle furnace for 2 hours at 550 °C
- After 2 hours, remove crucibles from furnace and let it cool for approximately 15 minutes and store in the a desiccator until needed
- Record the weight of the empty crucibles (in grams) on a four decimal scale (A)
- Measure approximately 20 ml of sludge into each crucible and record the weight (B)
- Dry crucibles in a drying oven overnight at 105 °C
- Remove the crucibles from the oven and let it cool down
- Record the weight of the dishes (C)
- Calculate the % TS as follow:

$$\%TS = \frac{C - A}{B - A} \times 100$$

- Once the weigh of the dried samples are recorded, transfer the crucibles to a muffle furnace and ignite for 3 hours at 550 °C
- After 3 hours, remove the crucibles from the oven and allow them to cool down for approximately 15 minutes and transfer it then to a desiccator and let it cool down completely for 2 hours
- Record the weight of the crucibles (D)
- Determine the % VS as follow:

$$\%VS = \frac{C - D}{B - A} \times 100$$

- Determine the % VS from TS as follow:

$$\%VS \text{ of } TS = \frac{VS}{TS} \times 100$$

C.3.5 Biogas composition

The biogas composition was determined with a Varian 3300 Gas Chromatograph. The GC had the following settings:

Table C-3 Specification of GC to determine biogas composition

Setting	Units	Value
Column i.d.	mm	2.0 x 3.0
Column packing		Hayesep Q (Supelco, Bellefonte, PA)
Mesh		80/100
Oven temperature	° C	55
Carrier gas		Helium
Flow rate of carrier gas	mL.min ⁻¹	30.0
Biogas injection volume	mL	0.4
Integration		Varian 4290 integrator

The following procedure was followed:

- Make sure there is sufficient gas in the cylinders
- Switch on “detector A”
- Extract 0.4 mL of biogas from the gas bags with a syringe
- Inject the sample into the GC and press “inject A”
- Wait for the Varian 4290 integrator to integrate and print the results
- Switch off “detector A” when finished

C.3.6 Biogas volume

The biogas volume was determined by withdrawing the gas in the gas bag manually with a 60 mL syringe. The following procedure was followed:

- Ensure that the valve is in the “closed” position
- Attach the valve to the inlet of the gasbag while ensuring that the clamp on the gas bag is still tightly closed
- Remove the clamp
- Insert the syringe to the other end of the valve

- Open the valve and withdraw 60 mL of biogas from the bag
- Close the valve while the syringe is still attached to the valve
- Once the valve is closed, remove the syringe and release the gas in the syringe
- Make a note on a piece of paper to determine the number of times gas was withdrawn from the gasbag ($n_{withdrawals}$)
- Repeat this until all the gas in the gasbag is removed
- During the last withdrawal, note the volume of gas left in syringe (V_{left})
- Determine the total volume of gas as follow:

$$Q_{biogas} = n_{withdrawals} \times 60 \text{ ml} + Q_{left}$$

- Determine the volume of methane as follow:

$$Q_{CH_4} = x_{CH_4} \times Q_{gas}$$

- Determine the volume of CO₂ as follow:

$$Q_{CO_2} = x_{CO_2} \times Q_{gas}$$

- Determine the total volume of biogas (methane + CO₂) as follow:

$$Q_{biogas} = Q_{CH_4} + Q_{CO_2}$$



Figure C-5 Varian 3300 GC used to determine the biogas composition



Figure C-6 Extracting biogas from the gas bags to determine daily volume biogas

Appendix D Daily results

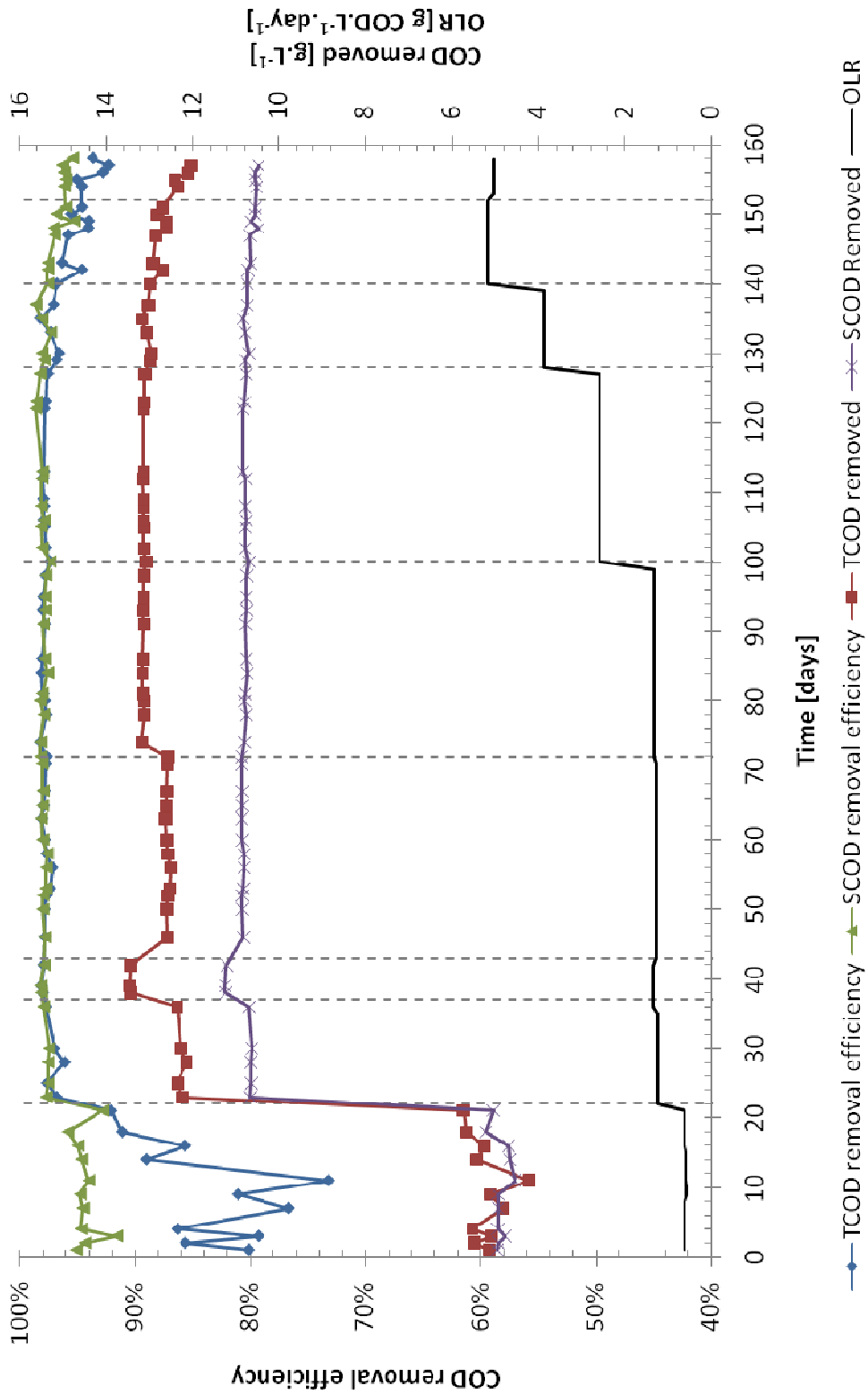


Figure D-1 COD removal and COD removal efficiencies at different OLRs throughout duration of operation of treatment of synthetic milking parlour wastewater in an ASBR at 35 °C (Reactor 1)

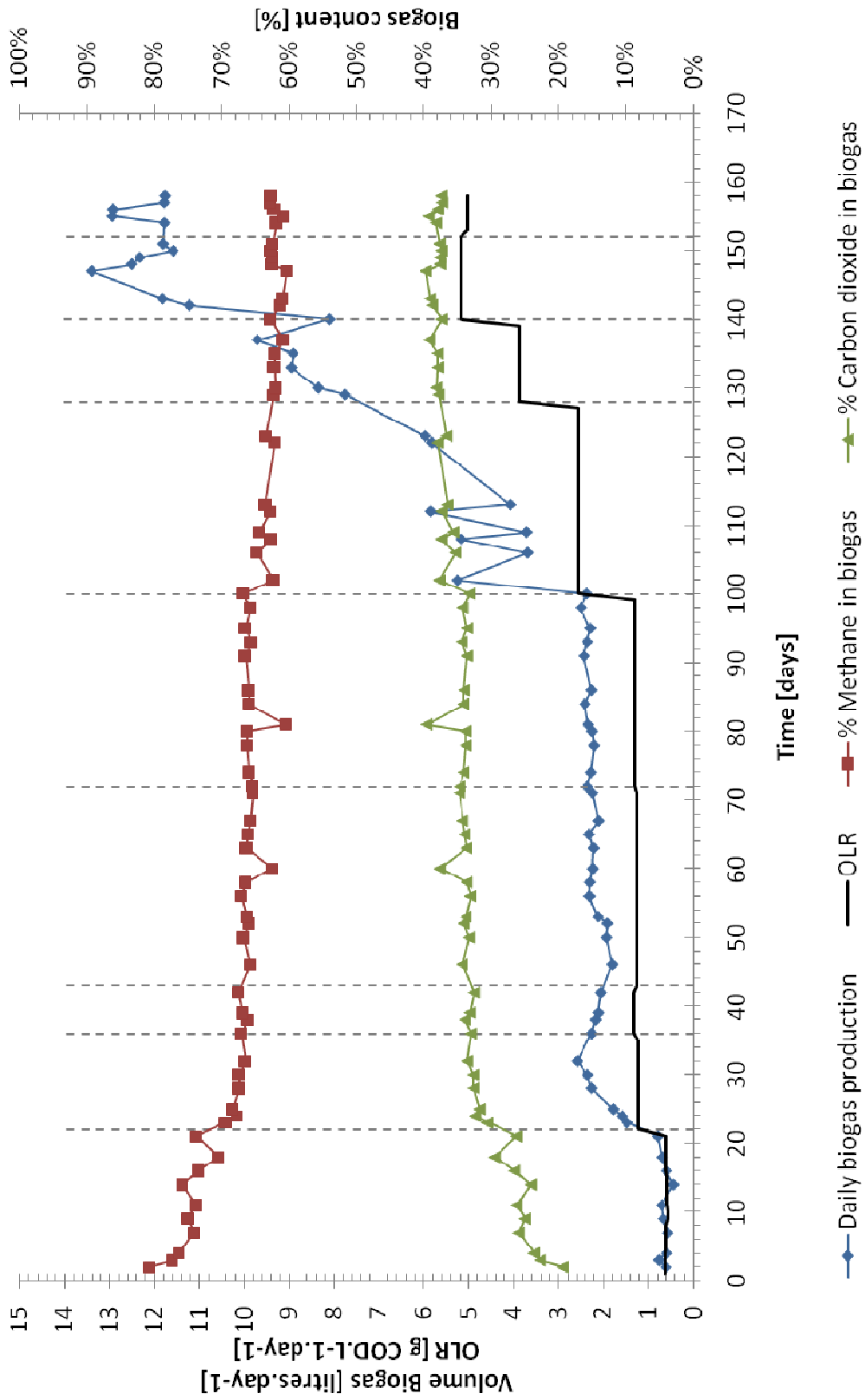


Figure D-2 Biogas production and biogas content at different OLRs throughout duration of operation of treatment of synthetic milking parlour wastewater in an ASBR at 35 °C (Reactor 1)

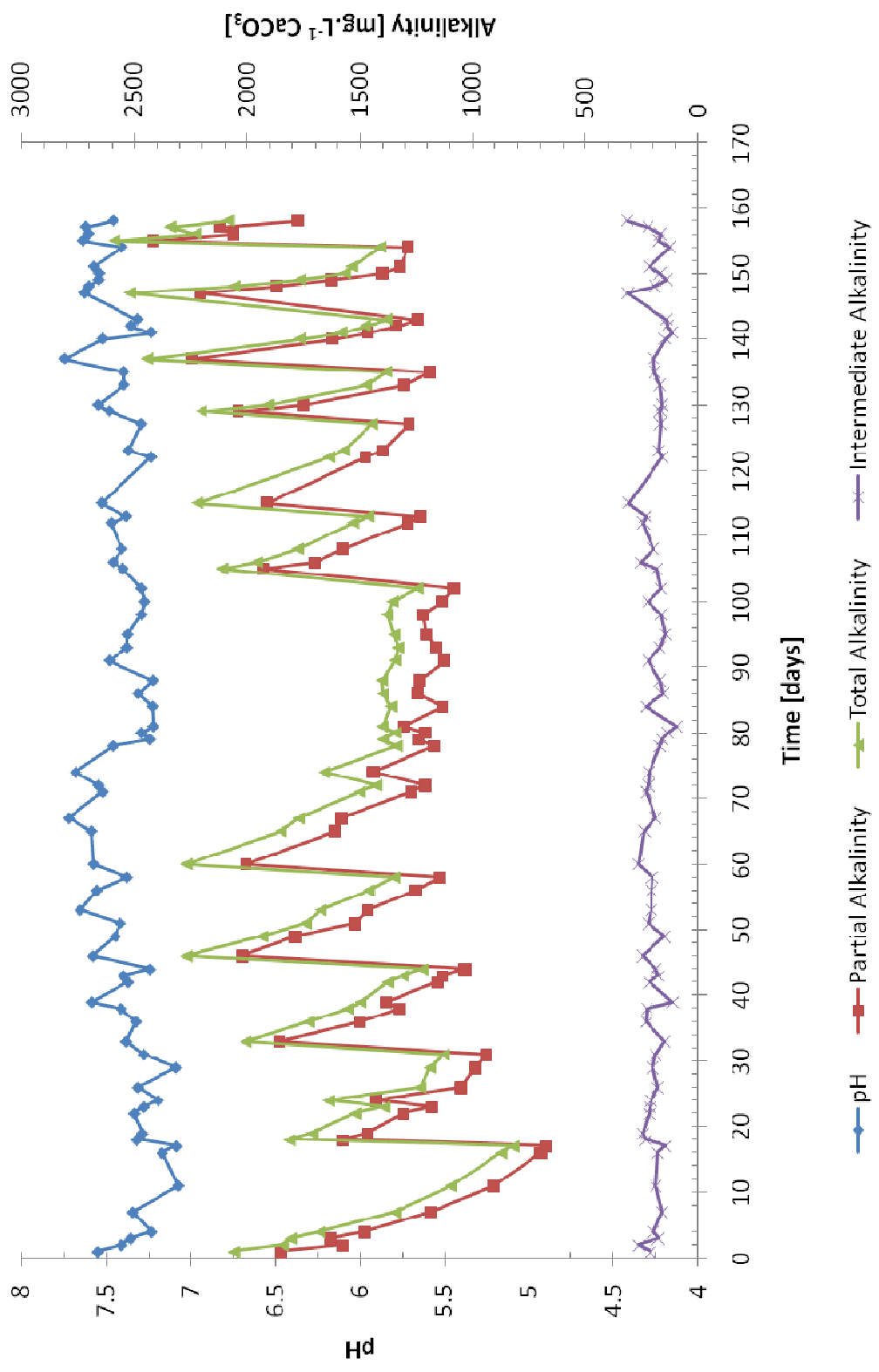


Figure D-3 pH and alkalinity of effluent *f* throughout duration of operation of treatment of synthetic milking parlour wastewater in an ASBR at 35 °C (Reactor 1)

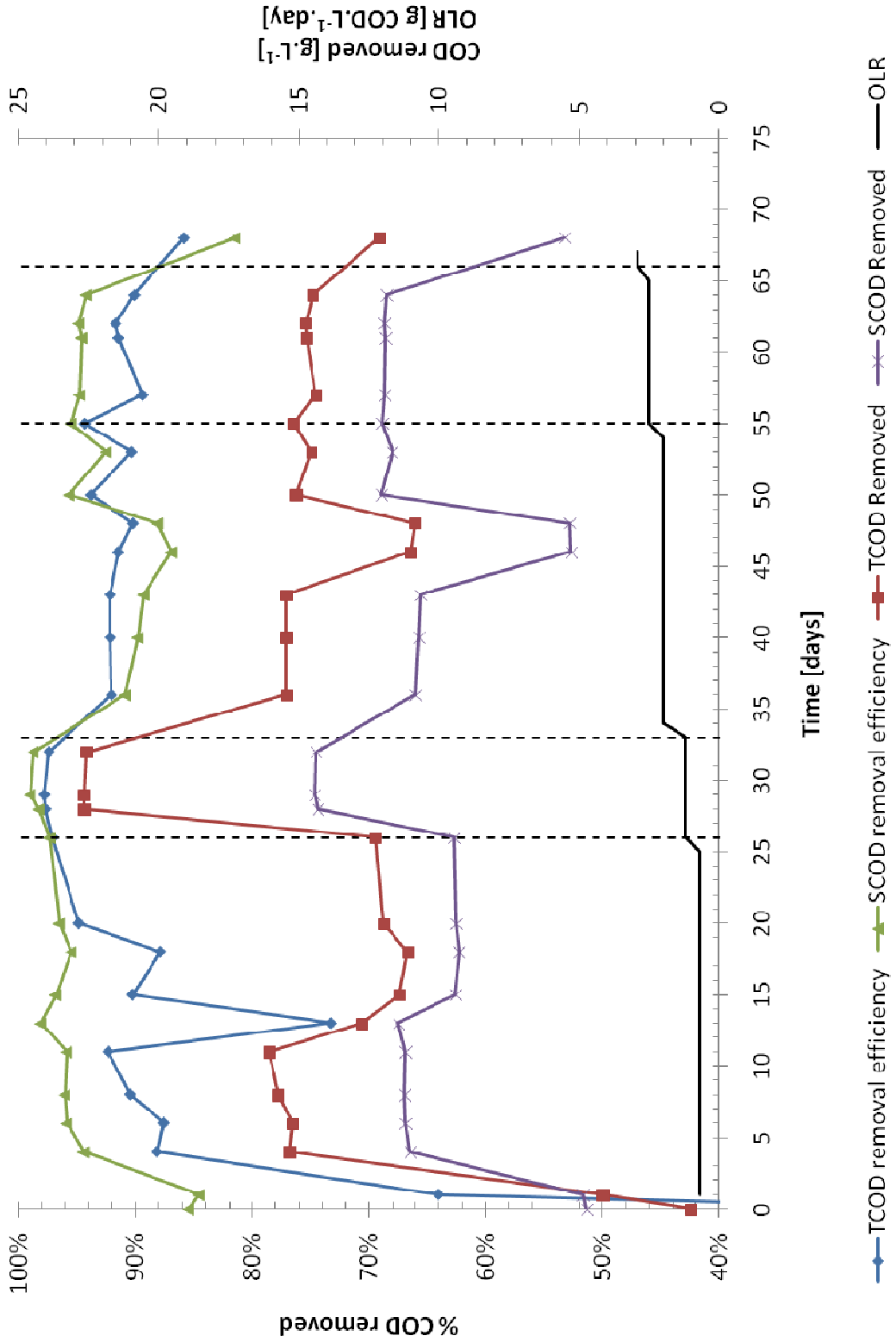


Figure D-4 COD removal and COD removal efficiencies at different OLRs throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 22.5 °C (Reactor 2 – first inoculation)

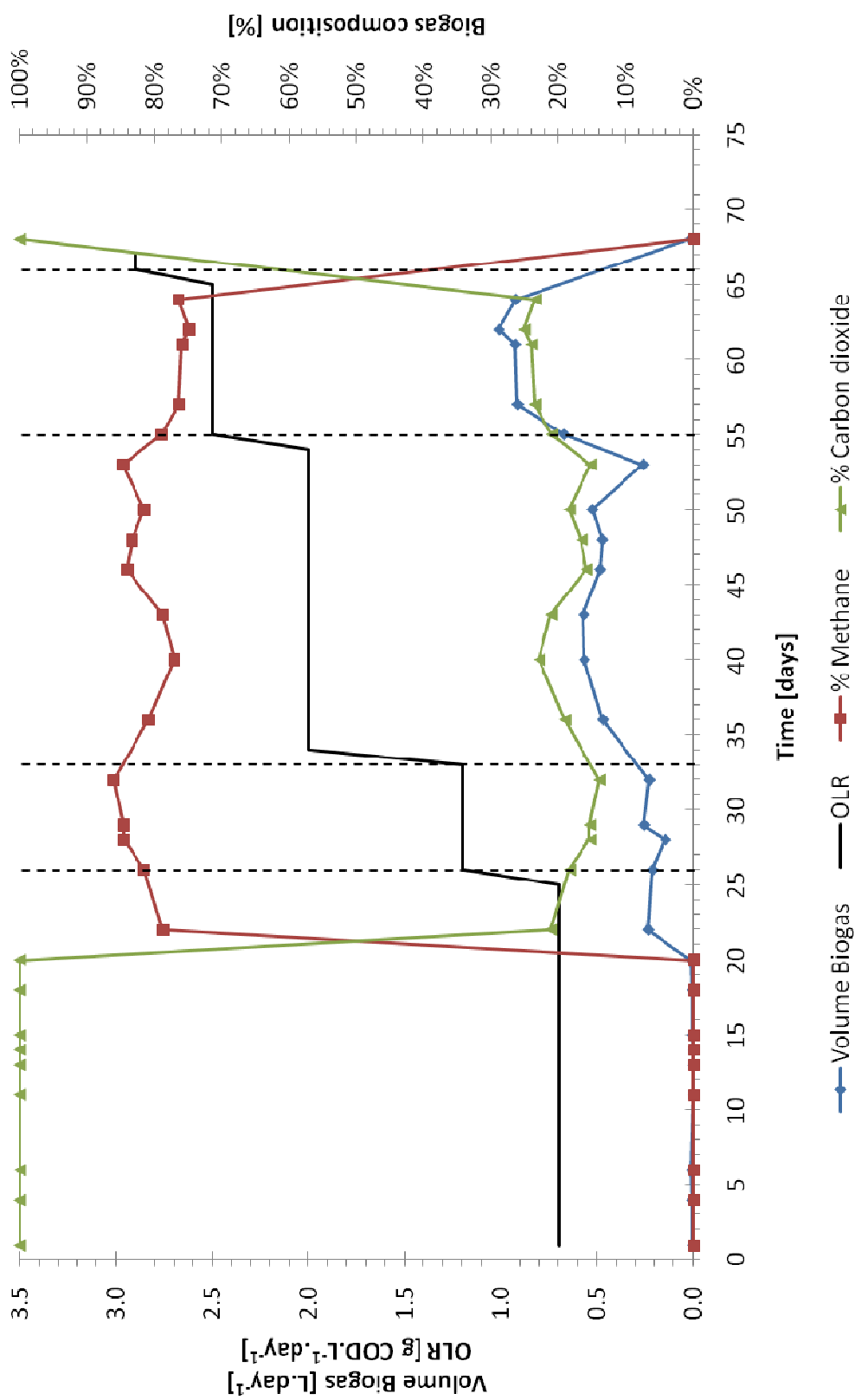


Figure D-5 Biogas production and biogas content at different OLRs throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 22.5 °C (Reactor 2 – first inoculation)

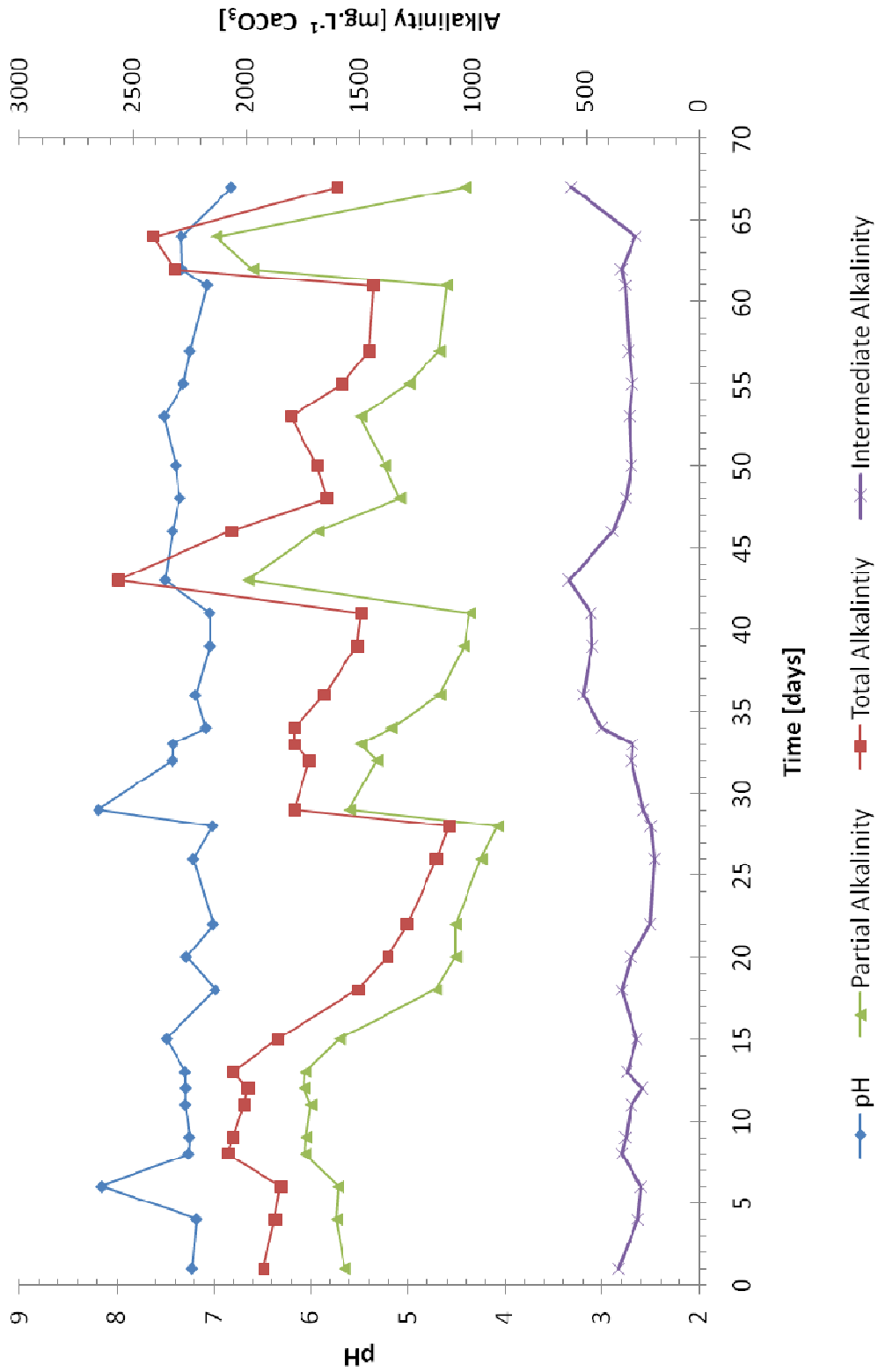


Figure D-6 pH and alkalinity of effluent throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 22.5 °C (Reactor 2 – first inoculation)

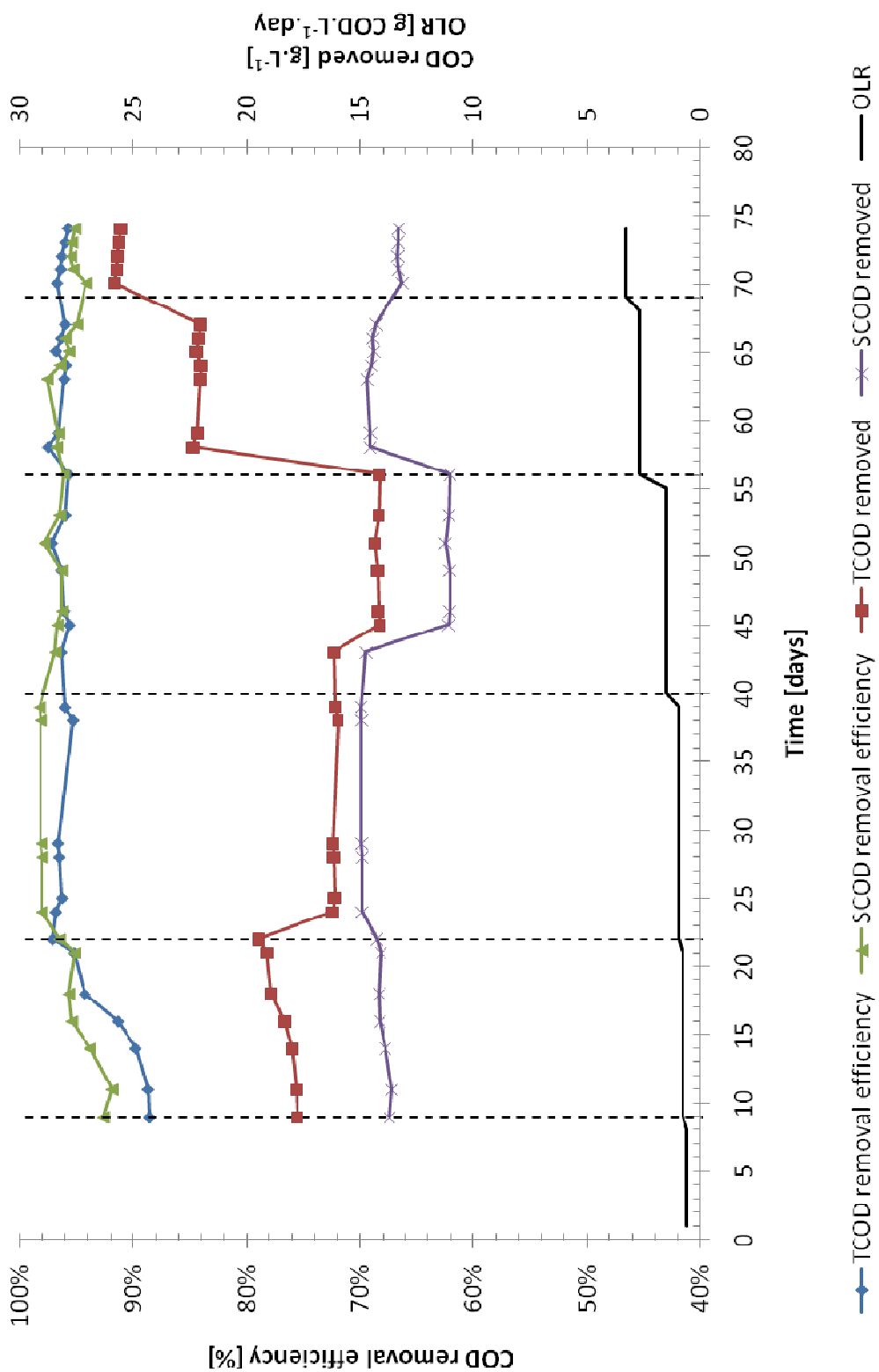


Figure D-7 COD removal and COD removal efficiencies at different OLRs throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 22.5 °C (Reactor 2 – second inoculation)

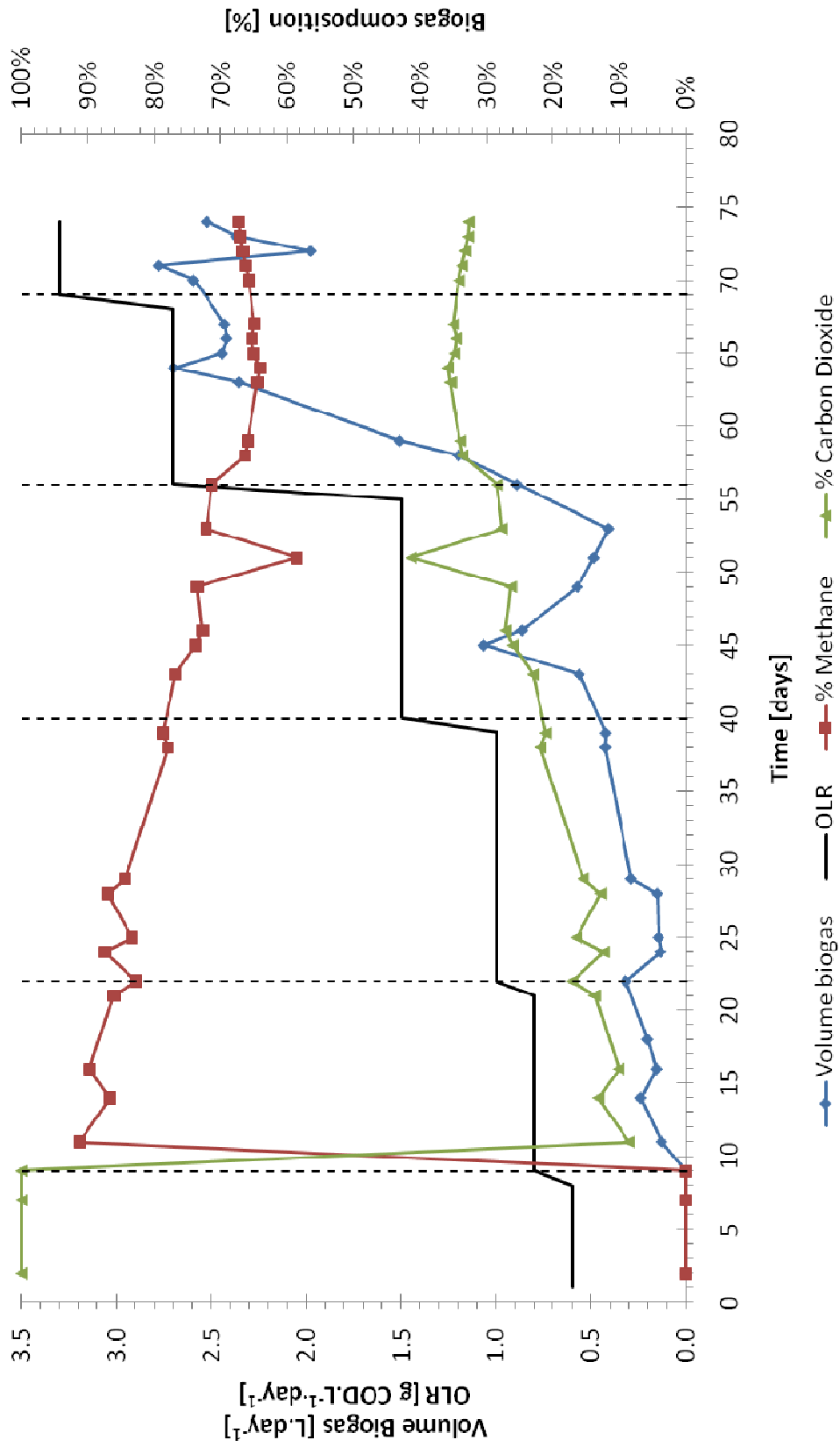


Figure D-8 Biogas production and biogas content at different OLRs throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 22.5 °C (Reactor 2 – second inoculation)

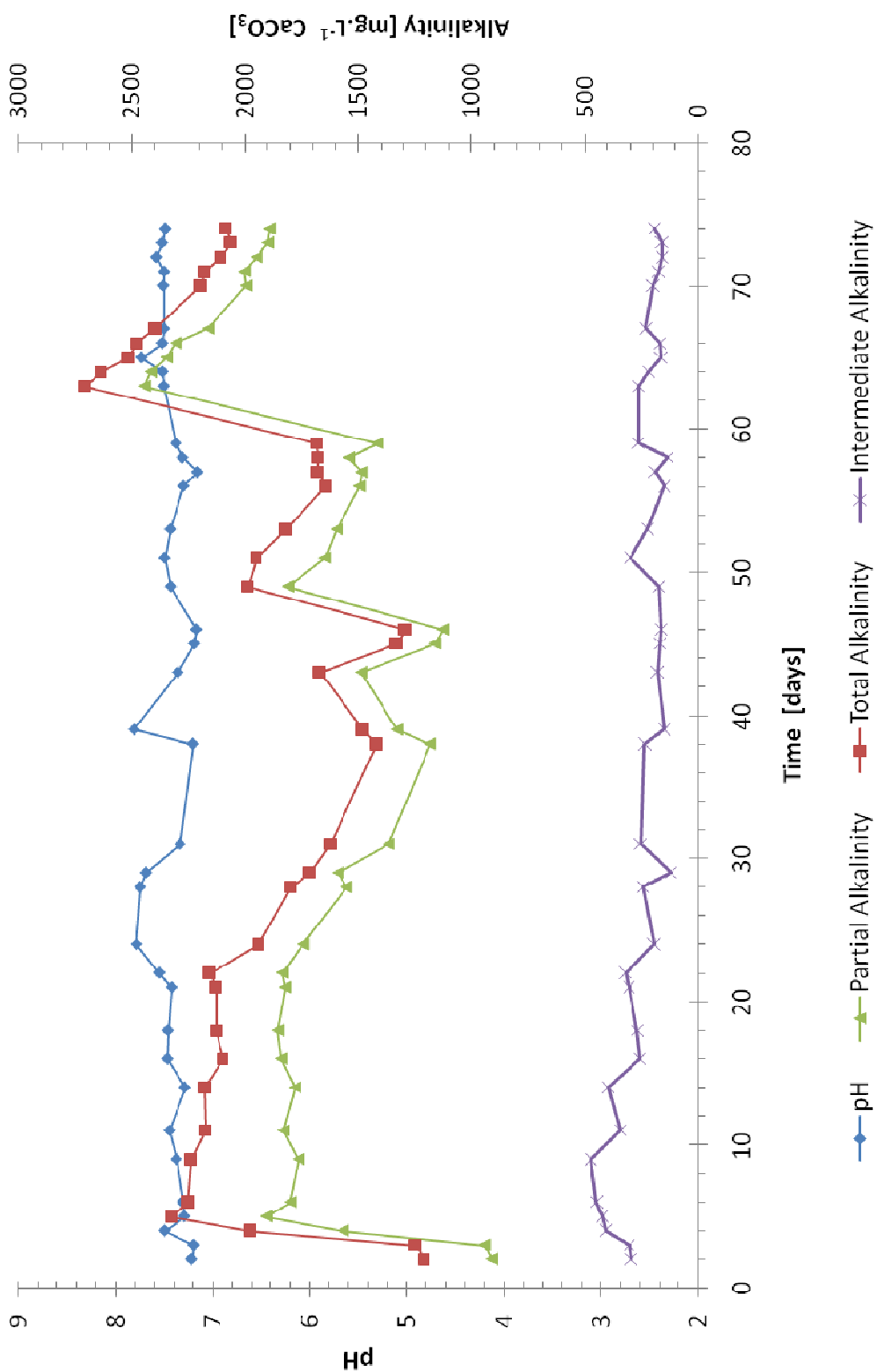


Figure D-9 pH and alkalinity of effluent throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 22.5 °C (Reactor 2 – second inoculation)

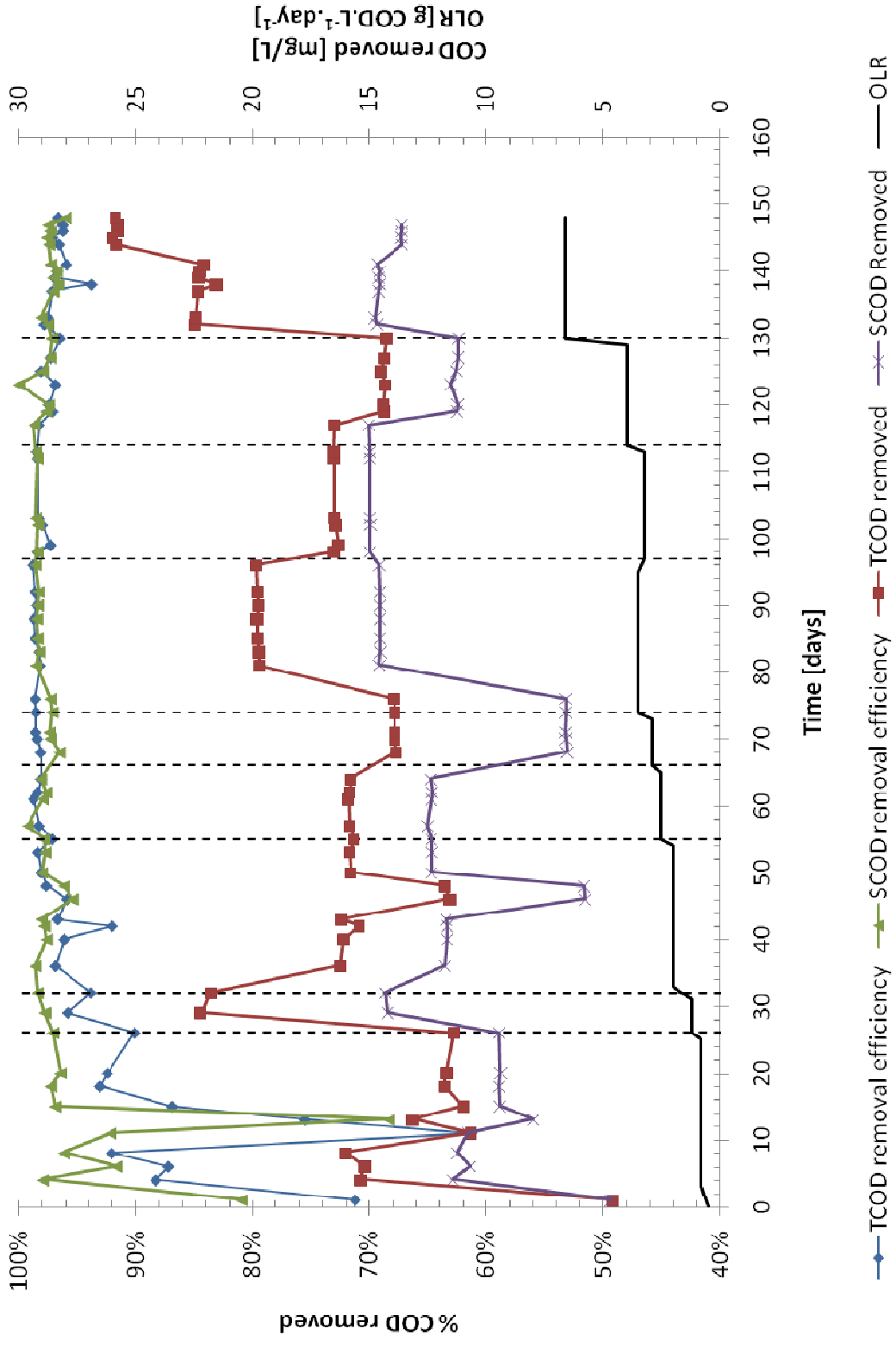


Figure D-10 COD removal and COD removal efficiencies at different OLRs throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 35 °C (Reactor 3)

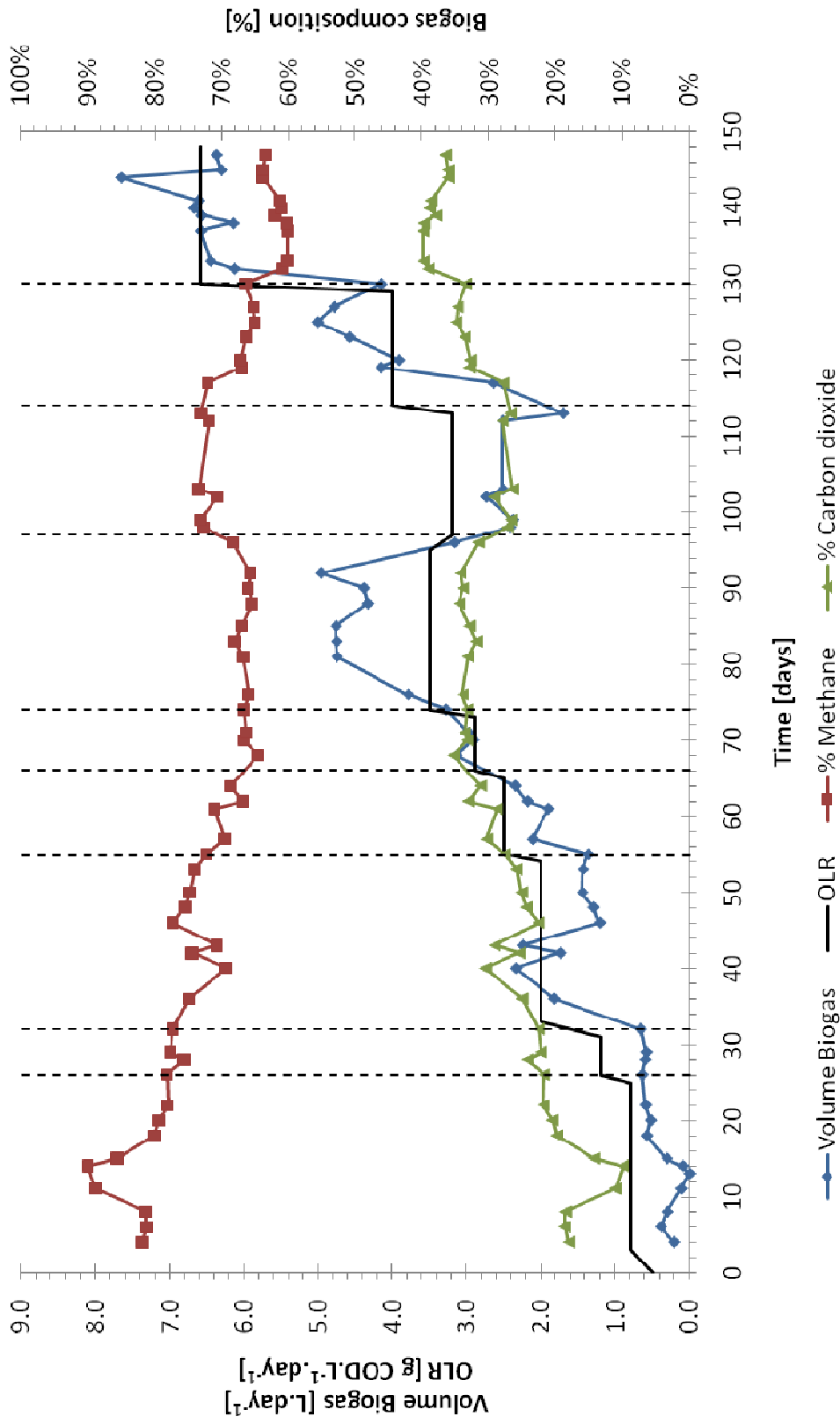


Figure D-11 Biogas production and biogas content at different OLRs throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 35 °C (Reactor 3)

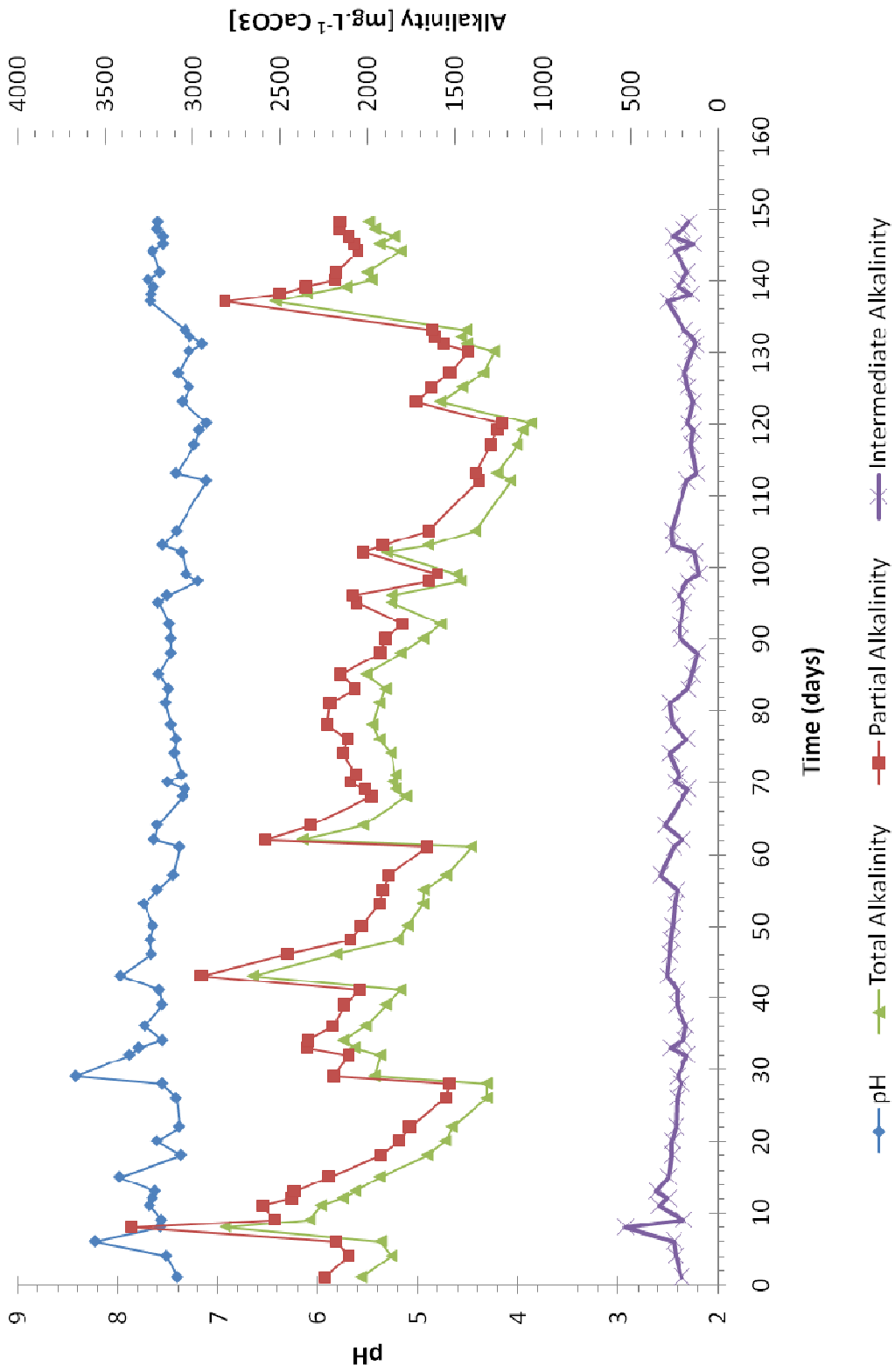


Figure D-12 pH and alkalinity of effluent *f* throughout duration of operation of treatment of real milking parlour wastewater in an ASBR at 35 °C (Reactor 3)

**Appendix E Recommended
sizing for an ASBR on a typical
South African milking parlour
to treat equipment wash water**

E.1 Sizing calculations

E.1.1 Wastewater volume and characteristics

According to the survey conducted on the five milking parlours visited, typically 6 L.cow⁻¹.day⁻¹ wastewater are generated from equipment washing. Thus, if an average of 500 cows per day in a milking parlour, 3 000 litres of wastewater will be generated from equipment washing daily. If the cows are milked 3 times a day, the volume of wastewater generated per milking event is 1 000 litres. The proposed design for the ASBR system will therefore be based on volume of 1 000 litres per milking event (corresponding to 3 000 litres per day) from equipment washing. Table E-1 propose the wastewater characteristics on which the design will be based. The characteristics of the wastewater are similar to those obtained during the experimental work in this study (see Table 5-4).

Table E-1 Wastewater characteristics for typical milking parlours as measured in this study (adapted from Table 5-4)

Parameter	Units	Average
Unfiltered COD	mg.L ⁻¹	20 000
Filtered COD	mg.L ⁻¹	10 000
Alkalinity	mg.L ⁻¹ CaCO ₃	520
TSS	mg.L ⁻¹	4 500
VSS	mg.L ⁻¹	4 400
Total Nitrogen	mg.L ⁻¹ N	400
Total Phosphorus	mg.L ⁻¹ N	40
Temperature (assumption)	°C	20
Flow rate	L.day ⁻¹	3 000

E.1.2 Reactor volume

According to the results obtained in this study, an ASBR should be able to operate comfortably at an OLR of 5 g COD.L⁻¹.day⁻¹. The reactor volume can therefore be estimated from:

$$V_{reactor} = \frac{COD_{inf} \times Q_{feed}}{OLR}$$

$$V_{reactor} = \frac{20\,000 \text{ mg} \cdot \text{L}^{-1} \times 3\,000 \text{ L} \cdot \text{day}^{-1}}{5\,000 \text{ mg COD} \cdot \text{L}^{-1} \cdot \text{day}^{-1}} \div 10^3 \text{ L} \cdot \text{m}^{-3}$$

$$V_{reactor} = 12 \text{ m}^3$$

(It must be noted, that if the ASBR can operate effectively at higher OLRs, the reactor volume can be reduced. For example, if the ASBR can operate at 10 g COD.L⁻¹.day⁻¹, the volume of the reactor can be reduced to 6 m³. More work on the maximum achievable OLR in the ASBR should therefore be done.)

Add 20 % extra for freeboard:

$$V_{reactor} = 12 \text{ m}^3 \times 1.2$$

$$V_{reactor} = 14.4 \text{ m}^3$$

If a L/D ratio of 2.4 is used (see page 67) , the reactor diameter (D) and reactor length (L) can be calculated as follow:

$$D = \sqrt[3]{\frac{4 \times 14.4 \text{ m}^3}{2.4 \times \pi}} = 1.97 \approx 2.0 \text{ m}$$

$$L = 2.4D = 2.4 \times 2.0 = 4.8 \text{ m}$$

The HRT of the wastewater in the reactor will therefore be:

$$HRT = \frac{V_{reactor}}{Q_{feed}}$$

$$HRT = \frac{12 \text{ m}^3}{3 \text{ m}^3 \cdot \text{day}^{-1}}$$

$$HRT = 4 \text{ days}$$

When compared to an HRT of 22 days used in the study by Upendrakumar *et al.* (92), a HRT of 4 days is acceptable.

E.1.3 Biogas production

If a 96 % COD removal efficiency is achieved, the effluent COD concentration will be:

$$COD_{effluent} = 20\,000 \text{ mg.L}^{-1} \times (1 - 0.96)$$

$$COD_{effluent} = 800 \text{ mg.L}^{-1}$$

At 35 °C and 1 atm, the maximum methane yield that can be achieved in an anaerobic reactor is $0.4 \text{ L}_{\text{CH}_4} \cdot (\text{g COD}_{\text{removed}})^{-1}$ (15). An efficiency factor of 0.7 is assumed (50). The total volume of methane production can therefore be calculated as follow:

$$Q_{\text{methane}} = E \times Y_{\text{CH}_4} \times Q_{\text{feed}} (COD_{\text{inf}} - COD_{\text{eff}})$$

$$Q_{\text{methane}} = 0.7 \times 0.4 \text{ L.g} \times 3\,000 \text{ L.day}^{-1} \times (20 - 0.8) \text{ g.L}^{-1} \div 10^3 \text{ m}^3 \cdot \text{L}^{-1}$$

$$Q_{\text{methane}} = 16.13 \text{ m}^3 \cdot \text{day}^{-1}$$

If it is assumed that the biogas consist of 65 % methane and 35 % carbon dioxide, the total daily biogas production will be:

$$Q_{\text{biogas}} = 16.13 \text{ m}^3 \cdot \text{day}^{-1} \div 0.65$$

$$Q_{\text{biogas}} = 24.8 \text{ m}^3 \cdot \text{day}^{-1} \text{ (at 35 °C and 1 atm)}$$

E.1.4 Energy content of biogas

At 35 °C, the volume occupied by 1 mole of methane can be calculated as follow:

$$V_{\text{methane}} \approx \frac{n \times R \times T}{P}$$

$$V_{\text{methane}} \approx \frac{1 \text{ mole} \times 0.082057 \text{ atm.L. (mole K)}^{-1} \times [(273 + 35) \text{K}]}{1.0 \text{ atm}} \times 10^{-3} \text{ m}^3 \cdot \text{L}^{-1}$$

$$V_{\text{methane}} \text{ for 1 mole methane} \approx 0.0253 \text{ m}^3$$

$$n_{\text{methane}} \approx \frac{24.8 \text{ m}^3 \cdot \text{day}^{-1}}{0.0253 \text{ m}^3}$$

$$n_{\text{methane}} \approx 980.8 \text{ moles} \cdot \text{day}^{-1}$$

$$m_{\text{methane}} \approx n_{\text{methane}} \times M_{\text{methane}}$$

$$m_{\text{methane}} \approx 980.8 \text{ moles} \cdot \text{day}^{-1} \times 16 \text{ g} \cdot \text{mole}^{-1}$$

$$m_{\text{methane}} \approx 15\,694 \text{ g} \cdot \text{day}^{-1}$$

The lower heating value (LHV) of methane is $50.1 \text{ kJ} \cdot \text{g}^{-1}$ and the energy produced per day in the form of combustible methane can therefore be estimated as:

$$W = 15\,694 \text{ g} \cdot \text{day}^{-1} \times 50.1 \text{ kJ} \cdot \text{g}^{-1}$$

$$W = 786 \text{ MJ} \cdot \text{day}^{-1}$$

$$W = 9.1 \text{ kW}$$

E.1.5 Heating requirements

It is assumed that the water temperature that enters the reactor will be approximately $20 \text{ }^\circ\text{C}$. The amount of heat that is required to heat up the reactor to $35 \text{ }^\circ\text{C}$ without heat recovery can therefore be estimated as:

$$W = \dot{m}_{\text{feed}} \times h_{\text{water}} \times \Delta T$$

$$W = Q_{\text{feed}} \times \rho_{\text{water}} \times h_{\text{water}} \times \Delta T$$

$$W = 3 \text{ m}^3 \cdot \text{day}^{-1} \times 1\,000 \text{ m}^3 \cdot \text{kg}^{-1} \times 4.186 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{K}^{-1} \times (35 - 20) \text{ K}$$

$$W = 188\,370 \text{ kJ} \cdot \text{day}^{-1}$$

$$W = 188\,370 \text{ kJ} \cdot \text{day}^{-1} \times \frac{1 \text{ day}}{86\,400 \text{ seconds}} = 2.2 \text{ kW}$$

E.1.6 Nutrient requirements

According to Cherinicharo (48), the optimum COD:N:P ratio for anaerobic digestion is 350:5:1. If the COD concentration of the influent wastewater is 20 000 mg.L⁻¹, then the N and P requirement can be estimated as:

$$\text{Daily mass of N required} = 20\,000 \text{ mg.L}^{-1} \times \frac{5}{350} \times 3\,000 \text{ L.day}^{-1} \times 10^{-3} \text{ g.mg}^{-1}$$

$$\text{Daily mass of N required} = 857 \text{ g.day}^{-1}$$

$$\text{Daily mass of P required} = 20\,000 \text{ mg.L}^{-1} \times \frac{1}{350} \times 3\,000 \text{ L.day}^{-1} \times 10^{-3} \text{ g.mg}^{-1}$$

$$\text{Daily mass of P required} = 171 \text{ g.day}^{-1}$$

According to Table E-1, the N and P concentrations in the wastewater are 400 mg.L⁻¹ and 40 mg.L⁻¹ respectively, which correspond to 1 200 g.day⁻¹ N and 120 g.day⁻¹ P (see calculations below).

$$\text{Daily mass of N in influent wastewater} = 400 \text{ mg.L}^{-1} \times 3000 \text{ L.day}^{-1} \times 10^{-3} \text{ g.mg}^{-1}$$

$$\text{Daily mass of N in influent wastewater} = 1\,200 \text{ g.day}^{-1}$$

$$\text{Daily mass of P in influent wastewater} = 40 \text{ mg.L}^{-1} \times 3000 \text{ L.day}^{-1} \times 10^{-3} \text{ g.mg}^{-1}$$

$$\text{Daily mass of P in influent wastewater} = 120 \text{ g.day}^{-1}$$

Hence, the nitrogen content in the wastewater is sufficient, but phosphorus is lacking. The mass of phosphorus needed per day will then be:

$$\text{Daily addition of P required} = 171 - 40 = 51 \text{ g.day}^{-1}$$

If phosphorus will be added in the form of K₂HPO₄, the daily mass of K₂HPO₄ required will be:

$$\text{Daily addition of K}_2\text{HPO}_4 = 51 \text{ g.day}^{-1} \times \frac{M_{\text{K}_2\text{HPO}_4}}{M_{\text{P}}} = 51 \times \frac{31}{174} = 287 \text{ g.day}^{-1}$$

E.1.7 Alkalinity requirements

According to literature, the minimum TA requirement to produce biogas with 35 % carbon dioxide, is $1\,500\text{ mg}\cdot\text{L}^{-1}\text{ CaCO}_3$. The alkalinity in the wastewater is $520\text{ mg}\cdot\text{L}^{-1}\text{ CaCO}_3$. Thus, the additional alkalinity required would be:

$$\text{Alkalinity addition} = 1\,500 - 520 = 980\text{ mg}\cdot\text{L}^{-1}\text{ CaCO}_3$$

The equivalent concentration of NaHCO_3 required is:

$$\text{Alkalinity} = \frac{84\text{ g}\frac{\text{NaHCO}_3}{\text{eq}}}{50\frac{\text{g}}{\text{eq}\text{ CaCO}_3}} \times 980\text{ g}\cdot\text{m}^{-3} = 1\,646\text{ g NaHCO}_3\cdot\text{m}^{-3}$$

The daily mass of NaHCO_3 required is therefore:

$$\text{NaHCO}_3 = 1\,646\text{ g}\cdot\text{m}^{-3} \times 3\text{ m}^3\cdot\text{day}^{-1} \times 10^{-3}\text{ kg}\cdot\text{g}^{-1} = 4.9\text{ kg}\cdot\text{day}^{-1}$$

E.2 Summary

Table E-2 shows a summary of the specifications for the proposed ASBR for the treatment of wastewater generated from the washing of the milking equipment. A proposed flow diagram for the process are shown in

The wastewater will first flow through a fat trap in order to reduce the FOG in the wastewater. After that, the water will flow to an equalisation tank in which alkalinity and nutrients will be added to the wastewater. The wastewater should be fed once a day and it is assumed that the temperature of the bulk wastewater generated per washing time will be approximately $30\text{ }^\circ\text{C}$. The equalisation tank should be big enough to keep wastewater for two days in case of a mechanical failure in the reactor. Figure E-1 shows a PFD of the proposed ASBR system to treat equipment wash water from a typical South Africa milking parlour. Heating to the reactor will be by means of hot water, flowing through coils that will be arranged around the reactor. The daily energy content of the methane produced will be approximately 2.2 kW. The methane can therefore be burnt and the heat can be used as energy source to heat the geyser that will provide hot water to control the temperature of the reactor. A control system will be implemented to control the temperature of the reactor contents by controlling the flow rate of

the hot water in the coils. The reactor should be insulated in order to prevent heat loss from the system.

Table E-2 Summary of proposed design for ASBR

Parameter	Units	Value
Number of cows		500
Predicted volume of water generated per cow for equipment washing	L.cow ⁻¹ .day ⁻¹	6
Number of milking times per day		3
Volume of wastewater per milking time		1
Volume wastewater generated per day	m ³	3
Total reactor volume	m ³	14.4
L/D		2.4
Reactor diameter (D)	m	2.0
Reactor length (L)	M	4.8
COD of wastewater	mg.L ⁻¹	20 000
Target COD removal efficiency	%	96
Total cycle length	hours	24
Duration of feed stage	hours	0.5
Feed flow rate	m ³ .min ⁻¹	6
Duration of react stage	hours	21
Duration of settling stage	hours	2
Volume decanted each day	m ³	3
Duration of decanting stage	hours	0.5
Operating OLR	kg COD.m ³ .day	5.0
Predicted volume biogas produced	m ³ .day ⁻¹	24.8
Predicted energy content of methane	MJ.day ⁻¹	786
Predicted energy content of methane	kW	9.1
Energy required to heat reactor content to 35 °C	kW	2.2
K ₂ HPO ₄	kg.day ⁻¹	0.29
Alkalinity requirement	kg.day ⁻¹ NaHCO ₃	4.9
Volume of equalisation tank	m ³	6

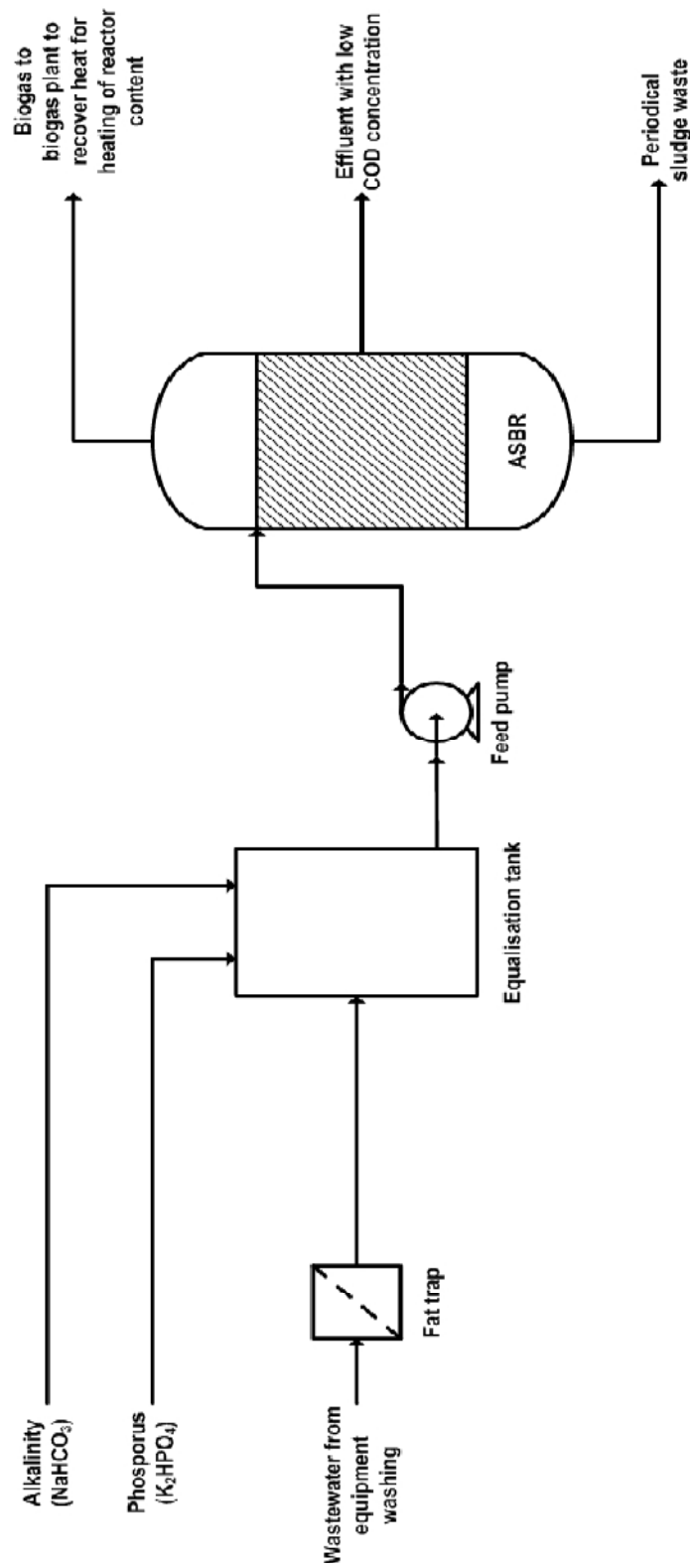


Figure E-1 Proposed PFD of ASBR system when treating milking parlour wastewater from equipment washing