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Potential biogas production by native cyanobacteria treating dairy farm wastewater at bench-scale

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Master of Science Thesis
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Abstract

Eutrophication of surface waters is a world-wide issue and since 10-15 years ago it has become the main environmental issue in Uruguay. Cyanobacteria blooms have become bigger in intensity and frequency. Dairy farms have been identified as one of the main contributors to eutrophication in the South of Uruguay. A university-owned dairy farm with a wastewater treatment system that discharged into a hyper-eutrophicated shallow lagoon (with a permanent cyanobacteria community in it) was taken as study case.

The first activity conducted was the assessment of the treatment system which included two monitoring campaigns. A second activity involved conducting batch laboratory experiments regarding the biogas production potential of the anaerobic co-digestion of the harvested native cyanobacteria with the milking parlor wastewater. Biogas production experiments were carried out in 500 mL bottles. Biogas was measured daily with an inverted graduated cylinder.

Results obtained in the monitoring campaigns showed that the waste stabilization ponds treating the milking parlor wastewater perform well despite being under designed. However, treatment is not enough to deliver an effluent quality which comply with the standards. Moreover, it was observed that the maintenance of the treatment system could be very much improved. This represents a cost-effective opportunity to enhance the performance of the system.

Laboratory experiments to determine the biogas production potential (or, considering the methane content in biogas, the biochemical methane potential, BMP) were successfully executed and monitored. The kinetics of the digestion could be followed with production and rate curves. It was found that native cyanobacteria is as much anaerobically degraded as milking parlor wastewater or as a mixture of them, all in the range of (538 ± 122) NmL_{biogas}/gVS to (607 ± 144) NmL_{biogas}/gVS.

Attention was put into the co-digestion of a 1:1 (volatile solids) mix of wastewater and cyanobacteria. Comparing biogas production with chemical oxygen demand (COD) removal, methane content in the biogas of the mix was estimated in 60% (v/v). For the 1:1 mix the ultimate biogas production potential was reported as (673 ± 48) NmL_{biogas}/gVS and the ultimate BMP as (404 ± 29) NmL_{CH4}/gVS.

The general conclusion is that the anaerobic co-digestion of milking parlour wastewater with natural-occurring cyanobacteria was successfully and thoroughly evaluated. The main conclusion of the wastewater treatment assessment is that further treatment is needed in order to comply with the standards. For the laboratory tests it was concluded that these results can be taken as a first approximation and should be repeated with more accurate methods.

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Abbreviations

AI	Anaerobic inoculum
AP	Anaerobic pond
BOD	Biological oxygen demand
BMP	Biochemical methane potential
CB	Cyanobacteria
CE	Cellulose
CN	Carbon to nitrogen ratio
COD	Chemical oxygen demand
CRS	Centro Regional Sur
CSTR	Completely stirred tank reactor
DO	Dissolved oxygen
EC	Electric conductivity
FC	Faecal coliforms
FP	Facultative pond
GL	Glycerol
HRAP	High rate algae pond
HRT	Hydraulic retention time
IN	Influent
IS	Inorganic solids
ISR	Inoculum to substrate ratio
LATU	Technological Laboratory of Uruguay
LD_{50}	Lethal dose 50%
MF	Microfiltered
ODM	Organic dry matter
OLR	Organic loading rate
ORP	Oxidation-reduction potential
pH	Hydrogen potential
SS	Suspended solids
SP	Soluble phosphorus
SBR	Sequencing batch reactor
SMY	Specific methane yield
SRT	Solids retention time
TA	Tajamar
TAN	Total ammonia nitrogen
TN	Total nitrogen
TP	Total phosphorus
TS	Total solids
TSS	Total suspended solids
UASB	Up-flow anaerobic sludge bed reactor
VFA	Volatile fatty acid
VS	Volatile solids
VSS	Volatile suspended solids
WSP	Waste stabilization ponds
WW	Wastewater

CHAPTER 1

Introduction & Objectives

1.1. Document presentation

This document presents the work performed by MSc candidate Lucas Martinez Arocena for his thesis titled "potential biogas production by native cyanobacteria treating dairy farm wastewater at bench-scale". The document follows a sequence of the following chapters: introduction & objectives, fundamental concepts, methodology, results, discussion, conclusions and references.

The Introduction & Objectives chapter serves to put this work in context. First the problems being addressed and the significance of this thesis are presented. Then the resources that were available for the completion of the thesis are described. Finally the objectives are defined.

Chapter 2 is Fundamental Concepts, where scientific literature is reviewed in order to introduce the reader into the areas of knowledge touched in this thesis. Sub-chapters include Eutrophication, Dairy farms in Uruguay, Anaerobic digestion and Cyanobacteria in wastewater treatment.

From Chapter 3 and on a division between the two parallel activities composing the thesis is done. It is presented first the milking parlour wastewater treatment system assessment, followed by the batch experiments performed in the lab regarding the determination of the biogas production potential of cyanobacteria and milking parlour wastewater co-digestion.

In the Methodology chapter the following questions are answered: What activities were held? What materials, devices, equipment, reagents were used? How were they used and to achieve what? What techniques, procedures and methodologies were followed?

It was decided to separate results from discussion for the sake of clarification. Therefore the Results (Chapter 4) is limited to presenting all the information gathered or created in this thesis in a systematic way. In the Discussion (Chapter 5) the following questions were addressed: Does results make sense? How they compare to the results obtained by other scientists? What can be learned or improved? Moreover this chapter includes most of the analysis of the information contained in this work.

Chapter 6 is the Conclusion chapter where the fulfilment of the objectives is verified, the most important results are summarized and extra conclusions are presented. Moreover a Perspectives section is included for future research and acknowledges are made. Finally references are listed in Chapter 7.

1.2. Problem addressed

Surface water quality in Uruguay has decreased over the years. In the beginnings of the 20th century the deterioration of the water quality was associated with industrialization and urban development. Nowadays agricultural activities are main contributors to the water quality deterioration, being specially associated to an increasing eutrophication (RAPAL, 2010). Among agricultural activities dairy farms have been identified as important contributors to water eutrophication in the southern Uruguay. The government has started demanding complete wastewater treatment for milking parlour's dairy farms (MVOTMA, 2013).

As a consequence of the eutrophication, situations like microalgae blooms have become bigger in intensity and frequency. Blooms as shown in Figure 1-1 cause damages to the environment, the public health and the economy. Hazard is increased when some type of cyanobacteria dominates the blooming community, due to their potential to produce cyanotoxins (a family of toxic compounds).

Figure 1-1 Photographs of a cyanobacterial bloom in Castillos Lagoon, Rocha, Uruguay in 2013 (Fabre, 2014)

Cyanobacteria can be mechanically removed from water bodies, as a mean for restoring aesthetics, for fighting the bloom, or for removing nutrients from the system (eutrophication control). Nevertheless, the resulting solid waste might be classified as hazardous due to cyanotoxins.

Nevertheless cyanobacteria occurrence it is not necessary linked mean cyanotoxin presence in water. Not every cyanobacteria specie has the capability of producing cyanotoxins. Moreover not always cyanotoxins are liberated (or produced) when cyanotoxin-producing cyanobacteria is present in the phytoplankton community. However, when human health is involved, just the potential of cyanotoxins presence water is enough to treat a cyanobacteria bloom as dangerous/unfavourable.

In addition Uruguay is moving towards a diversified energetic matrix putting the emphasis in renewable energy production. Energy microgeneration is now encouraged. The government-owned power company UTE is forced to buy exceeding microgenerated energy (MIEM-DNE, 2011). Biogas production from wastes (including wastewater) has become a growing technology in the electricity microgeneration market (Moreda, 2016).

1.3. Institutional resources

1.3.1. LATU

The Technological Laboratory of Uruguay (LATU), represented by Dr. Diana Míguez, is a UNESCO-IHE partner in Uruguay. Laboratory work was held in its facilities and LATU provided the personnel, vehicles, sampling equipment and laboratory analysis for the monitoring and material collection campaigns.

1.3.2. CRS

The Centro Regional Sur (CRS) is an agronomic research centre that belongs to the University of the Republic (UdelaR). It is located at latitude -34.606 and longitude 56.220, near Progreso city, at about 50 km away from Uruguay's capital Montevideo. Figure 1-2 shows CRS location framed in Montevideo; LATU location is shown as well.

One of the activities developed in the CRS is the production of milk. A dairy farm dealing with 185 milking cows on a daily basis. In the milking parlour (known as *tambo*), wastewater is produced as a consequence of the cleaning activity. Milking parlour wastewater is treated in waste stabilization ponds before discharging into a 5 -hectare shallow artificial lagoon (known as *tajamar*) which is hyper-eutrophicated and has a permanent cyanobacteria community.

In the CRS problems reported at the farm as follow.

1. Dairy farm wastewater treatment system was not properly designed leading to eutrophication of the receiving water body.
2. The eutrophicated *tajamar* presents a permanent presence of cyanobacteria which in summer time exhibit high cyanobacteria concentration ($1.7x10^7$ cell/mL).
3. Existing wastewater treatment system generates biogas but is not captured neither utilized.

Permission was given by Mr. Ruben Jacques, coordinator of the CRS, to use the dairy farm as case study. The main materials for this research were collected in there (wastewater, cyanobacteria and anaerobic sludge).

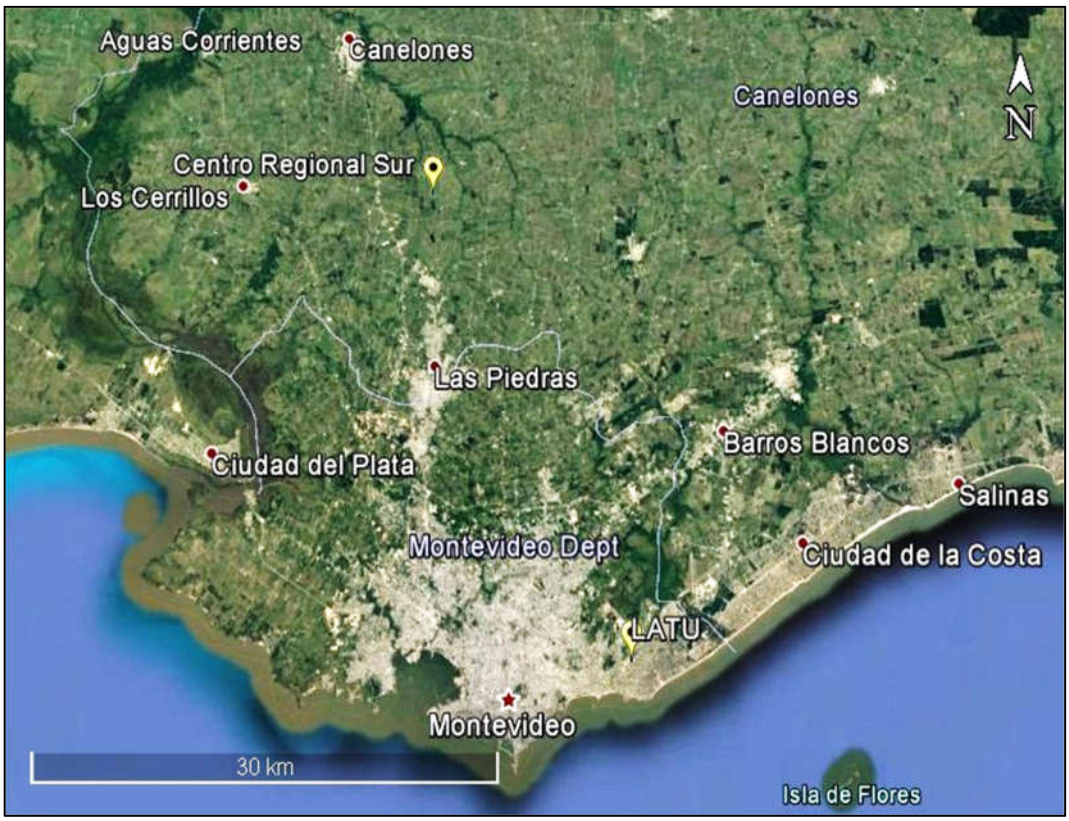


Figure 1-2 Location of CRS and LATU around Montevideo (the sea is the River Plate)

1.4. Thesis objectives

The general objective of this work is to evaluate the anaerobic co-digestion of milking parlour wastewater with natural-occurring cyanobacteria. In order to achieve that goal two specific objectives were raised:

- To analyse the wastewater production in the CRS dairy farm including a performance assessment of the existing wastewater treatment system.
- To conduct anaerobic digestion batch experiments in the laboratory for the determination of the biogas production potential of milking parlour wastewater, harvested cyanobacteria, and mixes of both.

1.5. Significance

Mechanical removal of cyanobacteria, although not always feasible, is a possible measure to reduce eutrophication in water bodies (Miao, et al., 2013). After having the cyanobacteria harvested, which is not an easy procedure, the challenge is to find alternatives for the cyanobacterial mass disposal which may have varying levels of cyanotoxin concentration. Anaerobic digestion is a promising alternative to treat this potentially hazardous waste while producing a valuable by-product (biogas).

Research on anaerobic digestion of phytoplankton has been centred in eukaryotic microalgae (typically Chlorella). Prokaryotic cyanobacteria digestion may differ significantly from microalgae due to difference in cell composition and lack of resistant cell wall in prokaryotes. Considering this, cyanobacteria digestion may lead to higher yields in biogas production compared to microalgae. Nevertheless there are uncertainties about the ability of digester's anaerobic community in degrading cyanotoxins.

Native Uruguayan cyanobacteria's characteristics have been thoroughly studied (Bonilla, 2009). However, anaerobic digestion of naturally occurring cyanobacteria in Uruguay has not yet to be thoroughly studied. No literature has been reported in scientific journals.

By taking dairy farm wastewater as the base for co-digesting cyanobacteria new ideas may be brought for the problematic of insufficient wastewater treatment in dairy farms. Moreover a set of wastewater treatment, ecological remediation and energetic biogas production could be achieved with this technology. It could be an integral solution for any agricultural activity that produce biodegradable wastes and want to remediate an eutrophicated water body.

This work fits well inside the water-energy-food nexus, because it deals with treating water originated in a food producing activity while generating renewable energy. Furthermore the three components of sustainable development are also included in the proposed work as follows:

- The environment: which is protected by reducing discharged loads into water bodies and by removing nutrients from eutrophicated aquatic ecosystems.
- The economy: which is benefited by generation of valuable biogas and better wastewater treatment;
- The society: by improving life quality of dairy farmers.

CHAPTER 2

Fundamental concepts

2.1. Introduction

This chapter contains a literature review that seeks to introduce the reader with the scientific concepts that this thesis is based on.

Firstly the eutrophication concept is introduced. This is because the research consists in considering eutrophicated water bodies as a resource rather than as a problem. Special attention is given to cyanobacteria. Their key role in eutrophication (when cyanobacterial blooms are triggered) and the existing experiences on anaerobic digestion of cyanobacteria are reviewed.

In the thesis work a Uruguayan dairy farm is taken as a case study. Therefore the situation of dairy farms in Uruguay is presented briefly, emphasising the wastewater impacts and management.

Anaerobic co-digestion with milking parlour wastewater is the studied technology to valorise cyanobacteria. A literature review is presented on anaerobic digestion of dairy farm wastewater, then on microalgae generally and cyanobacteria particularly. Lastly, specific experiences regarding anaerobic co-digestion of both substrates are also reviewed.

Finally, in order to introduce the reader to the complete picture regarding cyanobacteria and wastewater treatment, a section about photobioreactors is presented. It is worth mentioning that the original author's intention included the study of milking parlour wastewater treatment in photobioreactors but due to different reasons, mainly lack of time and resources, it could not be done.

2.2. Eutrophication

2.2.1. Introduction

Water eutrophication (from the Greek meaning "well nourishment") is the process in which an aquatic ecosystem gets richer in nutrients leading to excessive growth of phytoplankton and water quality loss (Yang, 2008). In essence water eutrophication is a natural process but human activities such as urbanization, industrialization and agriculture intensification greatly accelerates it.

Nutrients referred in this process are mainly nitrogen (N) and phosphorus (P). Equation 2-1 presents the stoichiometric relationship of phytoplankton growth. In spite of the relation

between N and P being 16:1, usually P isthe limiting nutrient in eutrophication. Nevertheless, the exact mechanisms of eutrophication processes have not been fully understood yet.

Equation 2-1 Phytoplankton bioplasm formation (energy and microelements not shown) (Yang, 2008)

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H^+ \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2$$

Investigations from the United Nations Environmental Programme indicated that about 30 to 40% of world's lakes and reservoirs have been somehow affected by eutrophication (Yang, 2008). Accelerated water eutrophication breaks out the intrinsic equilibrium of the aquatic ecosystem, leading to gradual degeneration of the water ecosystem's functions and affection of water quality and turbidity.

Moreover, eutrophication sets the basis for phytoplankton blooms, and a thick layer of "green scum" on water surface can become frequent. When the cyanobacteria are involved in a bloom they can produce significant amounts of toxins when they die. Toxins can be harmful to animals and humans.

To fight and prevent eutrophication the first measure is to control nutrient discharge into the water body, both from diffuse and point sources. Agricultural best management practices and good wastewater treatment in urban centres and industries are very much needed measures for eutrophication control. However, once the eutrophication condition is already installed in a water body, it may take decades to reverse the situation if only source-control measures are taken. *In situ* mitigation measures need to be taken in order to improve the situation in the short-term.

2.2.2. Phytoplankton and microalgae

Sometimes vague and/or contradictory terms are found in the literature to refer to microalgae, cyanobacteria and related terms. For the sake of clarification some terms used in this document are introduced as follows.

Phytoplankton is the autotrophic community of plankton, those tiny organisms that live in the water column and cannot swim against the current. Phytoplankton has a key role in aquatic systems as contributor to primary production through photosynthesis (Bonilla, 2009). Within the phytoplankton community the microalgae community is included.

Microalgae can be defined as single-cell microorganisms that live in water and exhibit photosynthetic metabolism. Based on this definition, both eukaryotic and prokaryotic organisms are part of the microalgae group. Including prokaryotic photosynthetic microorganisms (i.e. cyanobacteria) within the microalgae community is, however, controversial. This is because the term 'algae' was formerly classified within the plantae kingdom, limiting the concept to the Eukaryote domain.

Chlorophylls and accessory pigments are found in all microalgae, due to their autotrophic metabolism. However, heterotrophic and mixotrophic microalgae also exist. Heterotrophic ones consume organic compounds as primary energetic source and are independent of

photosynthesis. Mixotrophic microalgae are capable of both consuming organic compounds as fixing CO_2 via photosynthesis (Tijani, et al., 2015).

Eukaryotic microalgae evolved more than 1.5 million years ago from the symbiosis of a flagellated eukaryote with a primitive cyanobacteria. This set the basis for evolution of photosynthetic eukaryotes including land plants (Ball, 2005).

The primary endosymbiont subsequently led to three primary lineages: the glaucophytes (freshwater microalgae carrying primitive plastids), rhodophytes (also known as red algae), and the viridiplantae (the true plants). Within the last 6 phyla can be classified: chlorophyte (green algae), cryptophyta, dinophyta (dinoflagellates), euglenophyta, haptophyte and ochrophyta (which includes diatoms) (Tijani, Abdullah and Yuzir, 2015).

Finally cyanobacteria are prokaryotic phototroph microorganisms, which are Gram negative and have asexual reproduction. They are also called blue-greens, blue-green algae, blue-green bacteria, myxophyceaens, cyanophyceans, cyanophytes, cyanoprokaryote, etc. It is believed that cyanobacteria contributed to the oxygenation of the atmosphere, being the first oxygenic photosynthetic organisms on Earth, over 3 million years ago (Catherine, et al., 2013).

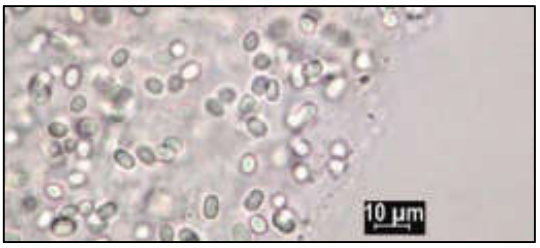
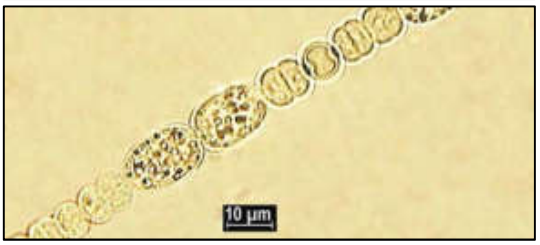
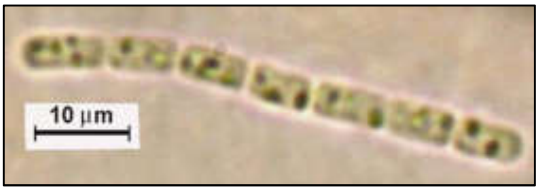
2.2.3. Cyanobacteria

Some cyanobacterial species produces toxins (called cyanotoxins). Cyanobacteria's toxins have been linked to: (i) mortality in aquatic macroinvertebrates, aquatic birds and other predators; (ii) to human health damage; and (iii) to generate adverse impacts on water treatment (Cheung, 2013). Examples of species of cyanobacteria are illustrated in Figure 2-1.

Cyanobacteria are oxygenic phototrophs with thylakoids with photosystems I and II, using chlorophyll-a and other pigments for photosynthesis. Phycobilins like phycocyanin are characteristic pigments of cyanobacteria, being responsible for their different colour that can vary from blue-green to red-violet. Phycobilins provide the ability to use a wide range of light spectrum which causes cyanobacteria to be able to absorb light very efficiently (Bartram, 1999).

Cyanobacteria can be found in single cells, colonies and multicellular filaments. Some species of the multicellular configuration present differentiated cells called heterocysts, capable of fixing atmospheric nitrogen, and akinetes, thick-walled dormant cells that serves as survival structure (Bartram, 1999).

Moreover many species of cyanobacteria have gas vesicles to adjust floatability giving them the possibility to enhance light and nutrient exposition and reduce losses by sedimentation. Other characteristics that give cyanobacteria advantages over green microalgae are a wide range of temperature resistance, the possibility of grow in environments with nutrient deficiency, and the capacity of synthetizing cyanotoxins as defence from predators (Bartram, 1999).

	• Specie: *Aphanotece sagnina* • Order: Chroococcales • Organization level: Colonial • Differentiated cells: No
	• Specie: *Dolichospermum viguieri* • Order: Nostocales • Organization level: Filamentous • Differentiated cells: Heterocysts and akinetes
	• Specie: *Pseudoanabaena moniliformis* • Order: Oscillatoriales • Organization level: Filamentous • Differentiated cells: No

Figures 2-1 Some examples of cyanobacteria aspect and morphology from (Bonilla, 2009)

2.2.4. Cyanobacterial blooms

A bloom is a sudden rapid growth of a community, driven by certain favourable environmental conditions. Generally this is correlated to a diminution in the ecosystem diversity (Merel, 2013). This phenomenon can last short periods, many days or even can have seasonal durations. Cyanobacterial blooms (also known as cyanobacterial harmful algal blooms) occur and have occurred naturally. However in the last decades due to human intervention and advanced on the eutrophication the frequency, severity and geographic distribution has increased (Cheung, 2013).

Depending on the dominant specie a bloom can occur in different configurations and appearances. As follows some figures are presented to give an idea of how a cyanobacterial bloom looks like. Figure 2-2 shows a cyanobacterial bloom with dispersed colonies. Figure 2-3 presents accumulative colonies forming a green scum. Finally Figure 2-4 shows blooms of green filamentous algae for comparison purposes.

Figure 2-2 Dispersed cyanobacterial blooms in A) urban lagoon, B) creek, C) reservoir (Bonilla, 2009)

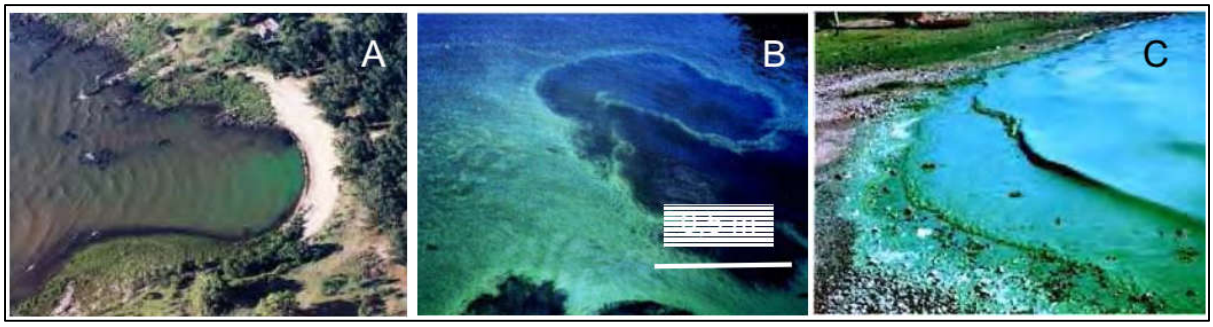


Figure 2-3 Accumulative cyanobacterial blooms in A) coastal beach, B) lentic system, C) reservoir (Bonilla, 2009)

Figure 2-4 Accumulative filamentous algae blooms in A) creek, B) pond, C) petri dish (Bonilla, 2009)

For a bloom to happen there must be a set of environmental and hydraulic factors (Molot, 2014). The first factor is light availability; the more light the more potential photosynthesis. Due to extra photosynthetic pigments cyanobacteria can grow in less optimum light conditions, limiting the development of other microorganisms and plants.

The ability to move in the water column through gas vesicles is also very important to seek for light and nutrients. Thus, still water favours blooms as strong currents might prevent cyanobacteria to float freely and may generate turbidity.

Temperature is another key factor as cyanobacteria reach maximum growth at 25-30 °C (Bonilla, 2009). Thus higher chances of cyanobacterial bloom formation is expected during summer in template regions. Moreover, global warming threatens to increase the frequency and

intensity of cyanobacterial blooms. When thermal stratification overturns in spring/summer in dimictic lakes, cyanobacterial blooms are more prone to occur not only for higher temperatures but also for making nutrients associated with the hypolimnion to be suddenly available.

Microalgae limiting nutrients include nitrogen, phosphorus and trace elements such as iron (Fe). There is still unanswered question about the exact relation between N, P and Fe concentrations and blooms. Some authors claim the optimum ratio is approximately 106:16:1 (C:N:P) known as the Redfield ratio (Bonilla, 2009).

Others suggest cyanobacterial blooms are better correlated to both total N and total P (Merel, 2013). A recent study (Molot, 2014) states that when the C, N and P concentrations are found in excess then the Fe^{+2} concentration determines if the bloom is dominated by microalgae or cyanobacteria.

Dilution by water flow is another factor affecting blooms (negatively). It does not involve losses but it can disperse the organisms through the water course and prevent high concentrations of cyanobacteria.

Cyanobacteria can also be predated by species located above in the trophic chain. Zooplankton species are the main predators of phytoplankton. However, many of these are inhibited or killed by cyanotoxins. Finally, cyanophage are virus that infects cyanobacteria but are very sensitive to UV radiation. Thus superficial booms are less suitable to be attacked by cyanophages (Bonilla, 2009). These existing ecosystem relations may open the door for biological control of cyanobacterial communities.

2.2.5. Cyanotoxins

Cyanobacteria produce a wide variety of secondary metabolites including toxic ones which are the cyanotoxins. Not all cyanobacteria produces cyanotoxins: there are toxic and not toxic species and, toxic and not toxic strains within the same specie (Cheung, 2013). Even a potentially toxic strain might not produce cyanotoxins if not activated by certain environmental factors.

The mechanisms for these toxins to act are diverse; their effect can be hepatotoxic, neurotoxic, dermatotoxic and inhibitors of protein synthesis (Merel, 2013). Figure 2-4 shows the chemical structure of microcystin, the most studied cyanotoxin presenting more than 80 structural variants, and saxitoxin, the most toxic with a median lethal dose (LD_{50}) of 10 µg/kg (rodents, 24h, intraperitoneal). Table 2-1 presents a list of the cyanotoxin produced by each cyanobacteria genera, together with the target organ.

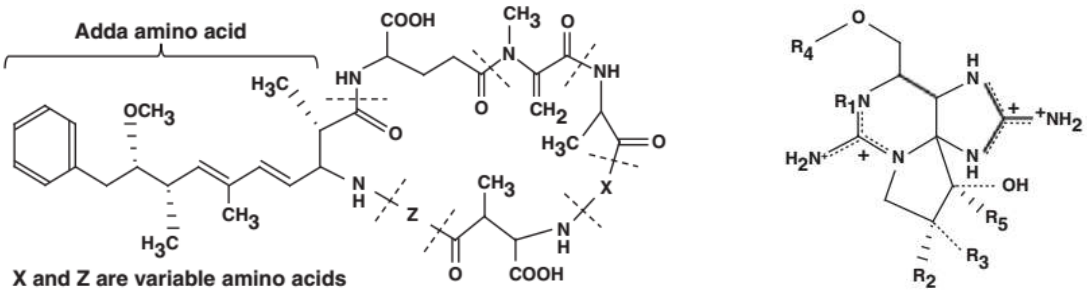


Figure 2-5 Microcystis and Saxitoxin, respectively, from (Merel, 2013)

Table 2-1 List of cyanotoxins, based on (Bartram, 1999, Bonilla, 2009)

Cyanotoxin	Compound type	# of variants	Primary target organ in mammals	Main genera
Anatoxin-a	Alkaloid	2	Nerve synapse	Anabaena, Aphanizomenon, Planktothrix (Oscillatoria)
Anatoxin-a (S)	Organophosphorus	1	Nerve synapse	Anabaena
Cylindrospermopsins	Cyclic guanidinic alkaloid	3 approx.	Liver	Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Raphidiopsis
Lipopolysaccharides (LPS)	Lipopolysaccharide	Many	Potentially irritant; affects any exposed tissue	All
Lyngbyatoxin-a	Alkaloid	1	Skin, gastro-intestinal tract	Lyngbya
Microcystin	Cyclic heptapeptide	More than 80	Liver	Anabaena, Anabaenopsis, Aphanocapsa, Hapalosiphon, Microcystis, Nostoc, Planktothrix (Oscillatoria)
Nodularin	Cyclic pentapeptide	8 approx.	Liver	Nodularia
Saxitoxin	Carbamate alkaloids non, mono and disulphated	20 approx.	Nerve axons	Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Raphidiopsis

Cyanotoxin production varies depending on microorganism's life cycle: the older the cyanobacteria population the more toxins it produces. Cyanotoxin production is also enhanced by environmental stress (Bartram, 1999). It has been stated that more global temperature and more eutrophicated state favors growth of toxic strains against non-toxic strains (Cheung, 2013).

The best available method to detect if a bloom has the potential to be toxic is identifying genes that contains information for cyanotoxin synthesis. However, identifying toxin producing genes does not mean that cyanotoxins are present, as specific environmental factors that might not be there are needed for triggering cyanotoxin production. The exact mechanism that triggers cyanotoxin production is not clearly understood yet.

2.2.6. Mechanical cyanobacteria removal

Cyanobacterial bloom mitigation measures applied in the punctual scale include chemical and physical methods. Chemical methods include application of herbicides and/or flocculants, although care must be taken because they can produce toxic by-products or enhance cyanotoxin liberation due to cell lysis.

Mechanical methods may or may not be preceded by flocculation. Slow sand filtration holds cyanobacteria cells before cyanotoxins are released, but it also can remove the cyanotoxins. When cyanobacterial scum accumulates, a second method is water suction by pumping and transfer to drying beds. If cyanobacterial bloom wants to be confined in a part of the water body, installation of containment booms is a feasible alternative (Bonilla, 2009).

There is an example of successful mechanical cyanobacterial removal in Lake Taihu, in southeast China: "Taihu blue algae (mainly consist of cyanobacteria) was salvaged from Taihu Lake every summer, as one of the most efficient measures to reduce Taihu Lake's eutrophication" (Miao, Lu, Zhao, Huang, Ren, Yan and Ruan, 2013).

2.3. Dairy farms in Uruguay

2.3.1. Introduction

Dairy farming is the activity of breeding mammals for harvesting fresh milk. Dairy cows are the most common animal for milk producing (particularly in Uruguay), although dairy farms with other mammals such as goats, sheep, buffalo, camels, etc. might be found in the world.

Milk is usually harvested twice a day and transferred through a milk pipeline to a bulk milk cooling tank installed within the dairy farm. Stored milk is daily picked up by pump trucks (milk hauler) to be carried to a dairy industry where the production of dairy products takes place. Dairy products include pasteurized milk, butter, yogurt, cheese, ice cream, powder milk, among many others.

In traditional establishments based on grazing in the field dairy farm wastewater is originated only in the milking parlour and waiting areas, if properly drained. After each milking session operators clean the floor with water producing an effluent that contains excrements, urine, milk

leftovers, detergents and other cleaning products. The risks of disposing untreated wastewater in dairy farms include: water eutrophication, soil oxygen depletion, diseases in animals and humans (MGAP, 2008).

2.3.2. Uruguayan milking sector

Milking production in Uruguayan dairy farms has grown steadily from the year 1975. The milking industrial sector plays an important role in the Uruguayan economy being one of the industrial sectors that adds most added value. The sector is composed by national and multinational companies that are continuously expanding the installed capacity. Local demand is broadly covered, with 70% of milking production being exported (UruguayXXI, 2015). The exportation of milk products is located in the fourth place, after soybean, beef and cereals, representing 8.3% of the total Uruguayan goods sells

In 2013 there were 4,291 dairy farmers in Uruguay, covering a total surface of 811,000 ha, using 440,000 milking cows (UruguayXXI, 2015). 87% of the harvested milk is forwarded to dairy industries for the processing of milk products. In relation to the number and extent of dairy farms, in 1986 there were 7,335 dairy farms in Uruguay while in 2013 it decreased to 4,291. Nevertheless the milk productivity went from 731 L/ha in the year 1977 to 2,370 L/ha in the year 2007.

Regarding the number and size of the milking establishments, dairy farms having between 50-199 ha represent 50% in number (and 21% in surface) and between 200-499 ha represent 22% of total (and 26% in surface). Establishments larger than 2,500 ha represent 0.8% of the total in number but 11% in surface (UruguayXXI, 2015). The so called 'Milking Basin' of Uruguay — where the highest density of dairy farms can be found— is placed mainly in the southwest of the country (Figure 2-6)

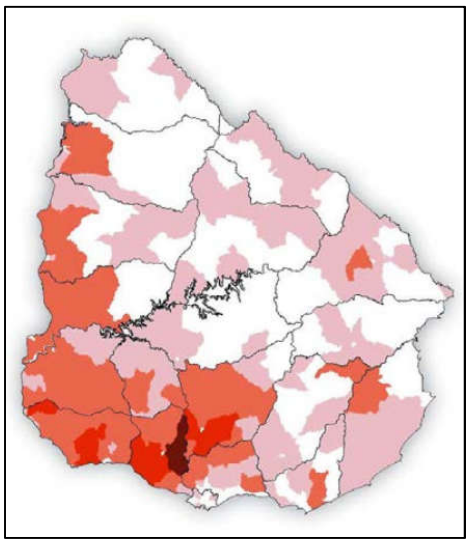


Figure 2-6 Map of Uruguay showing the amount of milking production in different political sub-divisions, for the year 2010/2011. The lighter colour represents 0-0.25% of total production, the next colour 0.25-2.5%, then 2.5-5%, and the darkest colour represents 5-7.1% (UruguayXXI, 2015)

A recent study (Moreda, 2016) estimated that the potential methane production of manure (including dairy farms and feedlots) in Uruguay is 1.5-2.3 millions of m^3/year, meaning 0.5-0.7 MW of electric power. The author states that this category is undergoing major changes and the methane potential can grow significantly. This is tendency is feasible due to stringer regulations and the intrusion in the market of new large-scale dairy farms with economic capacity to invest in modern wastewater treatment.

2.3.3. Common wastewater treatment practices

Dairy farm wastewater treatment in Uruguay is done mainly through waste stabilization ponds (WSPs), anaerobic and facultative (Figures 2-7, left). In many cases WSPs are overloaded and not properly maintained (Houlbrooke, 2015), which results in uncomplete treatment. WSPs systems are not very efficient in terms of removal of pollutants and area footprint. Moreover they have the disadvantage of not capturing the biogas produced in anaerobic ponds, causing the release to the atmosphere of a set of greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4). From these two, methane has a significant higher global warming potential.

Figures 2-7 Left: A polishing pond in a WSP system. Right: Direct discharge on terrain; from (Houlbrooke, 2015)

Direct discharge in terrain of dairy farm wastewater is common as well (and even recommended), but frequently is not properly done (e.g. right in Figures 2-7) (Houlbrooke, 2015). Terrain discharge should be spread in order to distribute nutrients spatially and thus avoid the risk of soil saturation which leads to surface runoff and leachates. Advanced technology for wastewater treatment and biogas collection is rare in Uruguayan dairy farms, especially in the medium and small scale.

Although not always implemented in Uruguay, it is a good practice to have liquid/solid separation steps, prior to the wastewater treatment system. The solid fraction of dairy farm effluent consists mainly in manure and can be stored in time for its use as fertilizer. However, waste piles generate leachate that should be redirected to the wastewater treatment system. A solid waste management plan is needed in order to prevent environmental impacts when solids are separated from the liquid fraction.

2.3.4. Uruguayan legal framework

In Uruguay the legal framework for water use and protection is centred in a law known as the Water Code (law № 14.859 from 1978) and in the constitution itself whose article 47 express that "the protection of the environment is of general interest", "water is an essential natural resource for life", among others.

The law is regulated by the Decree № 253 from 1979 which establishes national standards both for water quality and for discharge. The MVOTMA (Uruguayan Ministry of Housing, Territorial Planning and Environment) with a resolution released on the 25th of February of 2005 classified all water bodies as Class 3: "Waters intended for the preservation of fish in general and other members of the water flora and fauna, or also waters intended for the irrigation of crops whose product is not consumed naturally or, in those cases that are consumed in a natural way, irrigation that do not cause the product to wet". The national standards for Class 3 water bodies are presented in Table 2-2, metals not presented).

Table 2-2 National standards for Class 3 water quality (without metals and organic toxics)

Parameter	Unit	Value
Odor	-	Not perceptible
Floating materials and non-natural foam	-	Absent
Non-natural color	-	Absent
Turbidity	NTU	<50
pH	-	>6.5 and <8.5
DO	mgO/L	>5
BOD_5	mgO/L	<10
Oils and fats	mg/L	Virtually absent
Detergents	mg/L as LAS	<1
Phenolic substances	mg/L asC6H5OH	<0.2
Free ammonia	mgN/L	<0.02
Nitrates	mgN/L	<10
Total phosphorus	mgP/L	<0.025
Fecal coliforms	FC/100mL	<2000 in every of 5 samples (average <1000)

National standards for direct discharge of effluents into water courses are presented in Table 2-3 while national standards for sewages being disposed by infiltration on terrain are shown in table 2-4.

Table 2-3 National standards for effluents discharged directly into water courses (without metals and organic toxics)

Parameter	Unit	Value
Temperature	℃	<30 but cannot increase receiving water body's temperature above 2.
Floating materials and non-natural foam	-	Absent
pH	-	>6.0 and <9.0
BOD_5	mgO/L	<60
Total suspended solids	mg/L	<150
Oils and fats	mg/L	<50
Sulphur	mg/L	<1
Detergents	mg/L as LAS	<4
Phenolic substances	mg/L asC6H5OH	<0.5
Free ammonia	mgN/L	<5
Total phosphorus	mgP/L	<5
Fecal coliforms	FC/100mL	<5,000

Table 2-4 National standards for effluents being disposed by infiltration on terrain (without metals and organic toxics)

Parameter	Unit	Value
Temperature	℃	<35
Floating materials and non-natural foam	-	Absent
pH	-	>5.5 and <9.0
Settleable solids	mL/L	<10 (determined in Imhoff cone for 1 hour)
Total solids	mg/L	<700
Oils and fats	mg/L	<200

2.3.5. International standards

Word Health Organization (WHO) defines the water quality for irrigation water in order to protect the health of both consumers and agricultural workers. The Food and Agriculture Association (FAO) wastewater quality guidelines for agricultural use compiles the WHO guidelines for health protection with guidelines for maximizing crop production.

WHO 1989 guidelines for using treated wastewater in agriculture are presented in Table 2-5. The guidelines define three effluent categories depending on the irrigation type. FAO 1985 water quality guidelines for maximizing crop production also propose three categories for water irrigation, based on salinity, infiltration, specific ion toxicity, trace elements and miscellaneous effects. From all the regulated parameters, mostly agricultural, only electric conductivity, pH, bicarbonate and nitrates are normally analysed in wastewater monitoring programs (Table 2-6)

Table 2-5 WHO (1989) recommended microbiological quality guidelines for wastewater use in agriculture

Category	Reuse condition	Exposed group	Fecal coliforms (geometric mean no./100 mL)	Intestinal nematodes (arithmetic mean no. eggs/liter)	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks	Workers, consumers, public	<1000	<1	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees	Workers	No recommended standard	<1	Retention in stabilization ponds for 8-10 days or equivalent helminth and fecal coliform removal
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

Table 2-6 FAO (1985) guidelines for interpretation of water quality for irrigation

Degree of restriction on use	EC (mS/cm)	pH	Nitrates (mg NO_3-N/L)	Bicarbonate (me/L)
None	<0,7	Normal range 6.5-8	<5	<1.5
Slight to moderate	0,7-3,0		5-30	1.5-8.5
Severe	>3,0		>30	>8.5

2.4. Anaerobic digestion

2.4.1. Introduction

Anaerobic digestion is a fermentation process in which the organic matter is degraded producing biogas. Where organic material is available and the redox potential is low enough (that is, no oxygen is present) anaerobic digestion occurs naturally and spontaneously. Some examples are stomachs of ruminants, marshes, sediments of lakes, municipal landfills and even municipal sewers (Henze, 2008).

Anaerobic wastewater treatment has evolved into a competitive wastewater treatment technology. Different types of organically polluted wastewater can be treated with this technology. In countries like the Netherlands anaerobic reactor systems are used to treat almost all agro-industrial wastewaters and the application potential to other types of wastewater is growing. (Henze, 2008).

a) Anaerobic digestion as a source of renewable biofuel

In the world there is an ever increasing search for new and better clean technologies for energy production. In this line the production of biofuels from agricultural derived biomass is growing world-wide and anaerobic digestion is one of the technologies that are helping to achieve that. Based on the substrate utilized for biofuel it can be classified as within a first, second or third generation.

The first generation of biofuels are produced directly from food crops. However, this search for terrestrial based biofuel production can derive in other less obvious problems such as water eutrophication, resource depletion, reduced biodiversity and direct competition with current food crops (A. J. Ward, 2014).

The second generation of biofuels seeks to overcome the limitations of the first generation by using the residues from agricultural activities (including forestry). Biogas from dairy farm wastewater fits into this category.

The third biofuel generation uses non-food crops cultivated in designed, bioengineered systems with the aim to enhance production. Microalgae based biofuels (either from lipid extraction or from anaerobic digestion) are typical example of this relatively new category.

b) Closing loops

When organic substrates are anaerobically digested, reaction products such as biogas, effluent and sludge are produced. The methane in the biogas can be purified and generate biomethane which in turn can be converted into energy basically by two ways: direct combustion (e.g. for house coking), or electricity generation with gas turbines or steam generators. Biogas can also be upgraded to vehicle fuel or even injected into natural gas grids to regenerate them (Parajuli, 2011).

Moreover, the anaerobic effluent and the sludge are both rich in derivatives of organic nitrogen and phosphorus, in other words, rich in nutrients. Both the anaerobic effluent and the sludge can be processed in order to utilize those nutrients in agricultural applications in the form of organic fertilizers. Ammonium sulphate and struvite can also be harvested from anaerobic effluent to improve the quality of organic fertilizers (Tijani, Abdullah and Yuzir, 2015).

If an anaerobic digester wastewater is fed, the anaerobically treated effluent will be cleaner; particularly in terms of suspended solids (SS) and chemical oxygen demand (COD). The concentration of pathogens may also be reduced. However the total nutrient load is not significantly reduced. Organic and particulate-bond nutrient are hydrolysed and released as soluble N and P.

Considering the aspects mentioned above, it is clear that an anaerobic digester may receive wastewater (or wastes in general) and deliver cleaner water, organic fertilizer and energy. This is why anaerobic digestion can act as a key technology in closing the water, energy and nutrient loops in a wide array of economic activities.

2.4.2. Microbiology of anaerobic digestion

Anaerobic digestion is a process carried out by heterogeneous consortia of anaerobic microorganisms, with diverse biological and substrate affinities. There are five main groupings of anaerobic microorganisms as follows: fermentative bacteria, hydrogen-producing acetogenic bacteria, hydrogen-consuming acetogenic bacteria, carbon dioxide-reducing methanogens, and acetoclastic methanogens (Henze, 2008).

The microbial consortium ultimately mineralizes complex organic matter into CH_4, CO_2, NH_3, H_2S and H_2O. The digestion process is usually divided into four phases. A schematic view of the overall process is presented in Figure 2-8. The first one is the hydrolysis. Enzymes released by fermentative bacteria (exo-enzymes) convert particulate proteins, polysaccharides and fats into soluble compounds which can be incorporated into bacteria through the cell walls. Usually this is the slowest phase

Acidogenesis is the second phase where the anaerobic oxidation of amino acids, sugars, higher fatty acids and alcohols take place. Intra cellular dissolved compounds are converted and excreted (some are converted into new cell material). This phase produces volatile fatty acids (VFAs), alcohols, lactic acid, CO_2, H_2, NH_3 and H_2S.

The third phase is the acetogenesis. In this phase acetate, H_2 and CO_2 is produced, as well as new cell material. Sub-process involve in the acetogenesis include the formation of acetic acid and H_2 from mainly VFAs, and the formation of acetic acid from H_2 and CO_2 (known as homoacetogenesis).

The last phase is the methanogenesis where acetate, H_2, CO_2, fomate or methanol are converted into CH_4, CO_2 and new cell material. Methane can be produced in two ways as follows: either from acetic acid or from H_2 and CO_2. Generally 70% of the produced CH_4 originates from acetic acid. Methanogens are actually archaea and are obligate anaerobes with very narrow substrate spectrum. When the substrate is high in readily available organic matter and the hydrolysis phase is not the limiting step from the kinetic point of view, methanogenesis is the slowest phase.

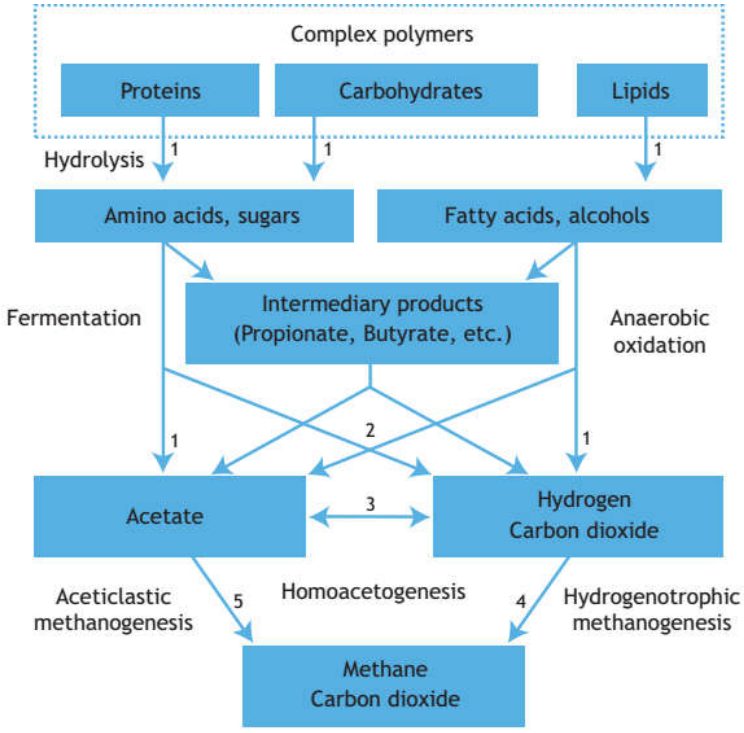


Figure 2-8 Scheme of the sub processes of anaerobic digestion. Numbers indicate bacterial groups involved: 1) Fermentative bacteria, 2) Acetogenic bacteria, 3) Homo-acetogenic bacteria, 4) Hydrogenotrophic methanogens, 5) Acetoclastic methanogens. From (Henze, 2008)

2.4.3. Theoretical methane production

There are three basic principles that govern biogas production from organic matter (Lier, 2016), listed as follows:

- Average oxidation state of C stays the same.
- Substrate C divides in two parts, one goes to form CH_4 (with C being completely reduced, oxidation state -4) and the other CO_2 (with C being completely oxidised, oxidation state +4).
- N and O stays completely reduced.

Buswell and Boruff reported in 1932 a stoichiometric relationship for biogas production from a CHON organic substrate, presented in Equation 2-2. It can be used to estimate the theoretical gas composition on a percentage molar basis, when the C, H, O and N composition of the substrate is known.

Equation 2-2 Stoichiometry of biogas production from organic substrate (A. J. Ward, 2014)

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a-b+2c+3d}{9}\right)CO_2 + (d)NH_3$$

Equation 2-3 includes the molar volume of CH_4 (V_m, which at 0 °C and 1 atm is 22.14 L) and can be used to calculate the volume of methane gas produced based on the amount of the volatile solids degraded during anaerobic digestion. However this theoretical equation overestimates the real biogas production as it assumes total conversion of volatile solids into biogas without considering intermediate products or the microorganism needs for cell maintenance and anabolism (A. J. Ward, 2014).

Equation 2-3 Methane yield (A. J. Ward, 2014)

$$CH_4\ yield\ \left({}^{L}/_{g\ VS\ degraded}\right) = \left(\frac{4a+b-2c-3d}{12a+b+16c+14d}\right) \times V_m$$

When CHON composition of organic substrate is not known (typical for complex environmental matrixes such as wastewater) CH_4 production of anaerobic digestion can be estimated based on known yields and rule of thumb: 5% of entering COD is converted to sludge (Figure 2-8). Depending on reactor's removal performance 10-20% of influent COD exits with the effluent. The rest (75-85%) of COD exits the system as CH_4 within biogas, resulting on a yield of 0.26-0.30 mL_{CH4}/mgCOD.

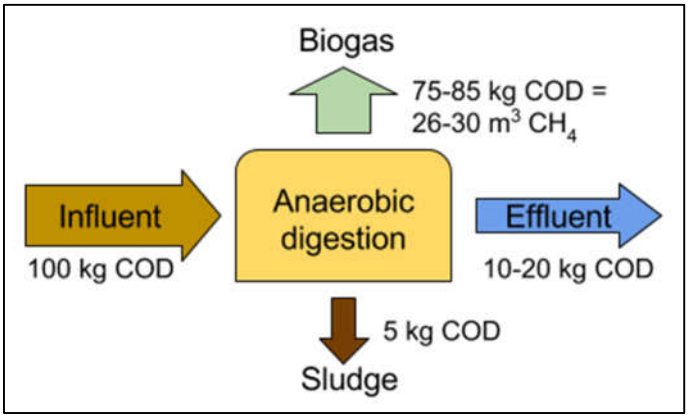


Figure 2-9 COD balance for anaerobic wastewater treatment, adapted from (Lier, 2016)

This relation comes from acetic acid combustion formula, which represents its biological oxidation (Equation 2-4). One mole of acetic acid (60 g) chemically demands two moles of oxygen (64 g).

Equation 2-4 Methane combustion stoichiometry

$$CH_4COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$$

On the other hand acetic acid is converted into methane and carbon dioxide by acetoclastic methanogens microorganisms (Equation 2-5) which are only Archaea (from what is known so far). Therefore 64 g COD of acetic acid produces 1 mole of methane, which at STP has a volume of 22.4 L. This in turns gives the used reference value of 0.350 $mL_{CH4}/mgCOD_{removed}$.

Equation 2-5 Acetoclastic methanogenesis

$$CH_4COOH \rightarrow CO_2 + CH_4$$

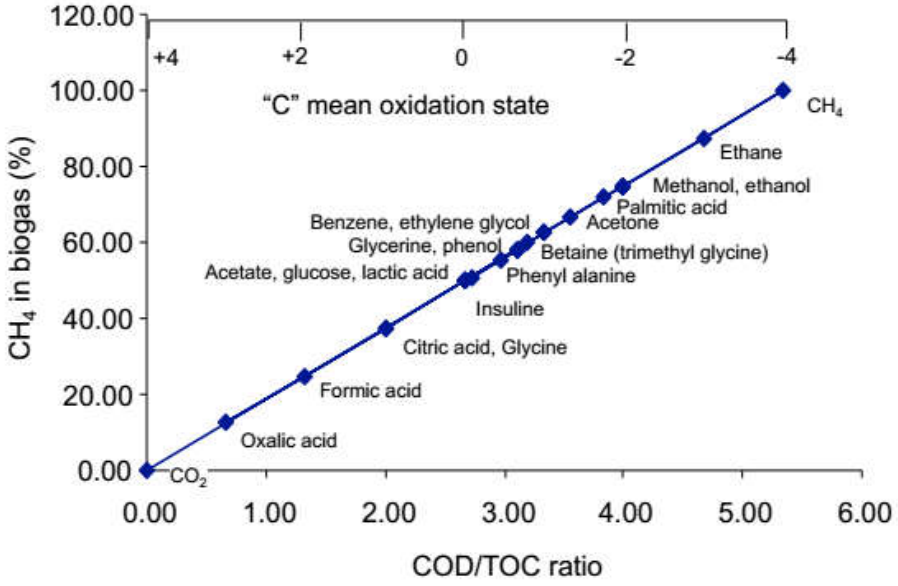
Moreover CH_4 content in biogas can be estimated if the quantity of reduced C relative to the total C is known, which can be estimated from the relation between COD and total organic carbon (TOC) (Equation 2-6) (Lier, 2016).

Equation 2-6 Stoichiometrich relation of COD and TOC

$$\frac{COD}{TOC} = \frac{8 \times (4a + b - 2c - 3d)}{12a}$$

Organic compounds have varying levels of COD/TOC ratio which represent differences in the expected CH4 content of biogas (Graph 2-1). The expected methane content in biogas can be calculated as 18.75 times COD/TOC ratio, if no organic S compounds are present.

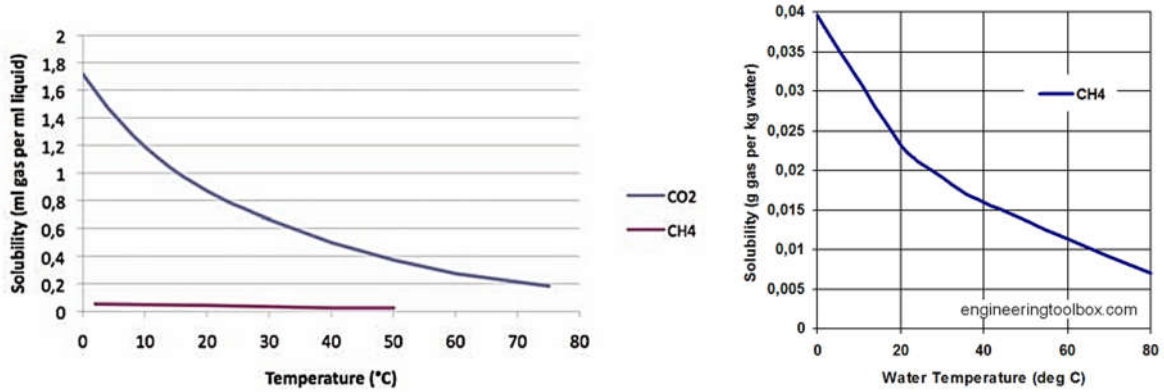
Graph 2-1 Relation between CH_4 percentage in biogas and COD/TOC ratio and "C" mean oxidation state (Lier, 2016)



However actual biogas composition in reality deviates from the theory. Fist the COD consumed due to cell growth (bacterial yield) is not considered. Moreover, alternative electron acceptors (SO_4^{2-}, NO_3^-, NO_2^-) 'consuming' COD and liberating H_2S and N_2 gases are frequently present in complex environmental substrates. In addition, organic matter may have limited biodegradability.

Finally CO_2 is about 40 to 60 times more soluble than CH_4 in water under anaerobic conditions (Parajuli, 2011). This increases the CO_2/CH_4 ratio of exiting biogas. Graphs 2-2 presents the difference in the solubility of CH_4 and CO_2 in water. At 30 °C, solubility of methane in water is 17.8 mg/L or 27.6 mL/L.

Graphs 2-2 Left: solubility of methane and carbon dioxide in water at 1 atm (Anneli Petersson, 2009). Right: detailed solubility of methane in water (ToolBox, 2017)



2.4.4. Real scale biogas production

The production of biogas in anaerobic digestion starts with the generation of bubbles in the slurry. The birth of a bubble can happen when there is enough pressure inside the bubble to balance the external pressure and surface tension. When the partial pressure of biogas bubbles exceeds the total pressure, the bubbles are released from the slurry into the headspace (Parajuli, 2011).

The digestion of organic materials produces biogas that typically consists of 55-65% methane, 35-45% carbon dioxide, 0-3% nitrogen, 0-1% hydrogen, and 0-1% hydrogen sulphide (B. Salam, 2009). When biogas gets richer in CH_4 the molecular weight of biogas as a total decreases (Parajuli, 2011).

The key operating parameter that influences the CH_4/CO_2 ratio of the biogas produced is the pH of the anaerobic reactor, as it drives the carbonate system to liberate or not CO_2. If pH increases, gaseous NH_3 is released elevating alkalinity, sequestering CO_2 and inclining biogas composition to CH_4. The generation of ammonia in the biogas is relatively enhanced by an increase in the protein content of the feed (Cantu, 2014). Moreover, protein rich in sulphurated amino acids may generate higher levels of H_2S in the biogas product (Tijani, Abdullah and Yuzir, 2015).

a) Operational parameters

In this section the main factors influencing the operation of anaerobic digestion are presented. The first to mention is temperature. Temperature regulates growth rate of all microorganisms in general. Psychrophilic (less than 15 °C), mesophilic (25-37 °C) and thermophilic (55-65 °C) anaerobic digestion can take place. In the psychrophilic range biogas production is very low (Parajuli, 2011) whereas thermophilic digestion may result in higher biogas production but demands extra heating energy that may make the technology unfeasible.

Alkalinity represents the substrate buffer capacity and is used to control pH. Different microorganisms have different optimum pH range, but generally anaerobic digestion is performed near neutrality. Methanogens have an optimum growth within the pH range of 6.6-7.4 (or a wider range of 6.0-8.0). However acid forming bacteria can still be active at pH lower than 4.5. Therefore continuous acid production can continue even if the methane production gets interrupted due to low pH (Parajuli, 2011).

Regarding the acid/base system in anaerobic digestion, there are two main compounds that regulate pH: carbonic acid and VFAs. The concentration of CO_2 in the headspace directly influences the carbonic acid system, as more gaseous CO_2 pressure results in more carbonic acid concentration and lower pH.

In the anaerobic process organic carbon is converted into methane and by products while organic nitrogen into ammonia. The fraction of free ammonia is regulated by pH. If the pH increases, toxicity by free ammonia can be triggered. For an adequate performance of the anaerobic system a total alkalinity of 1.5 g $CaCO_3$/L is recommended (Parajuli, 2011).

However, ammonia toxicity might result in a sudden drop in pH and possible cancelation of all digestion processes. Acetoclastic (acetate utilisers) methanogens are affected by free ammonia, forcing a shift to syntrophic acetate oxidation (CO_2 and H_2 production followed by hydrogenotrophic methanogens), which is slower and thus insufficient to compensate VFA production (Cantu, 2014).

Linked to the above, the carbon to nitrogen (C/N) ratio of the substrate is a significant factor for monitoring the performance and stability of an anaerobic digester (Tijani, Abdullah and Yuzir, 2015). The preferred C/N relations range between 20:1 and 30:1. Higher ratios indicate that the influent may be nitrogen deficient, and there is risk of bacteria washout. On the other hand, if C/N ratio is too low toxic levels of free ammonia can develop.

Mixing is other factor that affects anaerobic digestion, as it enhances contact between influent substrate and existing viable bacteria population. Apart from intensifying contact between microorganisms and substrate, mixing helps distributing the heat throughout the reactor, enhance gas release from the liquid, and prevents scum formation.

The hydraulic retention time (HRT) and the organic loading rate (OLR) are two key parameters in the reactor design. The HRT and the OLR are inversely proportional. HRT is the unit of time that the kinetics of the digestion processes have to fit. In general, the higher the retention time the better the quality of the effluent, but the bigger and more expensive the reactor.

Moreover, if the OLR is increased to a level higher than the optimum (due to reactor underdesign or overloading) the hydrolysis and volatile fatty acids (VFA) production processes will be accelerated —by hydrolytic and acidogenic bacteria— and the methanogenic capacity for processing all the VFA may be exceeded. If the OLR is not decreased, the VFA concentration increases causing a pH drop and finally inhibiting the methanogenic activity.

Finally it is worth to mention the existence of other potential inhibitors of anaerobic digestion that may be present in the substrate. Besides free ammonia these include heavy metals, organic pollutants, sulphides, and high salts concentration (Cantu, 2014).

b) Reactor design

An anaerobic digester consists in one or more airtight tanks fed with biodegradable material, where the digestion microbial process take place. Feeding can be done in batch (i.e. sequence batch reactor, SBR) or continuous. Contrary to aerobic processes, where all oxygen supplied is rapidly used and thus maximum permissible load is governed by maximum oxygen supply rate, in anaerobic processes maximum permissible load is the result of the amount of anaerobic bacteria in full contact with the wastewater constituents (Henze, 2008).

Figure 2-10 presents four different anaerobic reactor configurations. The first one is a completely stirred tank reactor (CSTR) where HRT and SRT are the same. This configuration is typically found in municipal wastewater treatment plants (WWTP) for anaerobically digesting activated sludge.

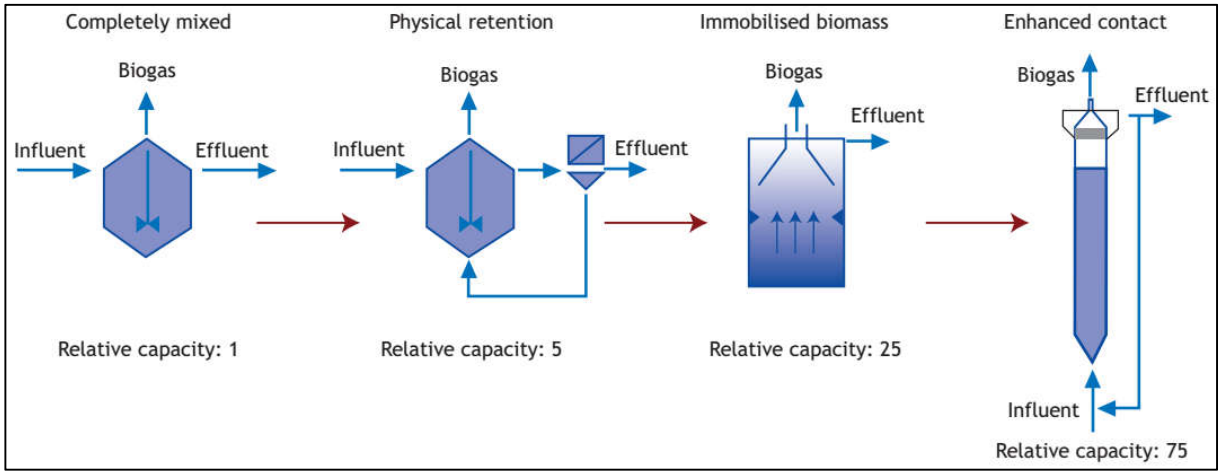


Figure 2-10 Different anaerobic reactors configuration, with their relative loading capacity, from (Henze, 2008)

The second reactor is known as the anaerobic contact process (ACP). It employs an external settler tank and sludge return to uncouple SRT from HRT and with that it achieves a relative loading capacity five times bigger than CSTR with no sludge return. Optionally the ACP is equipped with a flocculator or degasifier chamber before the settler to enhance sludge sedimentation like in Figure 2-10.

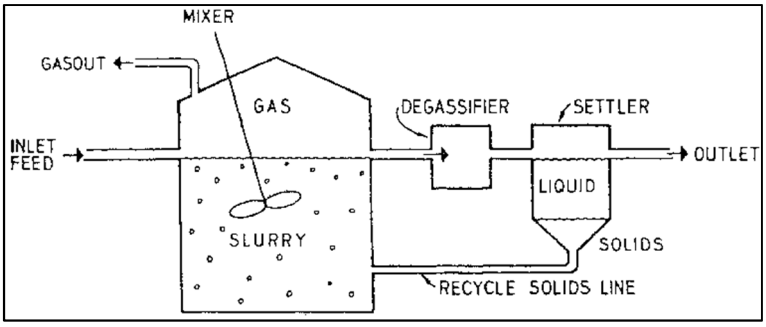


Figure 2-11 Anaerobic contact digester (Marchaim, 1992)

The anaerobic sludge bed reactors (third in Figure 2-9) enhance sludge retention (and increases relative loading capacity) by forming easily settling sludge aggregates in flocs or granules and being equipped with internal gas-liquid-solids separation systems. For the operation of these reactors good pretreatment is essential as suspended solids negatively affects granule formation.

Finally enhanced contact reactors are advanced versions of the sludge bed reactors, with the addition of carrier material or the formation of advanced granular sludge. Anaerobic filters base sludge retention on biofilm formation in carrier material that fills the reactor and are not shown in the figure.

2.4.5. Laboratory scale anaerobic digestion

Laboratory scale anaerobic digestion tests help to determine the ultimate methane potential of organic substances and their rate of biodegradation (Parajuli, 2011). Information for evaluation

of the anaerobic process is provided by measuring the gaseous end products as well as liquid products such as VFAs. Laboratory biogas analysis (i.e. biochemical methane potential, BMP) are usually performed by incubating the test substrate in suitable reactors at a given temperature (typically within the mesophilic or thermophilic range).

Biogas production is the key indicator of anaerobic digestion performance. Thus, in laboratory tests, it is very important to have a proper air-tight reactor with biogas collection and a coupled measurement device. Laboratory reactors can be either batch, continuous, or semi-continuous. Regarding the mixing provision they can be either intermittently mixed, continuously mixed or not mixed.

Errors in biogas measurement may have a big impact in the achieved results with laboratory scale experiments, which are very sensitive. This may cause difficulties to compare the technology with full scale plants. The main factors affecting the precision of biogas volume measurement are errors associated with varying temperatures, vapour content, solubility and pressure (Parajuli, 2011).

The biogas measurement itself can alter microbial anaerobic processes if it allows headspace pressure to grow, as high amounts of dissolved CO_2 can acidify the pH of the medium. Moreover, negative pressure in the headspace may also occur, which could drag outside oxygen-rich air into the anaerobic reactor. This can severely inhibit strictly anoxic methanogens. Procedures of biogas collection, slurry sampling and feeding should not allow atmospheric air diffusion into the reactor.

a) Biogas flow/volume measurement systems

Biogas measurement systems apply either volumetric methods (by providing constant pressure conditions and measuring the change in volume) or manometric methods (by keeping the volume constant and measuring the pressure increase) (Parajuli, 2011). There are many methods for measuring the volume of biogas produced, and some methods are presented next. An important requirement for these methods is to be able to maintain a low headspace pressure.

The biogas production can be determined indirectly by measuring the cumulative pressure in the headspace of the reactor via pressure transducers. In that case a blank consisting of tap water is used to account for abiotical pressure changes due to temperature and atmospheric pressure variations. Pressure data can be converted to volume of biogas using the ideal law of gases.

In the gas syringe method (Figure 2-12), a lubricated large syringe (i.e. 1,000 mL) may be used to measure biogas directly at specific intervals. The syringe is connected to the reactor by injecting the needle through the rubber/butyl seal or bung. If the plunger is drown out until pressure in the headspace reaches atmospheric pressure, the volume of gas in the syringe can be taken as a measurement of biogas produced (Pham, 2013).

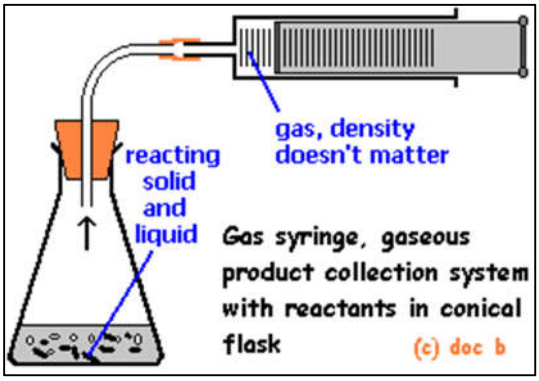
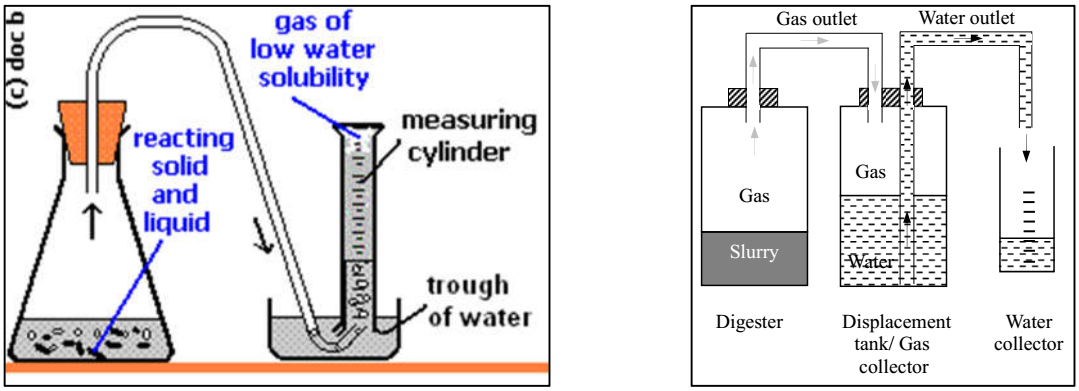


Figure 2-12 Gas syringe method for measuring biogas (Brown)

Biogas volumetric systems (or gasometers) use the liquid displacement method. In these systems the gas gets collected in vessels containing a suitable liquid which is displaced as the gas gets collected. These systems can be constructed with simple materials like jars or cylinders in many different creative configurations. Gasometers can be of the height type, where biogas volume is calculated from the measurement of change in liquid height, or can be of the weight type, in which the biogas volume is determined by weighting the displaced liquid. (Parajuli, 2011).

Two possibilities of the liquid replacement at intervals are shown in Figures 2-13. In the left example the headspace of the reactor is connected to a cylindrical graduated flask filled with the liquid. The opening of the graduated flask is connected with a hanging tube to a container of the same liquid. The biogas produced flows from the headspace up into the cylindrical flask and replaces the liquid; the hanging tube prevents the gas to flow from the cylindrical measuring flask to the liquid container (Pham, 2013). The right example is a variation of the system.



Figures 2-13 Two water displacement method for measuring biogas, left: (Brown), right: (B. Salam, 2009)

Automated anaerobic respirometers are electric devices such as the commercially available AMPTS® (Automatic Methane Potential Test System) and Yieldmaster ®, presented in Figure 2-14 and 2-15 respectively. AMPTS works with liquid replacement and buoyancy in a special unit, for on-line measurement of ultra-low biogas and biomethane flows produced in laboratory scale anaerobic digestion (BioprocessControl, 2015). Yieldmaster measures biogas flow with a pressure transducer named MilliGascounter®.

Figure 2-14 AMPTS II from Bioprocess Control. From left to right: sample incubation unit, CO_2 absortion unit, flow cell array and DAQ unit

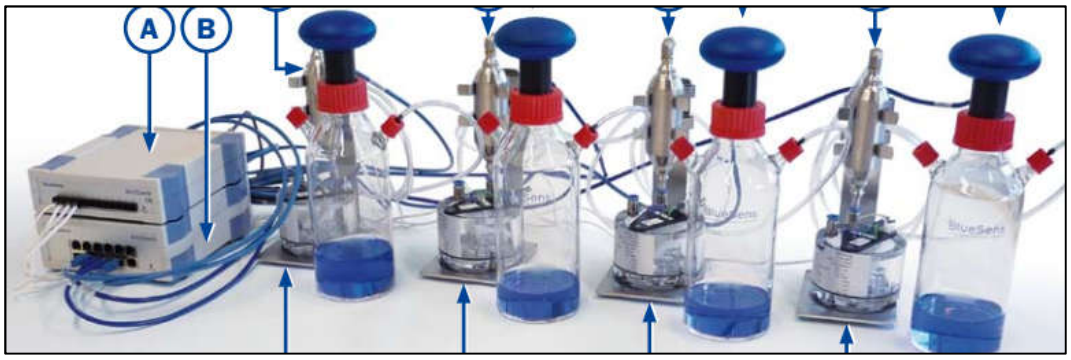


Figure 2-15 Yieldmaster® system for measuring CH_4 gas formation potential equipped with automatic CH_4 sensors and measurement of volume of biogas prduced by pressure (BlueSens)

Liquid displacement methods for measure biogas present a major drawback which is inaccuracy due to biogas solubility/diffusion through the barrier liquid. The evaporation of barrier solutions in long periods of time can also result in inaccuracies. Gas solubility errors can be eliminated by collecting biogas in gas bags and measuring the volume with liquid column meters at intervals. Acidified saturated NaCl solution is recommended as barrier solutions for using in liquid displacement methods due to its high resistance to CO_2 solubility (Parajuli, 2011).

b) Biogas composition analysis

Methane is the actual fuel in biogas, therefore the methane content in biogas needs to be measured. Biogas samples can be directly analysed in a gas chromatograph (GC) equipped with a suitable detector like thermal conductivity detector (TCD) or mass spectrometry (MS). The main advantage of GC is its capability of gathering both qualitative and quantitative information: identification of unknown components and determination of the concentration of each gas within the mixture) (Parajuli, 2011).

The concentration of CH_4 in the biogas can also be measured by a wet chemistry method using an alkaline solution for absorbing CO_2. The AMPTS uses this method. It can also be done manually as presented in Figure 2-16. Pham et al. (2013) found the manual wet chemistry method differed only slightly from GC and claimed its use is acceptable as an alternative to GC in laboratories with no access to chromatography technologies.

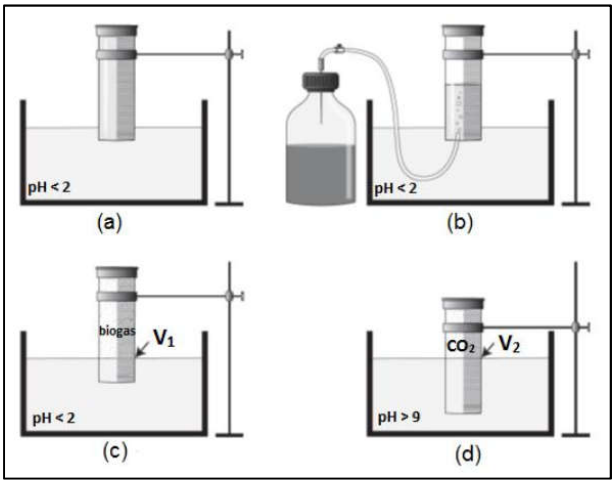


Figure 2-16 A wet chemistry method for determining CH4 concentration in biogas: a) liquid is 0.5 M HCl, b) clamp is open and biogas is collected within a time interval , c) liquid height is adjusted, d) KOH is added and liquid height is readjusted, from (Pham, 2013)

2.4.6. Anaerobic digestion of dairy farm wastewater

a) Introduction

The worldwide abundance of livestock, and particularly in Uruguay, makes manure a plentiful and renewable resource for biofuel production. Moreover ruminant manure have been established as suitable source for anaerobic digestion and biogas production (Manyi-Loh, et al., 2013). This is facilitated by rumen microorganisms that actually start anaerobic digestion in the digestive system of the animal. When manure is stored it spontaneously generates and release methane.

The technology has the potential of converting wastewater into valuable products like fertilizer and energy, and deliver a cleaner effluent in terms of organic matter content (COD). The digested liquid can be further processed to obtain concentrated fertilizers or to obtain clean water for recycling or irrigation. The controlled anaerobic digestion in a closed digester with biogas collection has been stablished as a proper way of managing animal wastes. Temperature control by heating might be present.

b) Composition of dairy farm wastewater

Dairy farm wastewater composition is high in solids, nutrients, organic matter and living microorganisms including pathogens (MGAP, 2008). Moreover it is relatively rich in nitrogen from urea and proteins, being suited for production of high concentrations of ammonia when

digested. In addition, cattle manure is characterized for high resistant fibre content of lignocellulosic nature which may hinder conversion (Cantu, 2014).

As already mentioned wastewater contains cow manure which was characterized by Labatut et al. (2011), results being presented in Table 2-7 and Table 2-8.

Table 2-7 Oxygen demand and solid characterization of cow manure (Labatut, et al., 2011)

Sample	BOD (g/kg)	COD (g/kg)	TS (g/kg)	VS (g/kg)	BOD/COD	VS/TS	VS/COD
Raw dairy manure	45.8	128.9	124.0	102.1	0.36	0.82	0.79
Manure separated liquid	33.2	71.0	57.5	40.5	0.47	0.71	0.57

Table 2-8 Chemical composition (% VS basis) of cow manure (Labatut, Angenent and Scott, 2011)

Sample	VFA	Protein	Lipids	Hemicelluloses	Cellulose	Lignin	Sugars, starch, pectin	Total
Raw dairy manure	3.5	5.7	16.1	9.6	32.6	13.8	16.5	97.8

Antibiotics are also commonly found in animal wastes due to the fact that most of them are designed to be quickly excreted from the animal to prevent resistance formation in microbial populations (Manyi-Loh, Mamphweli, Meyer, Okoh, Makaka and Simon, 2013). However, wastes from milking cows vary in chemical composition and physical characteristics due to differences in diet, stage of growth and the waste collection and storage systems.

c) Pretreatment

Due to high solids content that can deteriorate digester performance, including pretreatment is preferred. Screens and/or sedimentation chambers separate the solid fraction which can be managed more efficiently by other means.

d) Operational parameters

Livestock wastes are rich in nitrogen and produce alkalinity when degraded. However an increase in the OLR with the corresponding HRT decrease can result in accumulation of VFA and pH drop. In those cases if pH is not chemically adjusted there is a risk of killing methanogenic archaea.

Mesophilic anaerobic digestion is preferred to digest animal waste for many reasons. One of the reasons is the high protein content of the animal waste, which when thermophilically digested results in higher free ammonia concentration. Another important reason is the lower energy requirement of mesophilic digestion compared to thermophilic digestions.

Dairy farm digesters may have different configurations. If the solid content is high a continuously stirred tank reactors (CSTR) are preferred. If it is low fixed film digesters (with inert support for biofilm formation) can be considered. CSTR and plug-flow reactors for animal manure typically use 20-30 days HRT, while fixed film reactors usually need some hours to few days. Covered lagoons require higher times, up to 60 days (Manyi-Loh, Mamphweli, Meyer, Okoh, Makaka and Simon, 2013).

e) Previous results of reference

Theoretical methane yield from dairy cattle manure was estimated as 469 mL/gVS by (Møller, et al., 2004). Pham et al. performed ultimate biogas and BMP tests from cow manure with 3 different batch fermentation processes, namely Møller, Hansen and VDI (which stands for Association of German Engineers). Ultimate biogas and BMP values of 247 mL_{biogas}/gVS and 170 mL_{CH4}/gVS, respectively, were reported. CH_4 content of biogas was 69%.

Labatut et al. (2011) evaluated the specific methane yield (SMY, volume of methane produced by gram of organic matter consumed) of 47 individual manure samples collected from 6 different dairy farms at various periods of the year. The average SMY at mesophilic conditions was 243±60 mL CH_4/gVS_{added} and the range distribution was 127-239 mL CH_4/gVS_{added}.

The International Plant Protection Convention (IPPC) in the Guidelines for National Greenhouse Gas Inventories: Reference Manual (IPCC, 1997) includes the overall average distribution of BMP results which is 240 mL_{CH4}/gVS_{added}. This is similar to the 241 mL CH_4/gVS_{added} determined by Labatut, et al. (2011) using four CSTRs and three different HRTs (Labatut, Angenent and Scott, 2011).

A BMP production curve for a 40-day experiment was performed and is presented in Figure 2-17. It can be seen that dairy manure is a slowly-degradable substrate mainly attributed to the approximately 60% lignocellulosic composition (Table 2-9). After 25 days, the biogas production rate approaches zero, which indicates its biochemical methane potential.

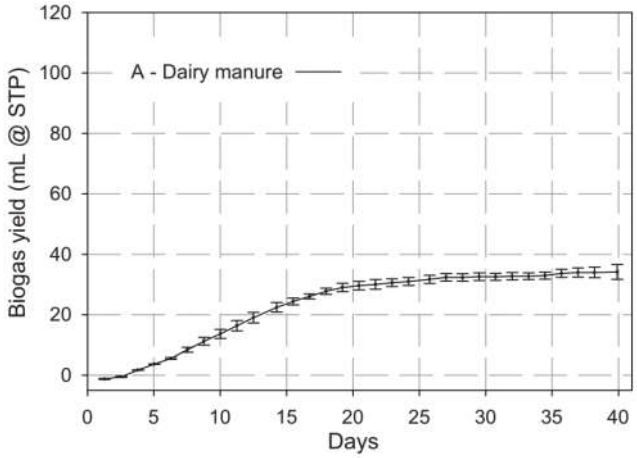


Figure 2-17 BMP assay curve of dairy manure; error bars represent replicates standard deviation (Labatut, et al., 2011)

Another article studying different volatile solid loads (VSL) in anaerobic digestion of manures concluded that operating the digester at low VSL results in better performance due to lower lag phase and higher hydrolysis rate (Yang, et al., 2016). The best results were obtained with 8 gVS/L.

Finally MSc Guillermo Zinola finished his thesis in 2016 in which Uruguayan dairy farm wastewater was anaerobically co-digested with glycerol for carbon balancing Co-digestion together with sludge recirculation enhanced biogas production rate by 87% (Zinola, 2016).

In stirred batch anaerobic digestion processes, using 1 L of reaction mixture at 30 °C, Zinola assessed the anaerobic biodegradability of dairy farm effluent and mixture of effluent and glycerol. For the first substrate 1500 mL of cumulative biogas volume after 15 days and a production rate of 140 mL biogas/gVS was obtained. For the mixture with glycerol, the cumulative biogas volume was 2397 mL and 240 mL/gVS of biogas production rate.

2.4.7. Anaerobic digestion of microalgae

a) Introduction

Biomass is a renewable source for energy production by processes like direct burning, anaerobic digestion, or production of biofuel. Anaerobic digestion of photosynthetic organisms can produce biogas from organic matter which was produced using sun light. Microalgae cultivation and further digestion is a new promising competitor to typical energetic crops.

The main advantages of microalgae cultivation are the possibility of using land that cannot be used for traditional agriculture and the possibility of constructing compact systems. Other advantages are high photosynthetic yield, fast growth and resistance to various types of contamination (Marcin Dębowski, 2013). Moreover, microalgae can be harvested from natural sources like eutrophicated and/or degraded water bodies with the benefit of removing nutrients from the aquatic system.

By applying pretreatment techniques and co-digestion processes together with suitable reactor configurations and operational strategies higher methane yields may be obtained. In 2015 it was established that the process of coupling anaerobic digestion with microalgae cultivation systems (in photobioreactors for example) that could establish complete utilization of all biomass components should be further explored (Tijani, Abdullah and Yuzir, 2015).

b) Composition of microalgae and effect on biogas production

Composition of microalgae biomass varies between species, but generally high content of proteins and lipids are found (Cantu, 2014). Table 2-9 presents the main macromolecular constituents of microalgae. The microalgae average composition is described as $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Tijani, Abdullah and Yuzir, 2015). Freshwater microalgal biomass contains low levels of sulphurated amino acids, therefore anaerobic digestion releases lower amounts of H_2S than other types of substrates (A. J. Ward, 2014)

Table 2-9 Fractions of macromolecules in microalgae cells (Tijani, Abdullah and Yuzir, 2015)

Macromolecular constituent	Function(s)	% of biomass
Proteins	Structure and metabolism	40-60%
Lipids	Structure and energetic reservoir	5-60%
Carbohydrates	Structure and energetic reservoir	8-30%
Nucleic acids	Genetic functions and cellular replication	5-10%

Macromolecular composition of microalgae is however strongly influenced by substrate feed and growth systems. For example it has been noticed that nitrogen starvation, within the viable limits, tend to favour lipid accumulation in detriment of carbohydrates (Tijani, Abdullah and Yuzir, 2015).

The ideal microalgae specie for maximum biogas production would be characterized for the following features: carbohydrate-based thin cell wall or absence of cell wall, large cytoplasm content, high growth rate in wastewater and high resistivity against toxic compounds. (Tijani, Abdullah and Yuzir, 2015). Angelidaki and Sanders calculated the latent methane productivity based on the carbohydrates/proteins/lipids composition of different microalgae biomass (Figure 2-18).

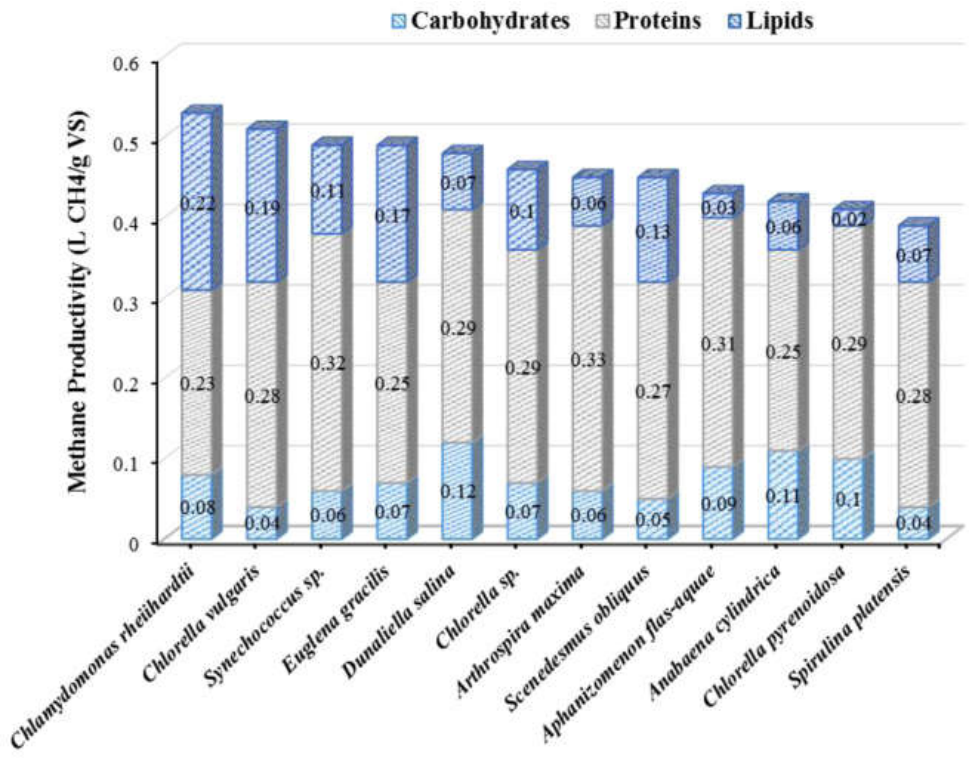


Figure 2-18 Latent methane productivity from the carbohydrates/proteins/lipids composition of different microalgae biomass, from (Tijani, Abdullah and Yuzir, 2015)

c) Pretreatment

Pretreatment steps that target cell lysis can enhance biogas production by releasing readily assimilable substrate from the interior of the cell. Pretreatment methods can be classified as mechanical (homogenization, autoclaving and sonication), physical (thermal hydrolysis), chemical (acid or alkaline treatment/ozonisation and enzyme addition) and biological (spontaneous fermentation).

Most of pretreatment research was conducted on eukaryotic microalgae due to the need to overcome cell wall hindrances in anaerobic digestion. When algae *Nanochloropsis oculata* slurry was thermally pretreated in an oven at different temperatures and exposure times (Marsolek, et al., 2014), the best results were obtained at 90 °C for 40 min. Nevertheless, after economic considerations, no pretreatment was the preferred alternative because energy required for heating was more than gained in enhanced biogas.

d) Challenges of microalgae anaerobic digestion

Ward et al. (2014) highlighted four problems usually found in anaerobic digestion of microalgae. The first is a low concentration of digestible substrate, or in other words a diluted feed. Therefore concentrating or harvesting microalgae biomass is a key step, particularly from the financial viability point of view.

To overcome this issue some actions may be taken, such as: incorporating a concentration steps (e.g. gravity settling, centrifuge), providing downstream settling tanks with sludge recirculation into the digester, designing more efficient anaerobic digesters (e.g. membrane reactors) and adding chemical coagulation followed by flocculation and centrifugation.

The second challenge is the cell wall degradability. The ability of microalgae to pass through an anaerobic digester and exit undigested has been demonstrated (A. J. Ward, 2014). Researchers found intact microalgae cells in digestate exiting a reactor operated at 30 d HRT. Higher biogas production were observed from microalgal species provided with either no cell wall or with a proteic cell wall, compared to microalgal species that had a carbonate-based cell wall (i.e. hemicellulose). Even lower biogas production were observed when using species with rigid cell walls. This is why pretreatment steps are preferred when digesting eukaryotic microalgae.

The third issue identified by Ward et al. (2014) is the C/N ratio, which was already covered in section 2.4.1.e. Finally, lipids may become a challenge for microalgae anaerobic digestion. Theoretically lipids have a higher methane production potential compared to proteins and carbohydrates. However lipids have low alkalinity and buffering capacity. In addition, anaerobic digestion of lipids produce intermediate products such as long chain fatty acids (LCFAs) and volatile fatty acids (VFAs) that can inhibit anaerobic bacteria.

It was reported that lipid concentrations of 5, 10 and 18% caused no inhibition, but concentrations of 31, 40 and 47% caused inhibition (A. J. Ward, 2014). This has consequences in microalgae cultivation establishments which, despite lipid high methane potential, may decide to extract lipids from microalgae to process separately

2.4.8. Anaerobic digestion of cyanobacteria

a) Introduction

Microalgae includes eukaryotic organisms, while cyanobacteria are a type of bacteria, prokaryotic organisms. The presence of a nucleus confining DNA might be regarded as the main difference between them but, however, for digestion purposes the main difference is the existence a cell wall, which gives extra resistance to hydrolysis by exoenzymes.

Methane yields from microalgae and cyanobacteria depends on their chemical composition. Thus, it is difficult to generalise about biogas potential of microalgae as a whole and studies over specific local conditions and species should be conducted. Nevertheless, differences between degradability of different species is attributed mainly to the presence and composition of the cell wall (Marcin Dębowski, 2013).

Considering the previous cyanobacteria have an advantage for anaerobic digestion over eukaryotic microalgae due to the lack of a cell wall. Nevertheless, cyanobacteria have a disadvantage over eukaryotic microalgae in the production of toxic compounds. It is not exactly clear in what extent anaerobic bacterial community is affected by cyanotoxins.

b) Previous results of reference

Dębowski, et al. (2013) reviewed the use of microalgae and cyanobacteria in processes of anaerobic digestion. Contrary to what it could be expected, studies comparing the anaerobic digestion of different species of microalgae and cyanobacteria did not reported a dependence between biogas production and taxonomic group. The biogas production ranged from 287 to 587 mL/gVS and methane production from 210 to 350 mL/gVS, with varying incubation periods, retention times, among others (Marcin Dębowski, 2013).

Another study compared batch laboratry anaerobic digestion tests of naturally occurring microalgae bloom from Taihu Lake naturally stored. Phytoplankton biomass was composed of both microalgae and cyanobacteria being the genera *Microcystis*, a cyanobacteria, predominant with 42.6% of biomass. Samples were stored in glasshouse at room temperature for 0, 7, 15, 30 and 60 days.

The storage led to cell death, microcystins release and volatile solids (VS) reduction by spontaneous fermentation. The best results were obtained at 15 days of storage, improving methane production and VS removal, which were 37% and 30% higher than those of fresh algae, respectively (Miao, Lu, Zhao, Huang, Ren, Yan and Ruan, 2013).

Microcystin anaerobic digestion was also studied by the previous authors. Anaerobic digestion process presented a high microcystins biodegradation efficiency, total removal rates ranged from 57% (fresh algae) to 81% (60 d of storage). Extracellular microcystins removal rates were considerable higher than intracellular, another proof of cell wall protection in anaerobic digestion.

Finally, Aramrueang et al. (2016) studied the effects of process parameters (HRT and OLR) on the digestion of cyanobacteria *Spirulina platensis*. In a CSTR operated at an OLR of 1.0 gVS/L/d and 25 days of HRT, biogas and methane yields were similar to batch tests, 502-514 mL/gVS and 342-370 mL_{CH4}/gVS, respectively (Aramrueang, et al., 2016). The authors recommended to increase OLR to 2.0 gVS/L/d and allowing a decrease in biogas and methane yields to 0.490 and 313 L/gVS, respectively, for economic considerations.

2.4.9. Anaerobic co-digestion of cyanobacteria with dairy farm wastewater

a) Introduction

Anaerobic co-digestion refers to digestion of many organic substrates simultaneously in one digester. Methane yield may be enhanced due to positive synergisms of the mixed materials, like bacterial diversity in different wastes and the supply of missing nutrients by the co-substrates (Manyi-Loh, Mamphweli, Meyer, Okoh, Makaka and Simon, 2013). In fact the process of co-digestion has been reported to exhibit steady correlation with biogas output.

Co-digesting animal wastes, microalgae, or any other N-rich substrate with C-rich co-substrate balances the C/N ratio and help to prevent inhibition from VFA and free ammonia (Tijani, Abdullah and Yuzir, 2015). It is not clear yet which is the ammonia threshold concentration that inhibits anaerobic digestion. (Manyi-Loh, Mamphweli, Meyer, Okoh, Makaka and Simon, 2013).

b) Previous results of reference

Panpong et al. (2015) evaluated a 64-day long batch anaerobic co-digestion tests of cannery seafood wastewater (WW) with almost pure (>99%) cyanobacteria Microcystis SP (CB), with and without glycerol waste (GW). The main results are presented in Table 2-10.

Anaerobic co-digestion of WW+GW+CB (94:1:5, v/v) increased biogas production by 14 mL_{CH4}/gVS, compared to anaerobic digestion of pure WW. One percent glycerol addition shifts C/N ratio from 9, which causes high release of total ammonia nitrogen, to 18, which is more balanced for anaerobic digestion.

Table 2-10 Batch biomethane production of co-digestion of CS with GW and CB (Panpong, et al., 2015)

Mixing ratio	CB/WW% (gVS/gVS)	C/N ratio	VS (g/L)	CH_4 yield (mL CH_4/gVS)	CH4 content (%)
WW (100%)	0%	11	7.76	278	59.0
GW (1%)	-	576	4.50	211	59.4
CB (5%)	100%	7	4.48	292	53.5
WW (94%) + GW (1%) + CB (5%)	3%	18	13.50	291	60.1
WW (89%) + GW (1%) + CB (10%)	6%	ND.	19.94	150	46.0
WW (84%) + GW (1%) + CB (15%)	10%	ND.	24.91	91	37.5
WW (95%) + CB (5%)	3%	9	12.74	192	46.8
WW (90%) + CB (10%)	6%	ND.	17.72	111	41.7
WW (85%) + CB (15%)	10%	ND.	22.69	81	34.6

Lake Taihu blue algae (mixture of cyanobacteria and microalgae, already presented in section 2.4.4.2) was also studied as co-substrate for anaerobic co-digestion (Miao, et al., 2014). Batch laboratory tests performed at 22 days of duration were carried at 35 °C, comparing two different inoculum: swine manure and anaerobic granular sludge from an external anaerobic digester.

Different inoculum/substrate ratios (ISR) were used and best results were achieved when swine manure mass (in terms of gVS) doubled blue algae mass (ISR 2.0). This led to an improved CH_4 yield of 213 mL/gVS, compared to 73.5 mL/gVS from digestion of blue algae with granular sludge (ISR 3.0).

Process parameters (pH, TAN, VFAs, enzyme variation) corroborated appropriate stability of process, suggesting anaerobic co-digestion of cyanobacteria with animal waste is a promising technology for both solid waste treatment and renewable-energy production (Miao, Wang, Zhao, Huang, Ren, Yan and Ruan, 2014).

Finally, another studied corroborated a positive impact of adding cyanobacteria (*Spirulina*) to cattle manure, in batch reactors at 35 °C. The experiment were conducted during 92 days (Cantu, 2014). Both pretreatment strategies and carbon balancing show to have a positive influence on the digestion process. Best results were found with thermally pretreated cyanobacteria (at 100 °C, 3 hours) with a C/N ratio of 20:1. Actually, co-digestion of non pretreated cyanobacteria resulted in worse performance compared to the control mixture (without cyanobacteria). Nevertheless, non-treated cyanobacteria showed higher CH_4/CO_2 ratio.

2.5. Cyanobacteria for treating wastewater

2.5.1. Introduction

Microalgae are nowadays considered a solution in wastewater treatment (Z. Arbib, 2015). They can grow based on sun light and nutrients, in shallow reactors with high light exposition called photobioreactors. Moreover photosynthetically grown microalgae grows with less rigid cell walls make phototrophic cultivation as the most sustainable approach for integration with biogas production systems (Tijani, Abdullah and Yuzir, 2015).

Biomass productivity of these systems is significantly lower than in heterotrophic microalgae systems, basically due to reduced cell growth rate and limitations in the gas-liquid mass transfer of CO_2. Nevertheless, phototrophic cultivation is still the most used approach for microalgae growth due to its simple reactor set up, easy scaling-up and reduced operational costs (Tijani, Abdullah and Yuzir, 2015).

When photobioreactors with photosynthetic microalgae are fed wastewater, heterotrophic bacteria grow based on the organic matter present in the wastewater. The co-existence of heterotrophs and phototrophs may generates positive synergisms as follows:

Heterotrophs need O_2 to grow based on the consumption of complex organic compounds and nutrients; when oxidising organic matter heterotrophs produce CO_2 and organic by products like vitamins. Phototrophs generate O_2 using energy from sun light while consuming nutrients, vitamins and CO_2 as a carbon source for growth.

Overall, this consortium consumes organic matter and it assimilates nitrogen and phosphorus delivering potentially high quality effluents low in COD, N and P. Moreover high energy

biomass (photoactivated sludge, a mix of bacteria and microalgae) is produced. This biomass can be in turn converted into biogas through digestion or processed to make organic fertilizer.

Finally, as CO_2 is the carbon source in photosynthetic organisms it might be a growth limiting factor. This is due to limitations in CO_2 transfer from the atmosphere to water and from low C/N ratios of sewage effluents. Therefore an external CO_2 supply with pH control might enhance productivity up to 100% (Tijani, Abdullah and Yuzir, 2015).

2.5.2. Integration with anaerobic digestion

Microalgae photobioreactors can be very efficient in biogas purification, due to microalgae cell inherent ability to screen for CO_2 in the flue gases. This represents one advantage of coupling photobioreactors with anaerobic digestion. However, microalgae inability to absorb other volatile gases than CO_2 may necessitate the application of extra purification steps of the biogas after microalgae filtration in industrial applications (Tijani, Abdullah and Yuzir, 2015).

Coupling anaerobic reactors with photobioreactors can be done in several ways. Photobioreactor may be placed upstream anaerobic digester, downstream, or even both. Depending in what is the purpose of the system what configuration is preferred.

a) Enhanced biogas production

If the goal of the photobioreactor is to produce photoactivated biomass for biogas production, then anaerobic digester is placed downstream the photobioreactor. The microalgae/bacteria biomass is cultivated with raw (filtered) wastewater or a synthetic medium and the photobioreactor liquor discharges into the digester.

b) Microalgae/bacterial wastewater treatment: photoactivated sludge

When wastewater treatment is the goal of the system or plant, photobioreactors are used as polishing steps of the effluent of a first anaerobic reactor. In this case, after a biomass concentration strategy (i.e. harvesting plant), photoactivated sludge is not recirculated back to the anaerobic reactor and instead it is put apart for further processing or disposing. This is done in order to not reintroduce nutrients again to the system, which would deteriorate overall nutrient removal efficiency from wastewater.

Photobioreactors with microalgae and bacteria consortium have great capability for complete wastewater treatment (in terms of COD, TN, TP and TSS), being able to reach wastewater quality levels that comply the European standard 91/271/EEC (Z. Arbib, 2015).

Harvested photoactivated sludge can be also converted into biogas. In this case the system would consist in a digester-photobioreactor-digester configuration which is also possible. This incorporation of anaerobic processes with microalgae wastewater treatment was studied by Z. Arbib et al. (2015) and they found a positive net energy generation (0.5 kWh/m^3). They claimed that “this approach changes the perspective of wastewater treatment to create a new concept of sustainability based on microalgae”.

Photobioreactors are suitable to follow an anaerobic digester instead of receiving raw wastewater for the following reasons: in the digestion process turbidity and suspended solids are reduced, which is good for light penetration and photosynthetic microorganism growth. Moreover, in anaerobic digestion soluble nutrients are released after hydrolysis of organic nitrogen and phosphorus, which can be easily assimilated by microalgae.

Nevertheless there are complications anaerobic effluent may cause to photobioreactors. Table 2-11 presents a list of those adverse effects. Mitigation alternatives to overcome these inhibitory effects are as follows: dilution of anaerobic feed, increasing CO_2 supplement to balance pH and ammonia concentration, and frequent biomass harvest (lower SRT) to avoid high microalgae concentrations (Tijani, Abdullah and Yuzir, 2015).

Table 2-11 Inhibitory features of anaerobic effluent on microalgae cultivation, from (Tijani, Abdullah and Yuzir, 2015)

Anaerobic effluent characteristic	Inhibitory effect(s) on microalgae cultivation
Anaerobic microorganisms community	Viable to disrupt the ecological organization of microalgae cells due to competition in nutrients and the sterility of the culture media is disrupted
Flocculants	Mutual shading, coagulation and clogging effects resulting in biomass sedimentation, mixing problems, and nutrient inaccessibility
Turbidity	Partial immersion of light resulting in shading effects
Nitrogen concentration	Toxicity of free ammonia
Volatile fatty acids concentration	Promotes the proliferation of heterotrophic microorganisms and thus affects sterility of the culture media significantly; long-chain ($>C_{14}$) fatty acids can be inhibitory for microalgae growth
Heavy metals	Cellular toxicity, disrupts the membranes transfusion and may lead to osmolysis
Organic trace elements	Latent cellular toxicity

2.5.3. Factors influencing microalgae cultivation

Microalgae is known for having a high growth rate compared to other biomass, with average production estimated in 19-25 g dry matter/m^2/d in common raceway pond (Cantu, 2014). Cultivation of microalgae in a bioengineered system such as a photobioreactor is governed by several parameters including: light intensity, nutrient composition, temperature and gaseous exchange rate.

The most important parameter in a photobioreactor is light availability, the central energy source of photosynthetic microalgae, which regulates oxygenation potential and biomass production rates (Z. Arbib, 2015). Shorter light intensities result in lower microalgal growth rates; however, too much light intensity may result in photo-inhibition that is a point where light receptors in the chloroplasts are damaged and photosynthesis activity is hindered. (Tijani,

Abdullah and Yuzir, 2015). In locations with seasonal solar radiation variation, shorter HRTs are applied in summer and longer in winter to regulate dilution rate and light availability.

Mutual shading is a common growth-restraining factor and involves a decreased light intensity in deeper waters of high cell density reactors. Shallow solar reactors or transparent tubular reactors may be designed to overcome this issue.

As with every biological process, temperature is a key factor that may limit microalgae productivity. Increasing temperature boost microalgae growth exponentially, within an optimum range of 20 °C to 35 °C. (Tijani, Abdullah and Yuzir, 2015)

Regarding nutrient demand, microalgae need macronutrients (N, P, K, Ca, S, Mg), vitamins and trace elements to attain high biomass productivity. Nitrogen and phosphorus relation 16:1 (N:P) is usually considered as optimum for microalgae growth. Trace elements that have been reported to improve microalgae growth are chelated salts of Fe, Ni, Mn, Se, Co and Zn.

Moreover microalgae consume CO_2 during photosynthesis and it has been reported to remove CO_2 from flue gas more efficiently than other lithotrophs. Carbon supplemented in the form of CO_2 accounts for approximately 50% of the biomass dry weight. Moreover, when O_2 is released during photosynthesis photo-oxidative damage can occur due to high O_2 concentrations. Pumping a CO_2 flux over the culture media helps to re-carbonate the media, control pH, and remove the extra O_2.

2.5.4. Photobioreactor design

There are a wide array of design options for photobioreactors. First microalgal mass production technologies can be classified in open and closed culture systems. The first type are mostly located outdoors, relying on natural sun light for photosynthesis activity. Closed photobioreactors may be located either indoor (with an artificial light source), or outdoor (with only solar light) or using both solar light and artificial light. The main design criteria include light availability, surface to volume ratio, gas exchange, orientation and inclination (Ojamäe, 2011).

Photobioreactors can be flat or tubular. Then, different positions are possible: horizontal, inclined, vertical or spiral. Finally it can be manifold or serpentine (Ojamäe, 2011). Typical photobioreactor designs are presented. The probably most used configuration, the so called high rate algae ponds (HRAP), are shown on Figure 2-19. Tubular reactors and flat plate reactors are presented respectively in Figure 2-20 and 2-21. Finally Figure 2-22 presents an interesting configuration for laboratory scale: the transparent bags.

Figure 2-19 Pilot scale (left) and prototype scale (right) of HRAPs, from (Z. Arbib, 2015)

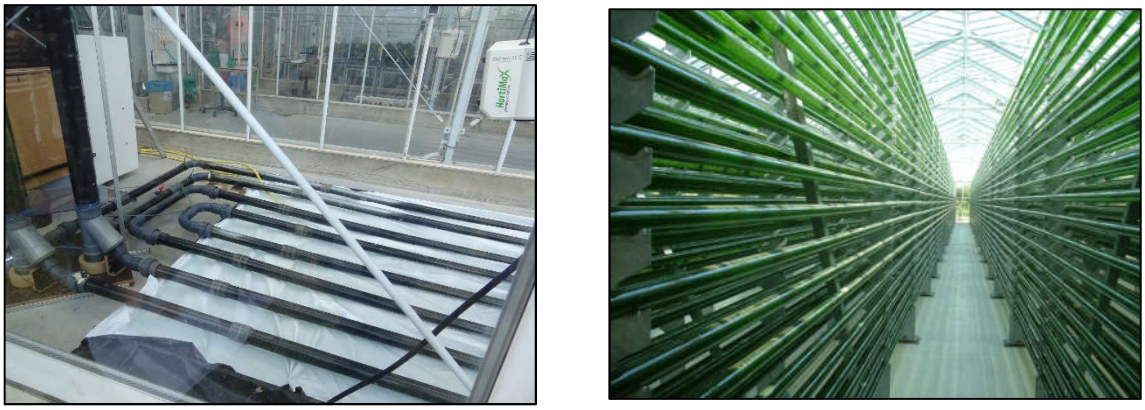


Figure 2-20 Tubular reactors. Left: pilot scale (own picture). Right: prototype scale from (Ojamäe, 2011)

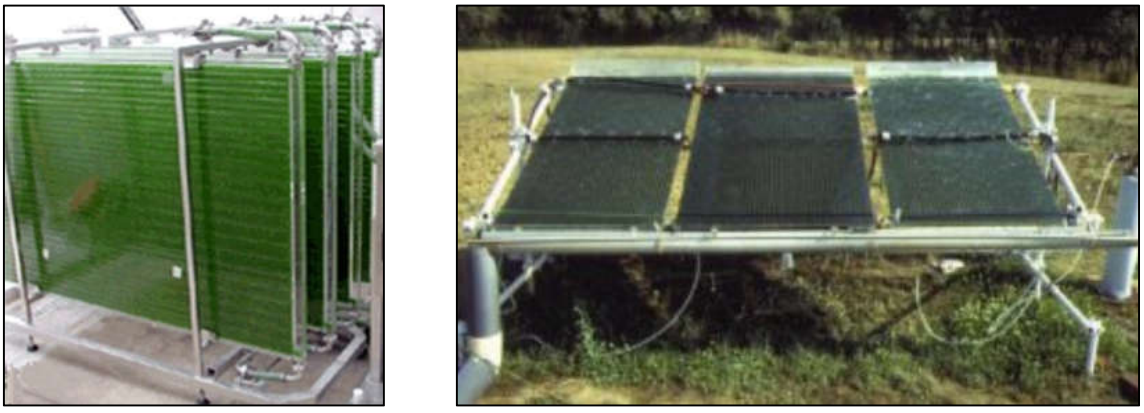


Figure 2-21 Flat plate photobioreactors, from (Ojamäe, 2011)

Figure 2-22 Lab scale transparent plastic bags, from Google Images

2.5.5. Harvesting step

Downstream the photobioreactor it may be necessary to install a solid-liquid separation step sometimes done in the so called harvesting plants. In this process suspended particles (cells) are removed and thus a clearer water is produced together with biomass concentrate. In some situations this process is crucial and can determine the economic feasibility of the overall process.

The settleability and other characteristics of the sludge determine is the harvesting strategy to incorporate. A normal settler (or clarifier or sedimentation tank) can be used with microalgal sludge of good settleability. If settleability is poor or microalgae tend to float, a dissolved air flotation (DAF) unit may be an efficient alternative.

Other alternatives are flocculation (chemo-flocculation, electro-flocculation or bio-flocculation), filtration or centrifugation (Tijani, Abdullah and Yuzir, 2015). Finally, microalgae may be harvested directly from the photobioreactor surface if it is an open reactor.

A portion of the harvested sludge may be recirculated to the photobioreactor in order to decoupling the hydraulic time with the solids time. This favours the growth of the active microorganism community and thus prevents washout. The rest of the photoactivated sludge, due to its nutrient content, may be further processed to incorporate in organic fertilizers,

2.5.6. Previous results of reference

Ruiz-Martinez et al. (2012) investigated the removal of N and P in semi-continuous laboratory-scale photobioreactor from an anaerobic membrane bioreactor effluent. Solids retention time (SRT) was 2 days and CO_2 addition was applied for pH control. Temperature ranged from 28-32 °C. The main microbial group in the microalgae community was eukaryotic microalgae Chlorococccales (an order from the Clorophyceae class), together with cyanobacteria.

Despite variations on the performance of anaerobic reactor, anaerobic effluent proved to be a suitable growth medium for microalgae. With an input of around 60 mg N/L and 8 mg P/L, mean biomass productivity was 234 mg/L/d and nutrient removal efficiency was good: 67% for NH_4^+-N and 98% for PO_4^{-3}-P. The authors stated that the use of this technology for domestic wastewater treatment could spread (Ruiz-Martinez, et al., 2012)

Hernández et al. (2013) used a treated liquid fraction of pig manure to feed a 5 L photobioreactor inoculated with microalgae *Chlorella sorokiniana* and aerobic bacteria. Reactor was operated at 24 ± 2.7 °C and 12 h per day of light supply. Biomass growth was 26.3 mg dry weigh/L/d. Removal efficiencies were 62% for COD, 83% for NH_4^+-N and 58% for soluble P.

In addition, batch anaerobic digestion tests of microalgae-bacterial biomass indicated that methane yield was determined by lipid content and by substrate/inoculum ratio. Best conditions delivered 518 $mLCH_4/gCOD_{added}$ were when using biomass with a lipid content of 30% and a substrate/inoculum ratio of 0.5 (Hernandez, et al., 2013).

Yang et al. (2016) reviewed a research in algae cultivation that obtained 0.95 and 0.67 mg/L/d of maximum removal rates for nitrogen and phosphorus, respectively, from anaerobic digester effluent of dairy wastewaters. (Yang, Ju, Li, Liu, Wang and Chang, 2016)

The project "ENERGY.2010.3.4-1: Bio-fuels from algae" tried to demonstrate on large scale the feasibility of integrating low cost microalgae cultures in wastewater treatment (Z. Arbib, 2015). Microalgae and bacteria consortium showed great capacity for complete wastewater treatment, reducing TCOD, TN, TP and TSS below European directive 91/271/EEC limits. Photobioreactor was tested both with raw screened wastewater and with effluent from UASB.

Microalgae-bacteria biomass was subject to mesophilic anaerobically digestion, giving a biomethane production of 173 mL CH_4/gVS. In that study, coupling anaerobic processes with microalgae wastewater treatment (UASB followed by photobioreactor followed by digester for harvested biomass) led to theoretical positive net energy balance of 0.5 kWh/m^3 wastewater (Z. Arbib, 2015).

CHAPTER 3

Methodology

3.1. Introduction

The methodology consisted both in performing monitoring and sampling campaigns at the CRS dairy farm, and in conducting biochemical methane potential (BMP) batch tests at LATU. In consequence thesis work consisted in desktop work, field work and laboratory work.

The desktop work involved data analysis, literature review and report writing. The field work consisted in conducting two monitoring campaigns (carried out in October and December), and two collection campaigns (one of them performed simultaneously with the second monitoring campaign in December 2016, while the other in February 2017). At the laboratory both the BMP tests were carried out, as well as some analytical determinations.

The thesis work can be organized in two components: (i) one focused on the dairy farm (wastewater treatment system assessment); and (ii) the other with the activities held and the results obtained at the laboratory (biogas potential tests).

3.2. Wastewater treatment system assessment

3.2.1. Specific objective

To analyse the wastewater production in the CRS dairy farm including a performance assessment of the existing wastewater treatment system.

3.2.2. Materials and equipment

Composite samples were taken using an automated water sampler (ISCO, Avalanche), whereas grab samples were taken with swing sampler (Figures 3-1). Plastic bottles were used to store samples in every case; however samples for microbiological analysis were collected in opaque amber glass bottles. Both Lugol solution and formaldehyde were used to preserve the samples for phytoplankton analysis. A plastic cooler with ice was employed for sample preservation during transportation.


Figures 3-1 Sampling the tajamar with swing sampler (left) and sampling the influent with automatic sampler (right)

3.2.3. Methodology

The first step was to gather all the available information about the CRS dairy farm including the milking parlour's wastewater treatment system. Contacts with the coordinator of the CRS (Mr. Ruben Jacques Martins) were established in order to get basic information of the facilities. The Google Earth® software was used to study the lay out of the system (Figure 3-2).

Afterwards, several visits to the CRS were conducted in order to observe the wastewater treatment system at the site. With all the above information the following four monitoring points were selected:

1. At the gutter that receives the wastewater after the solids removal chamber (that is, the influent to the biological system).
2. Anaerobic pond (after first biological treatment step).
3. Facultative pond (after second biological treatment step, that is, the effluent of the wastewater treatment system).
4. *Tajamar* (artificial shallow lagoon which is the receiving water body of the treated effluent).

Figure 3-2 Elements of the milking parlour wastewater treatment system (Source: Google Earth®)

Wastewater at the milking parlour is produced two times a day, each time over a period of 3 hours. In addition, the composition of wastewater varies in time due to the nature of the operation of the milking parlour. In consequence composite samples were taken to monitor the influent to the biological system.

In each opportunity, the sampling device was set to take 330 mL samples every 5 minutes over periods of 2 to 2.5 hours, depending that day specific cleaning operation. The two treatment ponds and the *tajamar* were assumed to be completely mixed, therefore grab samples were taken from those water bodies.

Two monitoring campaigns were held, the first one on 24/10/2016 (middle spring) and the second one on 14/12/2016 (late spring/beginning of summer). During the first campaign nine parameters were analysed in the laboratory as follows: dissolved oxygen (DO), electric conductivity (EC), chemical oxygen demand (COD), 5-day biological oxygen demand (BOD_5), total nitrogen (NT), faecal coliforms (FC), microcystin-LR, saxitoxin and phytoplankton.

The second campaign pretended to be more thorough, so 11 parameters were added to the already mentioned parameters as follows: total solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS), microfiltered/soluble COD (COD_{mf} or SCOD), ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), soluble phosphorus (SP) and total organic carbon (TOC).

For the collection and preservation of the samples the Standard Methods for the Examination of Water and Wastewater, 22nd Edition (APHA, 2012) were followed. Based on results of monitoring campaigns a critical assessment of the wastewater treatment system was done including suggestions for re-designing the treatment ponds.

3.3. Biogas potential determination

3.3.1. Specific objective

To evaluate the mesophilic anaerobic co-digestion of dairy farm wastewater with natural occurring cyanobacteria.

3.3.2. Materials and equipment

Cyanobacteria (CB) were collected using a 0.25 μm pore opening phytoplankton net (Figure 3-3). Composite wastewater (WW) samples were collected using an automatic sampler as described in section 3.2.3. Sludge from the anaerobic pond of the wastewater treatment system was collected using a swing sampler and served as the anaerobic inoculum (AI).

Figure 3-3 Phytoplankton net of 0.25 μm of pore opening.

Figure 3-4 presents a photograph showing the biological materials used in this research. The microcrystalline cellulose (CE, CAS 9004-34-6) for positive control was obtained from the Chair of Pharmacotechnics of the Faculty of Chemistry of the UdelaR, while glycerine (GL) was bought from a local drugstore (Figures 3-5).

Figure 3-4 Biological materials, from left to right: anaerobic inoculum, cyanobacteria, wastewater

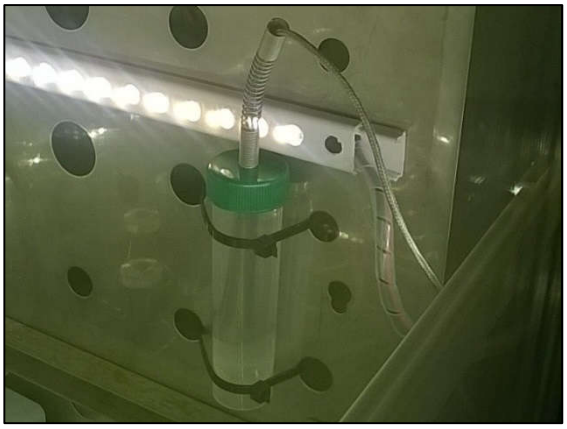
Figures 3-5 Chemical materials. Left: Glycerine. Right:Microcrystalline cellulose

The batch tests were carried out in opaque amber glass bottles of 500 mL sealed with butyl rubber septum and an aluminium cap of 30 mm of diameter (Figure 3-6). A crimper machine was borrowed from local a firm Dilvan to mechanically seal the aluminium caps (left in Figures 3-7).

Figure 3-6 Left: Glass opaque ambar 500 mL bottles. Right:30 mm aluminium cap and butyl rubber seal

An environmental chamber (Forma Scientific) was used to control the temperature at 30 °C (right in Figures 3-7). The actual temperature in the chamber was registered by a PCIM temperature and humidity control system (Figures 3-8).

Figures 3-7 Left: Crimper machine. Right:Environmental chamber for temperature control.



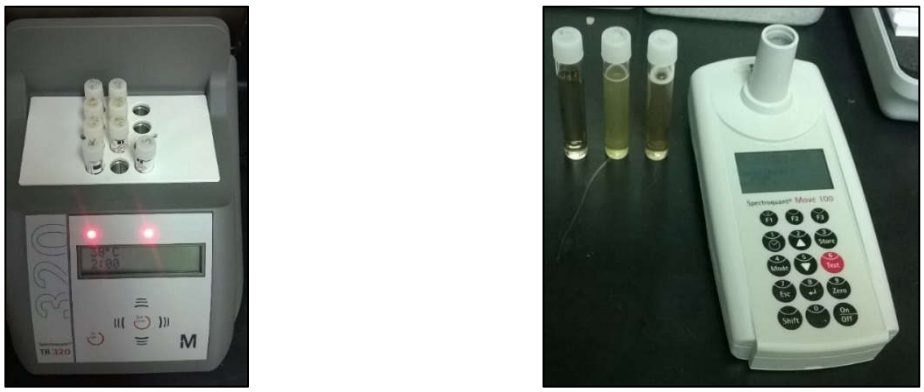
Figures 3-8 PCIM temperature and humidity control system

The biogas measuring device consisted in an inverted graduated glass cylinder and a plastic tube with needles in both ends connecting the inverted graduated cylinder with the sealed bottled as shown in Figures 3-10. The volume of the plastic tube was 13 mL.

The measuring device was used with distilled water in the first round of BMP tests, and with a saturated NaCl solution in the second round of BMP tests. For the wet chemistry analysis of biogas, a basic NaOH solution was used; thymol blue was employed as a colour indicator to assure alkaline conditions (pH > 9.6).

Figures 3-9 Biogas measuring system employed.

A Spectroquant® TR 320 and a Move 100 were used for thermal digestion and spectrophotometric analysis of COD, TN and TP (Figures 3-11) using Spectroquant® Cell Test Kits. For solids analysis an oven at 105 °C and a muffle furnace at 550 °C were used for getting TS and IS/VS respectively. An analytical scale was provided for weighting the samples. The pH was measured with a pH-meter probe.



Figures 3-10 Spectroquant® TR 320 and Move 100

Chemical reagents used during this research included thymol blue indicator (Merck®), hydrochloric acid fuming 37% (Merck®), sodium hydroxide pellets (Merck®), sodium chloride (domestic use bought in a local shop) and distilled water (produced at LATU facilities).

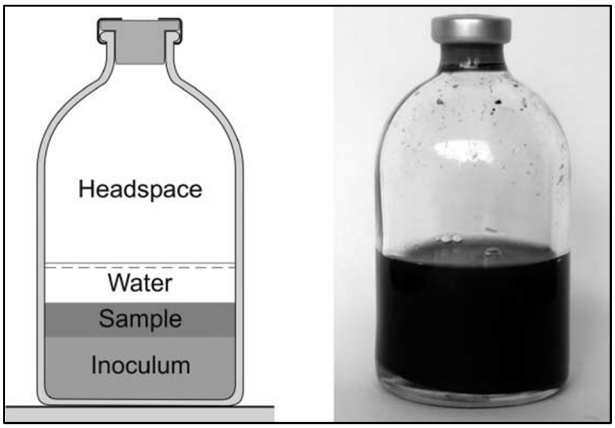
3.3.3. Methodology

a) Experimental design

Batch experiments were conducted as BMP tests. There are many possibilities when designing a BMP tests; literature about that is abundant. Therefore, it was decided to take the following recent publication "Towards a standardization of biomethane potential tests" (Holliger, 2016) as main reference for designing the experiment.

The main reason for basing the work on that publication is its credibility and standardisation approach. It was the outcome consensus of a roundtable discussion held by forty "BMP experts" on June 2015, supported by the Anaerobic Digestion Specialist Group of the International Water Association (IWA).

Optimal biogas production conditions were sought in the relation amount of inoculum/cyanobacteria/wastewater/glycerine. In each BMP round the amount of inoculum was maintained the same within each bottle, while the quantities of the substrates changed from triplicate to triplicate. Figure 3-11 schematises how the BMP vessels were filled.



Figures 3-11 Schematic representation of prepared BMP vessels (Angelidaki, 2009)

The variables evaluated in this research were the following:

- Proportion in the co-digestion of cyanobacteria and wastewater (CB:WW in mg of VS)
- Inoculum-to-Substrate Ratio (ISR), also in mg of VS, meaning that ISR 2 has the double mass of inoculum than substrate
- Carbon to nitrogen ratio (C/N) of the substrate in mg TOC/mg TN

When studying CB:WW proportion and C/N ratio, an ISR of 3 was used. The day after material collection campaign, the VS content of each material was measured in order to estimate how many mL of WW and CB, and g of AI, were needed in the bottles to achieve the desired configurations.

BMP tests were conducted by triplicate. The C/N ratio was "balanced" with glycerine. In order to know how much GL needed to be added for C/N balancing at the desire ratio, TOC analysis was carried out for both the WW and CB samples. The glycerine TOC was calculated from the molecular formula of glycerine $C_3H_8O_3$ (39.1% of its weight as C).

Blank essays were conducted in every round of experiments without the addition of substrate; only the anaerobic inoculum and distilled water were added to blank controls. As stated by Holliger, et al. (2016), a positive control with microcrystalline cellulose was conducted in the second round of BMP test. Table 3-1 summarises the experiment design for the BMP tests. A more detailed description is presented below.

Table 3-1 Batch experiments configurations

Round	Experimental variables	Configurations	Bottles used
1	WW:CB proportion	100:0, 75:25, 50:50, 25:75, 0:100; blank	6*3=18
2	ISR and C/N ratio	ISR 2, 3 (also C/N 2.5) and 4; C/N 5 and 10; blank; positive control	7*3=21

It is important to mention that no buffer and/or nutrient solution was added in the BMP essays. This was decided, against what is recommended on the literature, in order to obtain real field results. The inclusion of any of those materials in wastewater treatment is unlikely to be done by dairy farmers due to the increasing treatment costs. Moreover complex biological materials like the ones used already contain nutrients and some buffer capacity.

Tables 3-2 and 3-3 presents the detailed configuration for BMP tests. In the first round of tests, the best proportion of the co-digestion of cyanobacteria and wastewater was studied. The second round pretended to be a confirmation step regarding the biogas potential of the 1:1 (wastewater:cyanobacteria in gVS) co-digestion; therefore, the other variables were studied in this second round such as ISR and C/N.

Table 3-2 Details of first round of BMP tests

Parameter	Unit	Blank	100% WW	25% CB	50%	75% CB	100% CB
Experiment variable							
ISR	-	∞	3	3	3	3	3
WW:CB	mgVS: mgVS	0:0	1:0	3:1	1:1	1:3	0:1
Substrate C/N	-	N.A.	2.0	2.2	2.4	2.8	3.4
Actual amounts							
Inoculum	mgVS	988	988	988	988	988	988
Cyanobacteria	mgVS	0	0	82	165	547	329
Wastewater	mgVS	0	329	247	165	82	0
Inoculum+ substrate	mgVS	988	1317	1317	1317	1317	1317
Distilled water	mL	225	153	116	79	42	5
Headspace	mL	250	250	250	250	250	250

As it can be seen the first round of tests resulted more concentrated (in terms of gVS per test) than the second round (988-1317 mgVS vs. 633-950 mgVS). Main reasons are explained as follows.

The cyanobacteria in the first round of experiments was more concentrated than in the second round (0.15% VS vs. 0.90% VS) however the cyanobacteria was less concentrate than the wastewater (0.46% VS in first round and 0.31% VS in second round). Therefore, in the first round, when different proportions between cyanobacteria and wastewater were tested, cyanobacteria was the limiting material in terms of volume and the one that set the amount of inoculum (which depended on ISR).

For example, in the first test 100% CB was composed of 220 mL of cyanobacteria (representing 329 mgVS with a 0.15% of VS) and 25 g of inoculum (containing 988 mgVS due to a VS% of 3.95%). Because 988 is three times 329 (approximately), ISR is three. 5 mL of distilled water f were added to leave a headspace of 250 mL. The other tests worked with the same amount of inoculum (25 g) and the same amount of substrate VS. The more wastewater in the configuration, the more distilled water to be added in order to leave the same headspace.

Table 3-3 Details of second round of BMP tests

Parameter	Unit	Blank	Positive	ISR2	ISR3	ISR4	CN5	CN10
Experiment variable								
ISR	-	∞	3	2	3	4	3	3
WW:CB	mgVS: mgVS	0:0	0:0	1:1	1:1	1:1	1:1	1:1
Substrate C/N	-	N.A.	∞	2.5	2.5	2.5	5.2	10.1
Actual amounts								
Inoculum	mgVS	633	633	633	633	633	633	633
Cyanobacteria	mgVS	0	0	158	106	79	49	26
Wastewater	mgVS	0	0	160	106	78	50	25
Cellulose	mgVS	0	213	0	0	0	0	0
Glycerol	mgVS	0	0	0	0	0	113	160
Inoculum+ substrate	mgVS	633	846	950	845	790	844	844
Distilled water	mL	239	239	0	79	120	165	200
Headspace	mL	250	250	250	250	250	250	250

In the second round of experiments cyanobacteria and wastewater were always mixed 1:1 (VS). The test that set the amount of inoculum for the others was the one with a higher amount of substrate with the limit of 250 mL of liquid. This test was the ISR2 test which contained no distilled water and 188 mL (158 mgVS) of harvested cyanobacteria, 51 mL (160 mgVS) of wastewater, 11 g (633 mgVS) of inoculum. In this round ISR=3 tests contained around 845 mgVS, ISR=2 tests contained 950 mgVS and ISR=4 tests contained 790 mgVS.

b) Material collection campaigns

Two rounds of BMP tests were conducted; each round required the collection of fresh biological material before starting the experiments. The material collection campaigns involved sampling wastewater from the milking parlour and harvesting cyanobacteria from the *tajamar*. Anaerobic inoculum (sludge from the anaerobic pond) was collected individually before the campaigns.

A first attempt for material collection was made on 30/11/2016 after all the necessary laboratory set up was accomplished. However, it failed. The wastewater could not be sampled because cyanobacteria harvesting took longer than expected; therefore, the wastewater production period was missed.

The second attempt was executed correctly on 14/12/2016 and led to the first round of BMP tests started on 16/12/2016. The second round of BMP tests was started on 07/12/2016 after a material collection campaign was carried out on 03/12/2016.

c) BMP launch

The anaerobic inoculum was collected 2 to 5 days before the material collection campaign. The inoculum was stored in the chamber at 30 °C for degassing and acclimatisation, in accordance with references (Angelidaki, 2009, van Loosdrecht, 2016). Before starting the BMP experiments a leakage test was carried out to the measuring system by injecting a known amount of air into a sealed bottle (Figure 3-12).

Figure 3-12 Tightness test on the biogas measuring system

For starting up the BMP tests care had to be taken to minimise aerobic conditions. Thus, the bottle filling was conducted as efficient and quick as possible. First all the bottles to be used were put on the table. Then, the biological materials were added: AI, CB and WW. Later on, distilled water was added. Afterwards, chemical materials (CE, GL) were added.

The amounts were previously calculated in order to leave the same headspace volume in every bottle (250 mL). N_2 gas was injected in the headspace to replace the air and make an inert atmosphere inside the vessel. All the bottles were sealed with the crimper machine (Figure 3-13). Finally, bottles were placed inside the chamber.

Figure 3-13 Replicates from the second round of experiments after preparation (already sealed). Materials used (from left to right: CB, WW, AI, CE, GL) are also shown.

d) Biogas production monitoring and mixing

Every day (generally the amount of biogas produced by each replicate was measured. When measuring biogas is important to set the ambient temperature to a known temperature because thermal expansion of gases is significant and may add uncertainty to the measure. In consequence, biogas measurements were carried out inside the temperature chamber, at 30 °C. Afterwards volumetric measures (mL) were normalized to 25 °C (NmL).

The biogas was measured by puncturing the rubber seal with the needle connected to the tube and to the graduated cylinder. Immediately after puncturing, bubbles would come out of the other end of the tube thus making the headspace of the inverted graduated cylinder larger. The measure was done by writing down the time the puncture was made, the initial volume of the headspace, and the final volume.

Care was taken to maintain the same height of the water column in the graduated cylinder relative to the water in the beaker between both volume readings (same head). The amount of biogas produced was considered as the difference between final and initial volume. A temperature correction was made in order to normalize volumes.

The frequency of the readings varied along the tests. In the first BMP round, the bottles were punctures twice a day during the first 7 days, trying to separate in time those readings as much as possible (i.e. reading in the morning and in the late afternoon). Afterwards, the biogas production decreased in intensity; therefore, the frequency was changed to one reading per day, and sometimes alternating with one reading every two days.

The final days the frequency came back to one per considering the BMP test as finalized after three consecutive days with an observed biogas production of less than 1% of the accumulated production (Holliger, 2016). In the second round of BMP tests a criteria of reading just once per day was used (even during the first days of the experiments).

Mixing of the vessels was done manually after measuring biogas production, in accordance to main reference (Holliger, 2016). In Figures 3-14 the importance of mixing can be seen: before mixing three layers exist (scum, liquor and sediments), a situation that makes the contact between bacteria and substrate more unluckily and thus slower.

Figures 3-14 Effect of mixing the vessel. Left: before mixing with layers. Right: after mixing without layers.

e) Wet chemistry biogas analysis

The methane content of biogas (sometimes called "biomethane") is an essential parameter to evaluate in a BMP test. Methane is the actual valuable gas within biogas that can be burned (which is not the case for CO_2, H_2S or N_2). Moreover the BMP tests are expressed in NmL_{CH4}/gVS,

Normally biogas analysis is done with a gas chromatography (GC) which is simpler, faster and gives more reliable results. Unluckily that kind of analysis was not available in the laboratory where this research was conducted. Therefore basic wet chemistry analytical techniques were conducted to measure the amount of CH_4 within the biogas.

This techniques use the high solubility of CO_2 and H_2S in alkaline solutions to absorb those gases and measure a volume which is representative of what is left in the biogas. If the absorption worked well, the measure should indicate just the remaining CH_4 in the gas phase.

It was important not to interfere with the ongoing biogas monitoring. In other words, the biogas measure of that day had not to be disturbed because of the analysis of the methane content in the biogas. This prevented employing the probably most straightforward way which would be using the same gas measuring system but changing the neutral measuring liquid with an alkaline solution while making sure the biogas bubbled through the solution. If this had been done, the biogas measure of that day would be lost.

Three different techniques were attempted to estimate the methane content in the produced biogas. The first one was inspired in Pham, et al. (2013) and is presented in Figure 3-15. It was chosen first because it could be done with the same setup used for monitoring the biogas production.

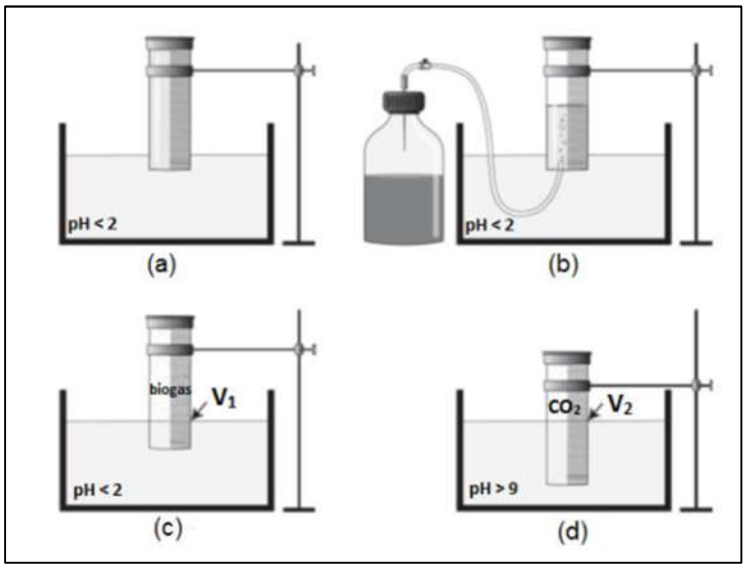


Figure 3-15 Wet chemstry biogas analysis by Pham, et al. (2013)

The actual procedure executed is described as follows; a picture of the process is shown in Figure 3-16:

a) Start with the inverted graduated cylinder filled with distilled water with thymol blue indicator (yellow colour).
b) Puncture the needle connected to the graduated cylinder into the rubber septum of the desired BMP bottle.
c) Measure volume difference (biogas production).
d) Add NaOH pellets mixing with magnetic stirring, confirm change in colour to blue and leave 10 min. Measure new volume (methane only).

Figures 3-16 Wet chemstry biogas analysis: adding NaOH into the measuring liquid

The second approach was to take with a plastic syringe 3 mL of biogas from the BMP vessels prior the reading. Therefore 3 mL were added to the reading to obtain the actual volume of biogas produced. Those 3 mL in the syringe were bubbled into an inverted bottle containing just basic solution (with thymol blue indicator to assure pH>9.6).

The bottle was closed with a rubber septum punctured with a second needle, which served as way out for the excess volume. The displaced solution was collected in a graduated tube and the measured volume would correspond to the volume of CH_4 in the biogas (Figures 3-17, left)

Finally during the second BMP round the measure was done directly over the headspace of an inverted pipette with alkaline solution in it. Like the previous one, biogas was subtracted with syringe before the normal reading, this time the volume being 5 mL. It was slowly liberated inside the pipette and the difference in volumes of the headspace was recorded (Figures 3-17, right).

Figures 3-17 Wet chemistry biogas analysis. Left: displacing a basic solution. Right: measuring the headspace.

f) Physicochemical analysis

The day after collecting the materials from the dairy farm, solids analysis (TS and VS) were carried out to design the amount of material to be added to the bottles, as everything was based on mg of VS.

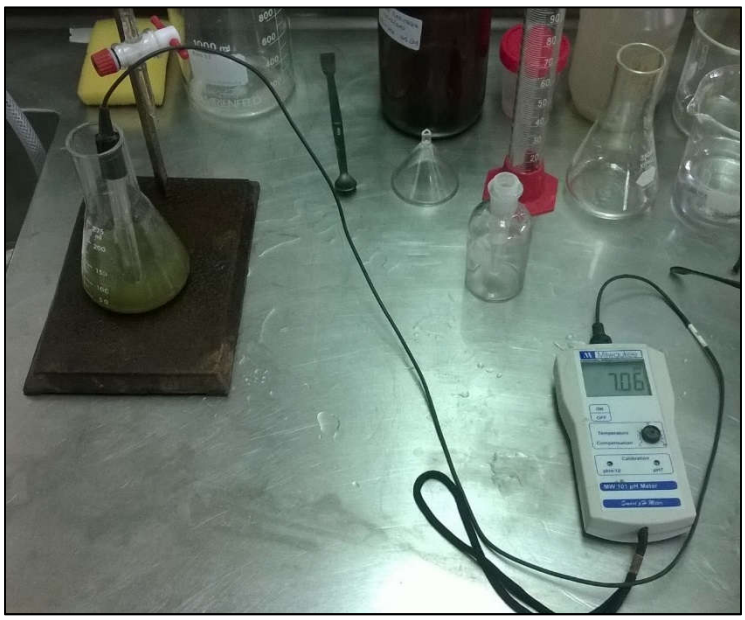
The Standard Methods for the Examination of Water and Wastewater, 22nd Edition (APHA, 2012) were followed, specifically methods 2540 B, E and G, and measures were done in duplicate. To ensure complete drying, samples were left overnight in the oven at 98 °C (Figure 3-18) before the steps of 103-105 °C (for TS) and 550 °C (for IS/VS).

Figures 3-18 Solids analysis. Samples of AI, CB, WW, CE and GL (in that order) after an overnight drying.

In the second BMP round of experiments, the COD of AI, CB and WW was measured before the tests were started. After completion of the BMP tests, the COD was measured from the resultant liquor: raw and filtrated with 0.45 µm Minisart® syringe filters (Figure 3-19).

Figures 3-19 Filtering resultant licqour of BMP tests.

The pH of AI was measured with pH-meter according to method 9045D of EPA (EPA, 2004). Alkalinity of AI and of a 50% WW:CB mixture were measured by titration with HCl 0.05 N until a pH of 5.75 for bicarbonate alkalinity and from then until pH 4.3 for alkalinity due to VFA. The pH of the titration was followed with a portable pH-meter (Figure 3-20).



Figures 3-20 Alkalinity titration.

g) Phytoplankton analysis

In the second material collection campaign a sample of harvested cyanobacteria was sent for phytoplankton analysis at the LATU laboratories. The objective was to know what phytoplankton organisms were being digested.

Taxonomic identification was performed with Olympus CKX41 inverted optical microscope with a magnification of 1000X. Cell counts were made following the methodology Utermhöl (1958). Sedgewick-Rafter 1 ml sedimentation chambers were gridded at 1 µL. Random quadrants were counted and at least 100 cells of the most abundant species, so that the confidence interval was 95%, with a counting error of less than 20% (LATU, 2017).

The biovolume of each species is estimated with the average of the cellular measurements (length, width, thickness) taken for each of the taxa (n = 5-30) based on the approximation of its geometric form according to Edler (1979) and Hillebrand et al. (1999). The calculated biovolume was corrected to biomass as cell carbon (µg C cel-1), using the equations of Menden-Deuer & Lessard (2000). The total biomass was calculated by adding the cell biomass multiplied by its density (LATU, 2017).

In the second round of BMP tests the digestion of cyanobacteria was followed with microscope. On days 0, 2, 6 and 10 of the experiment a 3 mL sample of water was taken from the replicate ISR3-1 and analysed with microscope by Graciela Ferrari from LATU. Photos could be taken on days 0, 2 and 6.

h) Statistics

For every triplicate measure standard deviation (SD) and relative standard deviation (RSD) was calculated with Microsoft Excel® software. SD for accumulated biogas production was calculated as the sum of the reported SD. The SD for the biogas production rates was calculated by dividing the reported SDs by the time; thus, it was assumed time had no associated error.

The SD of the corrected accumulated biogas production (blank subtracted) was calculated as the sum of SD of the test's accumulated biogas production and the blank biogas production. For calculating the SD of the biogas/biomethane yields (NmL/gVS) SD the SD of the corrected accumulated biogas production was divided by the mass of VS (assuming mass weight has no SD).

The value of graph error bars was taken as SD (half of the value up and half down). Results were expressed as (Value ± 1SD) when more than one measure was performed.

3.4. Schedule

A Gantt chart with the schedule followed on this Master thesis is presented in Figure 3-20. In summary within a total of 31 weeks preparing the thesis proposal took 5 weeks, setting up the lab took 11 weeks, monitoring BMP tests took 12 weeks and writing final report took 3 weeks. Four visits to the CRS dairy farm with the sampling team were made.

Schedule	2016 - 2017																													
	September				October				November				December					January				February				March				April			
Week #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Thesis proposal																																	
Reading and writing	x	x	x	x																													
Thesis proposal handing in				x																													
Thesis proposal defence					x																												
CRS																																	
Visits				x					x												x												
Monitoring campaigns								x							x																		
Material collection campaigns												x			x							x											
Batch experiments																																	
Lab set up					x	x	x	x	x	x	x	x	x	x							x												
Batch 1: WW:CB proportions															x	x	x	x	x	x		x	x	x	x	x	x						
Batch 2: C/N ratio																																	
Thesis writing																																	
Data collection and processing															x	x	x	x	x	x	x	x	x	x	x	x	x						
Reading and writing					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Thesis handing in																														x			
Thesis defence																															x		

Figure 3-21 Gantt chart with the schedule executed

CHAPTER 4

Results

4.1. Wastewater treatment system assessment

4.1.1. Previous knowledge

a) Wastewater characterization

In the year 2015 a single analysis of a wastewater sample to the facultative pond was executed; the results where shared and presented by the coordinator of the CRS (Table 4-1).

Table 4-1 Laboratory analysis results of milking parlour wastewater sample

Parameter	Unit	Result	Analysis date
$cBOD_5$	mg O_2/L	704	30/09/2015
COD	mg O_2/L	1780	01/10/2015
Soluble P	mg P/L	1.42	30/09/2015
Total P	mg P/L	53.2	13/10/2015
Total N	mg N/L	258	09/10/2015

b) *Tajamar* characterization

In the same *tajamar* (artificial shallow lagoon) where cyanobacteria were collected, a project named LGSonic was developed by Míguez and Boccardi and titled "Ultrasonic control of cyanobacteria and harmful algae" (Boccardi, 2015).

The objective of LGSonic was to demonstrate the efficiency and environmental effects of an ultrasound commercial equipment in eutrophicated water bodies. In autumn 2015 *in situ* measurements of temperature, electric conductivity (EC), oxygen, turbidity and pH were done in the *tajamar*. Results are presented in Table 4-2.

Table 4-2 Average values of physicochemical properties of water column in the tajamar on the 28/04/2015

Parameter	Unit	Value
Temperature	°C	21.3
EC	µS/cm	504
DO	%	156
DO	mgO/L	13.97
Turbidity	NTU	158.7
Turbidity (Secchi disk)	cm	10
pH	-	8.82

The same day two samples from different points in the perimeter of the *tajamar* were collected for the analysis of nutrients, cyanotoxins and bioassays. The results are presented in Table 4-3.

Table 4-3 Results of chemical and biological analysis of two samples from the tajamar taken on the 28/04/2015

Parameter	Unit	Sample 1	Sample 2	Average
TOC	mgC/L	42.5	43.8	43.2±0.9
TN	mgN/L	11.7	10	10.9±1.2
NO_3^-	mgN/L	<0.017	<0.017	<0.017
NO_2^-	mgN/L	<0.030	<0.030	<0.030
TP	mgP/L	3.1	3.21	3.16±0.08
SP	mgP/L	1.51	1.67	1.59±0.11
TSS	mg/L	126	70	98±40
Chlorophyll-a	µg/L	142.5	321.2	231.9±126.4
Pheophytin	µg/L	826.5	736.5	781.5±63.6
Microcystin-LR	µg/L	<0.73	<0.73	<0.73
Saxitoxin	µg/L	<0.30	<0.30	<0.30
Bioassay with *Daphnia magna*	%	>100	>100	>100

Finally, phytoplankton analysis was done for samples taken during winter on 25/06/2015, before the ultrasonic device started to operate. It was confirmed that cyanobacteria largely dominates phytoplankton community of the *tajamar* with a cyanobacteria proportion of 99.8% and a total density of 7,655,000 cells/mL.

4.1.2. Visits and interviews

Seven visits to the CRS were made during the thesis including the monitoring and material collection campaigns. During visits it was possible to visually inspect the wastewater treatment system, take photos, and to converse with both the responsible and the operators of the dairy farm.

a) Surface waters

As introduced in Section 1.4.2 the dairy farm of the Centro Regional Sur (CRS) is located in Canelones department 25 km away from the shoreline of the River Plate. The general basin of the dairy farm is the Santa Lucia River, where most of the dairy farms are located. The Santa Lucia River is suffering a concerning eutrophication problem aggravated because it serves as drinking water source for half of the Uruguayan population. The sub basin is the hypereutrophic river Canelón Grande and the local basin is the creek De La Lana. The local basin has an area of 32 km^2 and a population of 5,452 inhabitants (MVOTMA, 2017).

b) The milking parlour

The dairy farm consists in 185 cows, which are milked in a milking parlour twice a day, in the early morning and in the afternoon. Milk is sold to CONAPROLE (standing for National Cooperative of Milk Producers) whose trucks come daily to pick the milk up. Ground water is the source of water in the milking parlour. Chemical products are used for cleaning activities and include sodium hypochlorite, acid detergent and basic detergent. Moreover, cows are fed during milking extraction with feed ration in a continuous way.

Each milking operation lasts between 3 to 4 hours. During that time, the cows defecate and urinate, and milk and feed are spilled on the floor, etc. In consequence, cleaning is started right away operations start, and it is continued until the end when a thorough cleaning is done to leave the establishment in good hygienic conditions. The floor is washed down by the operator with a pressure hose (Figure 4-1) while the milking equipment has a self-washing program. As a result of the cleaning operations wastewater is produced.

Figure 4-1 Operator cleaning the floor of the exit esplanade

Infrastructural elements of the milking parlour and its wastewater treatment system are shown next. Figures 4-2 present the way in for cows into the milking parlour. It can be seen that the path is not paved therefore wastewater generated in that part is not collected and it just runs off to the soil.

Figures 4-2 Entrance to the milking parlour (tambo in Uruguayan local language) (left: 04/11/2016, right: 14/12/2016)

The milking room consists of 10 milking machines and feed dispenser (Figures 4-3). All wastewater generated there is centrally collected in a chamber and delivered to the solids chamber by a 6" PVC sewer.

Figures 4-3 Milking room with 10 places (04/11/2016)

After the milking activities, cows go out and wait in a cement esplanade were more wastewater is produced (Figures 4-4). The collected milk is stored in a refrigerated tank at 4 °C which is emptied ever day or every other day. Figures 4-5 also show that besides underground water the milking parlor is provided with a rainwater collection system and a solar-water heating system.

Figures 4-4 Exit esplanade with wastewater sewer system (left: 21/09/2016, right: 04/11/2016)

Figures 4-5 Left: Milk storage room from inside. Right: Milk storage room from outside with a rain water collection system and solar water heating (04/11/2016)

c) Amount of wastewater generated

In Uruguay it is estimated that 50 to 65 litters of wastewater is produced per milking cow (CONAPROLE, 2008, MGAP, 2008). For 185 cows and twice a day this represents a flow of 18.5-24 m^3 per day.

Regarding the variation of the flow within time, the wastewater is not evenly generated. Before the milking operation begins (05:30 and 14:00 hours) the influent gutter to the biological ponds can be found dry. After the operation starts, the wastewater starts being generated with a minimum flow.

When all the cows were milked the workers start to clean the floor which generates the maximum wastewater production. The flow peak lasts only 10-20 minutes and after the cleaning activities have finished, the flow in the influent gutter can be seen as just a trickle product of remainder up-flow overspills.

d) Elements of the wastewater treatment system

The first element of the wastewater system is the wastewater generator. In this case those are the milking room, the storage room and the exit esplanade which all have concrete floors with drains and gutters to collect and conduct the wastewater.

A rectangular solids chamber with a depth of 2 m serves as pre-treatment for coarse solids. The settled solids are taken with a backhoe from the side every week (verbal communication from the workers). The chamber has three inlets receiving the milking parlour's effluents (right in Figures 4-6) and one overspill outlet (bottom of right picture in Figures 4-6). The wastewater with less coarse suspended solids exits the chamber by a concrete open gutter (left in in Figures 4-10) and it is the influent to the biological treatment system.

Figures 4-6 Photos of the solids chamber (left: 21/09/2016, right: 03/02/2016)

The biological system is composed of two wastewater stabilization ponds, an anaerobic pond (with nominal size of 3 m of depth and 20 x 24 m of surface, Figures 4-7) and a facultative one (same surface but shallower, 1.5 m of depth, Figures 4-8).

Figures 4-7 Photos of the anaerobic pond (21/09/2016)

Figures 4-8 Photos of the facultative pond (21/09/2016)

The facultative pond discharges into an area where seems that a plug-flow polishing pond used to exist, although it was not constructed in concrete. Figures 4-9 present photos of it on spring. In summer time it was observed completely dry. An effluent of all the elements was observed through a channel dug in the soil (right in Figures 4-10). The area that the effluent travels it was observed to be floodable.

Figures 4-9 Photos of the polishing ponds (21/09/2016)

Figures 4-10 Photos of wastewater channels. Left:Concrete open gutter carrying the ingluent. Right: Excavated-on-soil channel carrying the effluent. (21/09/2016)

The *tajamar* (artificial shallow lagoon) is the last element of the wastewater treatment system as the receiving water body. The volume of the *tajamar* can be estimated from a surface of 5.17 hectare (measured with Google Earth Pro® software) and an assumed average depth of 0.5 m, resulting in 25,850 m^3.

Due to the nature of the last channel (floodable, excavated on soil) the discharge of the wastewater treatment system into the *tajamar* is somewhere between punctual and diffuse (Figures 4-11).

Figures 4-11 Photos of discharge zone next to the tajamar (21/09/2016)

As summer drew nearer *tajamar* colour turned greener (Figures 4-12). Origin of eutrophication may come from dairy farm wastewater discharge but also from runoff of the surrounding agricultural activities that include swine production, beekeeping production, horticulture production and fruticulture production (Fagro).


Figures 4-12 The tajamar in summer (left: 14/12/2016, right 03/02/2016)

e) Irrigation

Irrigation pumps installed in the facultative pond and in the tajamar were observed (Figures 4-13). The CRS' coordinator expressed that irrigation was applied on pastures; the water was taken from both water bodies.


Figures 4-13 Pumps installed in the system, left: facultative pond (4/11/2016), right: tajamar (24/10/2016)

4.1.3. Laboratory analysis results

Results from samples taken on the first campaign (24/10/2016) are presented in Table 4-4. It's worth to mention that the idea was to measure more parameters, but internal communication problems within LATU resulted in the analysis of only a portion of the initially planned analysis. Moreover, the anaerobic pond was not possible to sample in this opportunity due to lack of liquid phase (it was full of sludge to the top). Therefore, only the influent wastewater, the facultative pond and the *tajamar* were sampled.

Table 4-4 Results of first monitoring campaign in October (24/10/2016)

Parameter	Unit	Influent wastewater	Facultative pond	*Tajamar*
Physicochemical				
DO	mgO2/L	0.10	0.07	5.17
EC	µS/cm	5,760	3,623	378
Organic matter				
COD	mgO_2/L	16,100	1,290	149
BOD_5	mgO_2/L	9,650	445	95
Nutrients				
TN	mgN/L	665	210	6.27
Pathogens				
FC	MPN/100mL	>1,600,000	920,000	N.A.

A second monitoring campaign was executed the 14/12/2016. All the planned monitoring points were sampled correctly and all the requested parameters were analysed correctly. Results are presented in Table 4-5.

Table 4-5 Results of second monitoring campaignin December (14/12/2016)

Parameter	Unit	Influent wastewater	Anaerobic pond	Facultative pond	*Tajamar*
Physicochemical					
pH	-	8.39	7.6	8.38	9,68
EC	µS/cm	10,090	6,520	4,700	494
Solids					
TS	mg/L	10,300	3,920	3,040	455
TSS	mg/L	6,850	742	236	159
VS	mg/L	4,400	1,330	855	216
VSS	mg/L	3,860	608	192	114
Organic matter					

Parameter	Unit	Influent wastewater	Anaerobic pond	Facultative pond	*Tajamar*
COD	mgO_2/L	8,840	1,850	874	243
COD_{mf}	mgO_2/L	4,840	631	404	108
BOD_5	mgO_2/L	4,420	444	192	39
Nutrients					
TN	mgN/L	719	373	140	10.6
NH4+	mgN/L	719	373	140	2.27
NO2-	mgN/L	0.14	0.11	0.19	0.35
NO2-	mgN/L	0.23	<0.018	0.09	<0.018
TP	mgP/L	52	49	27.1	2.52
SP	mgP/L	12.5	28.7	9.55	1.25
TOC	mgC/L	1,439	N.A.	N.A.	N.A.
Pathogens					
FC	MPN/100mL	>1,600,000	920,000	24,000	200
Cyanotoxins					
Microcystin-LR	µg/L	N.A.	N.A.	N.A.	<0.34
Saxitoxin	µg/L	N.A.	N.A.	N.A.	<1

4.1.4. Data processing

Some relations that can give more information about the wastewater and the treatment system are presented in table 4-6.

Table 4-6 Relations between measured parameters for different points

Unit	Wastewater	Anaerobic pond	Facultative pond	*Tajamar*
October				
BOD_5/COD	0.60	-	0.34	0.64
TN/COD	0.04	-	0.16	0.04
December				
BOD_5/COD	0.50	0.24	0.22	0.16
BOD_5/SCOD	0.91	0.70	0.48	0.36
TN/COD	0.08	0.20	0.16	0.04
TP/COD	0.006	0.026	0.031	0.010
C:N:P	28:14:1	-	-	-
C/N	2.0	-	-	-

4.2. Batch experiments

4.2.1. Solids measurement results

The following are the results corresponding to the measurement of solids content (total, volatile inorganic) of cyanobacteria (CB), wastewater (WW), anaerobic inoculum (AI), cellulose (CE) and glycerol (GL). Measurements were carried out for total solids (TS), inorganic solids (IS) and volatile solids (VS) after each material collection campaign.

The failed material collection campaign (the one wastewater was missed) served to study at least the solids content of the cyanobacteria filtrate and the anaerobic inoculum (Table 4-7). This allowed to estimate how much volumes were needed for the second attempt to harvest cyanobacteria.

Table 4-7 Solids of materials collected the 30/11/2016

Material	%Water	%TS	%VS	%IS	%VS in TS	%IS in TS
CB	99.9%	0.11%	0.08%	0.03%	73%	27%
AI	63.8%	36.2%	3.60%	32.6%	10%	90%

Table 4-8 presents the results of solids analysis on the materials that were used to launch the first round of BMP tests.

Table 4-8 Solids of materials collected the 14/12/2016

Material	%Water	%TS	%VS	%IS	%VS in TS	%IS in TS
CB	99.8%	0.18%	0.15%	0.03%	81%	19%
WW	99.1%	0.89%	0.46%	0.44%	51%	49%
AI	83.4%	16.5%	3.95%	12.6%	24%	76%

The results of solids analysis on the materials for the second round of BMP tests are shown in Table 4-9. It also includes the results of TS for a sample of the *tajamar* (TA), that is like CB but taken directly without filtering it with the phytoplankton net. It can be seen that the laborious harvesting process only duplicates the concentration of VS compared to taking water directly.

Table 4-9 Solids of materials collected the 03/12/2016

Material	%Water	%TS	%VS	%IS	%VS in TS	%IS in TS
CB	99.9%	0.13%	0.09%	0.04%	67%	33%
TA	99.9%	0.07%	-	-	-	-
WW	99.4%	0.59%	0.31%	0.28%	53%	47%
AI	58.7%	41.3%	5.75%	35.5%	14%	86%
CE	3.12%	96.9%	96.8%	0.09%	100%	0%
GL	5.94%	94.1%	94.1%	0.00%	100%	0%

4.2.2. Chemical analysis results

a) By LATU

On the second monitoring campaign the biological materials for the batch tests were harvested. Samples were analysed and results are shown in Table 4-10.

Table 4-10 Results of analysis made on milking parlour wastewater and harvested cyanobacteria

Parameter	Unit	Influent	Cyanobacteria
Physicochemical			
pH	-	8.39	7.52
EC	µS/cm	10.09	0.87
Solids			
TS	mg/L	10,300	1,340
TSS	mg/L	6,850	840
VS	mg/L	4,400	1,010
VSS	mg/L	3,860	770
Organic matter			
COD	mgO_2/L	8,840	1,930
COD_{mf}	mgO_2/L	4,840	277
BOD_5	mgO_2/L	4,420	517
Nutrients			
TN	mgN/L	719	94.9
NH4+	mgN/L	719	72.1
NO2-	mgN/L	0.14	0.08
NO2-	mgN/L	0.23	<0.018
TP	mgP/L	52	14.3
SP	mgP/L	12.5	5.46
TOC	mgC/L	1,439	325

The same proportions between measured parameters that were done for the results of the wastewater treatment system can be applied to these two materials (Table 4-11).

Table 4-11 Proportion between analysed parameters of biological materials used

Relation	Wastewater	Cyanobacteria
BOD_5/COD	0.50	0.27
SCOD/COD	0.55	0.14
COD/TOC	6.14	5.94
TN/COD	0.08	0.05
TP/COD	0.006	0.007
C:N:P	28:14:1	23:7:1
C/N	2.0	3.4

b) Own analysis

On the second round of tests analysis of pH, COD and alkalinity were performed on the biological materials used. Results are found in Table 4-12.

Table 4-12 Results of own analysis done in the biological materials used in the second BMP round (Feb/2016)

Parameter	Unit	AI	CB	WW	50% (CB:WW)
pH	-	8.5	6.6	6.9	7.1
COD	mgO/L	67,300±9,899	1,358±3	6,110±42	2,043±25
Total alkalinity	$mgCaCO_3/L$	2,900	N.A.	N.A.	623
Bicarbonatic alkalinity	$mgCaCO_3/L$	813	N.A.	E	354
Alkalinity due to VFA	$mgCaCO_3/L$	3,713	N.A.	N.A.	269

Also in the second round, after depletion of biogas production the resultant liquor of three replicates were analysed. Results are presented in Table 4-13 and include COD, nitrogen and phosphorus.

Table 4-13 Results of own analysis done in the licqour of BMP bottles after biogas production ceased

Parameter	Unit	ISR2	ISR3	ISR4
COD	mgO/L	3,270±42	3,470±42	2,570±14
SCOD	mgO/L	203	287	159
TN	mgN/L	240.0±14.1	25.0±7.1	160.0±0.0
SN	mgN/L	87.0±7.1	67.0±7.1	46.0±0.0
TP	mgP/L	26.4±22.0	33.0±0.4	29.3±0.4
SP	mgP/L	26.1±0.1	21.6±0.1	18.1±0.2

4.2.3. Phytoplankton results

a) *Tajamar* samples

During the monitoring campaigns (October and December) and during the second collection campaign (February) phytoplankton analysis was requested over water samples from the tajamar. Results are presented in Table 4-14.

Table 4-14 Phytoplankton community evolution during sampling campaigns

	October (cell/mL and %)		December (cell/mL and %)		February (cell/mL and %)	
Total cyanobacteria	**1,065,994**	**100.0%**	**8,610,333**	**100.0%**	**16,606,000**	**100.0%**
CYANOPHYCEAE					16,582,000	99.9%
Ciano s/d	1,101	0.1%		0.0%		0.0%
Aphanizomenon sp.		0.0%	273,333	3.2%	59,333	0.4%
Raphidiopsis		0.0%	3,107,000	36.1%		0.0%
Oscillatorial s/d	127,987	12.0%		0.0%		0.0%
Planktothrix sp.	860,914	80.8%	5,230,000	60.7%	442,667	2.7%
Raphidiopsis/Cuspidothrix	75,992	7.1%		0.0%	15,720,000	94.7%
Limnothrix cf. redekei		0.0%		0.0%	88,000	0.5%
Pseudanabaena sp.		0.0%		0.0%	272,000	1.6%
CHLOROPHYCEAE	0		0		24,000	0.1%
Clorophyceae s/d					4,000	0.0%
Monoraphidium arcuatum					6,000	0.0%
Monoraphidium contortum					6,000	0.0%
Monoraphidium griffithii					8,000	0.0%

Moreover, for the February sample, the determination of biovolume and biomass was request. Results are presented in Table 4-15.

Table 4-15 Phytoplankton analysis report from sample taken on 03/02/2017

Taxon	Density (cell/mL)		Biovolume (µm³/mL)		Biomass (ngC/mL)	
Cyanophyceae	**$1.66x10^7$**	**99.86%**	**$4.15x10^5$**	**98.85%**	**$7.44x10^4$**	**98.07%**
Aphanizomenon sp.	$5.93x10^4$		$1.57x10^3$		$2.78x10^2$	
Limnothrix cf. redekei	$8.80x10^4$		$2.07x10^3$		$3.69x10^2$	
Planktothrix sp.	$4.43x10^5$		$1.06x10^4$		$1.89x10^3$	
Pseudanabaena sp.	$2.72x10^5$		$2.14x10^3$		$1.16x10^3$	
Raphidiopsis/Cuspidothrix	$1.57x10^7$		$3.99x10^5$		$7.07x10^4$	
Chlorophyceae	**$2.40x10^4$**	**0.14%**	**$4.85x10^3$**	**1.15%**	**$1.47x10^3$**	**1.93%**
Clorophyceae s/d	$4.00x10^3$		$9.81x10^1$		$1.74x10^1$	
Monoraphidium arcuatum	$6.00x10^3$		$2.13x10^3$		$6.42x10^2$	
Monoraphidium contortum	$6.00x10^3$		$2.10x10^2$		$7.30x10^1$	
Monoraphidium griffithii	$8.00x10^3$		$2.41x10^3$		$7.35x10^2$	
TOTAL	**$1.66x10^7$**	**100%**	**$4.20x10^5$**	**100%**	**$7.59x10^4$**	**100%**

b) Microscopic monitoring of cyanobacteria digestion

Pictures from the samples taken to follow the digestion are shown in Figures 4-14 to 4-16 for day 0, 2 and 6 respectively. On day 0, abundant cyanobacteria cells can be observed of *Planktothrix sp.* (left picture) and *Raphidiopsis sp.* and *Aphanizomenon sp.* (right picture).

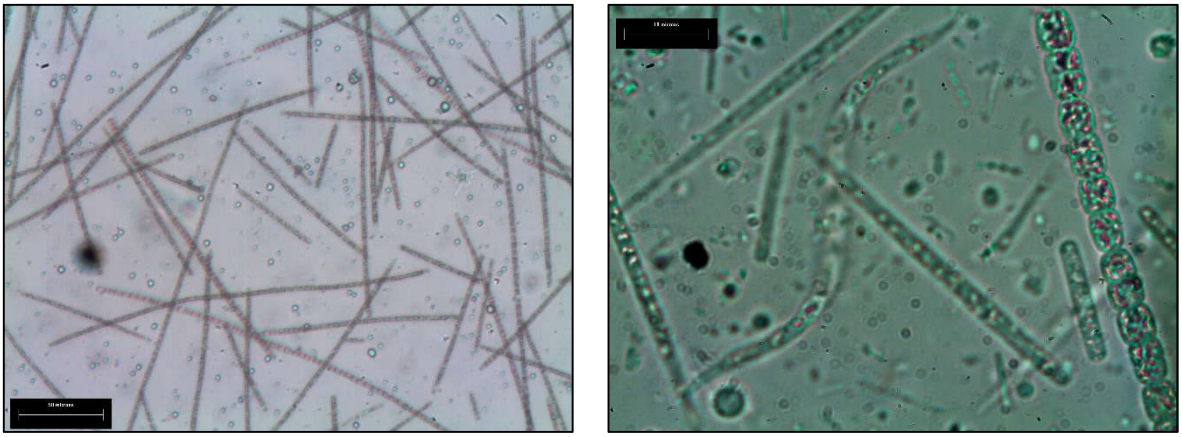


Figure 4-14 Microscopic view of day 0. Left: 20X. Right: 100X.

Already on day 2 concentration of whole cells of cyanobacteria has fell dramatically and detritus is more present. On day 6 it was difficult to find cyanobacteria cells and it was treated as the first negative result. Although on day 10 it was not possible to take pictures, cyanobacteria cells were not found and it was considered as a second negative result.

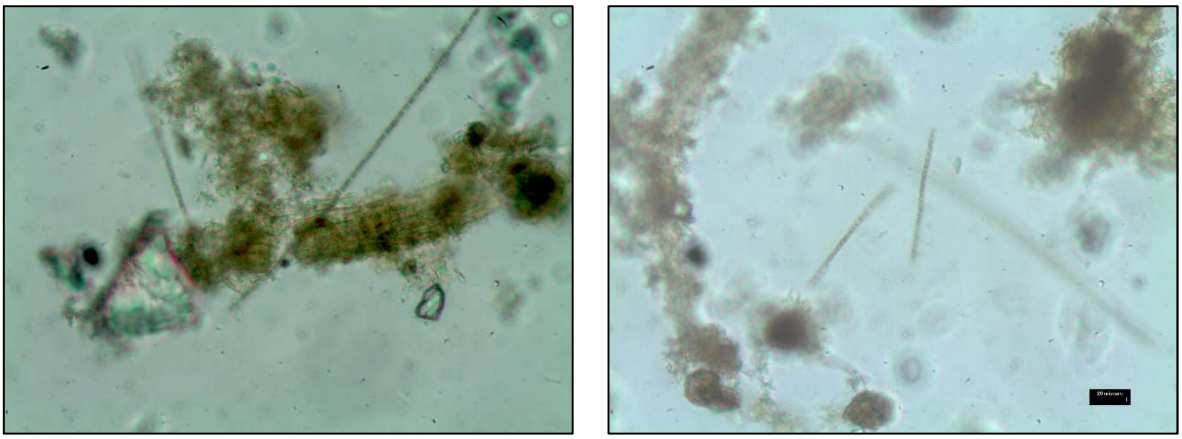


Figure 4-15 20X microscopic view of day 2

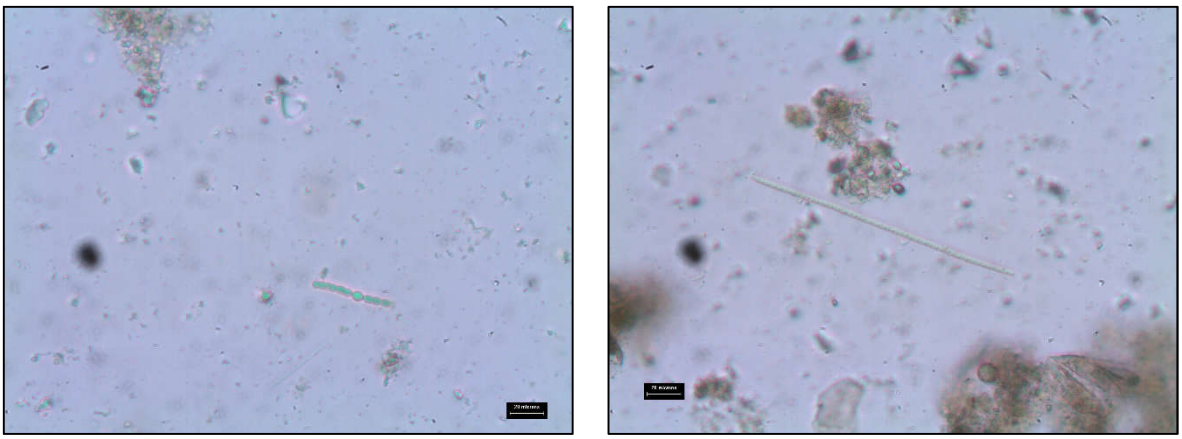


Figure 4-16 20X microscopic view of day 6

4.2.4. Biogas production curves

The first result of biogas production monitoring consists of one table for each BMP bottle with the time the measured were done (day and hour), the initial volume of the graduated cylinder (Vi), the final volume of the graduated cylinder after capturing biogas (Vf), the difference between both volumes indicating the total volume of biogas produced in that measure (Vt), the normalized (to 25 °C) total volume of biogas measured (NVt) and the accumulated normalized biogas production (Acc. NVt).

Table 4-16 shows the results of biogas measurement obtained for replicate 1 of test "50% WW:CB" which is one of the 39 replicates done (13 tests in triplicate).

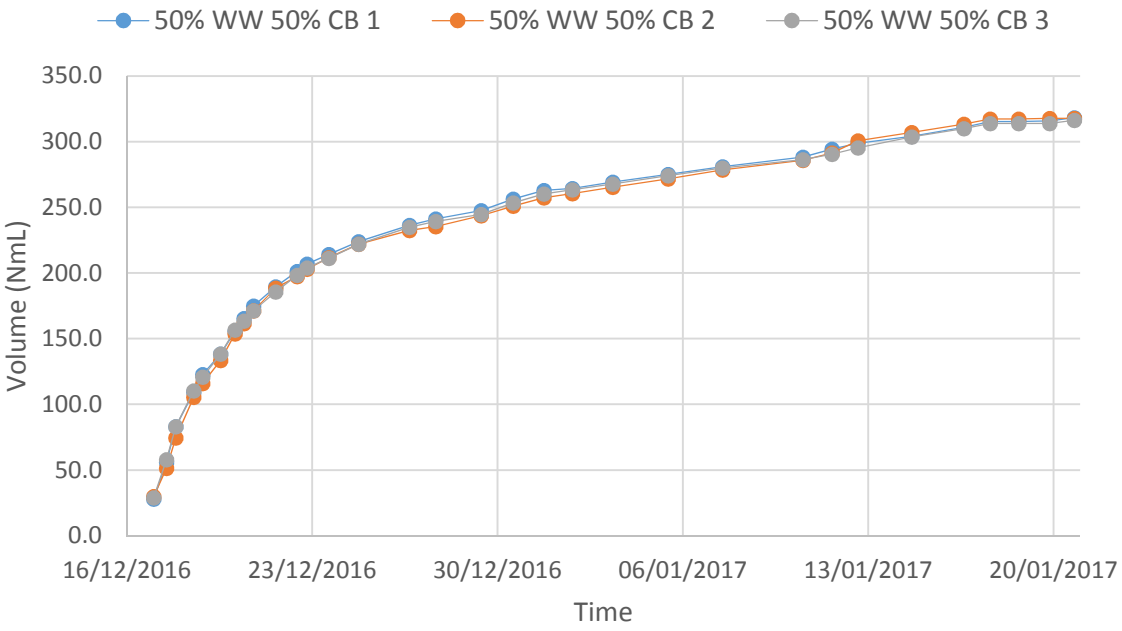
Table 4-16 Example of a biogas monitoring table (replicate 1 of test 50% WW:CB)

#	Day	Hour	Vi (mL)	Vf (mL)	Vt (mL)	NVt (NmL)	Acc. NVt (NmL)
1	17-Dec	00:23	13	42	29	28.0	28.0
2	17-Dec	11:51	44	73	29	28.0	56.1
3	17-Dec	20:11	38	66	28	27.1	83.2
4	18-Dec	12:32	71	98	27	26.1	109.3
5	18-Dec	20:47	14	28	14	13.5	122.8
6	19-Dec	13:01	12	28	16	15.5	138.3
7	20-Dec	01:56	13	31	18	17.4	155.7
8	20-Dec	10:12	40	50	10	9.7	165.4
9	20-Dec	18:53	19	29	10	9.7	175.0

#	Day	Hour	Vi (mL)	Vf (mL)	Vt (mL)	NVt (NmL)	Acc. NVt (NmL)
10	21-Dec	14:54	20	35	15	14.5	189.5
11	22-Dec	10:17	44	56	12	11.6	201.1
12	22-Dec	19:16	48	54	6	5.8	206.9
13	23-Dec	14:53	7	14.5	7.5	7.3	214.2
14	24-Dec	17:56	5.5	15.5	10	9.7	223.9
15	26-Dec	16:06	9	22	13	12.6	236.4
16	27-Dec	15:51	30	35	5	4.8	241.3
17	29-Dec	09:14	5.5	12	6.5	6.3	247.6
18	30-Dec	14:11	5	14	9	8.7	256.3
19	31-Dec	18:03	9	16	7	6.8	263.0
20	01-Jan	19:50	5.5	7	1.5	1.5	264.5
21	03-Jan	08:18	8	13	5	4.8	269.3
22	05-Jan	10:39	7	13	6	5.8	275.1
23	07-Jan	12:00	11	17	6	5.8	280.9
24	10-Jan	12:41	5	12.5	7.5	7.3	288.2
25	11-Jan	15:10	35	41.5	6.5	6.3	294.5
26	12-Jan	14:27	18.5	23	4.5	4.4	298.8
27	14-Jan	15:11	7	12.5	5.5	5.3	304.1
28	16-Jan	14:43	24	31	7	6.8	310.9
29	17-Jan	14:21	14.5	19	4.5	4.4	315.2
30	18-Jan	16:13	26	26	0	0.0	315.2
31	19-Jan	20:26	21.5	22	0.5	0.5	315.7
32	20-Jan	18:40	35.5	38	2.5	2.4	318.1

As each bottle led to one of the tables like Table 4-15, each set of triplicates led to a graph like shown in Graph 4-1, where the single accumulated biogas production of each bottle is presented.

Graph 4-1 Accumulated biogas volume for each of the triplicates of 50% CB:WW test



With the data of each set of triplicates it was possible to calculate averages, standard deviations (SD), relative standards deviations (RSD) and rate of biogas production (expressed in NmL/h and representing the amount of biogas produced in the time periods between measures).

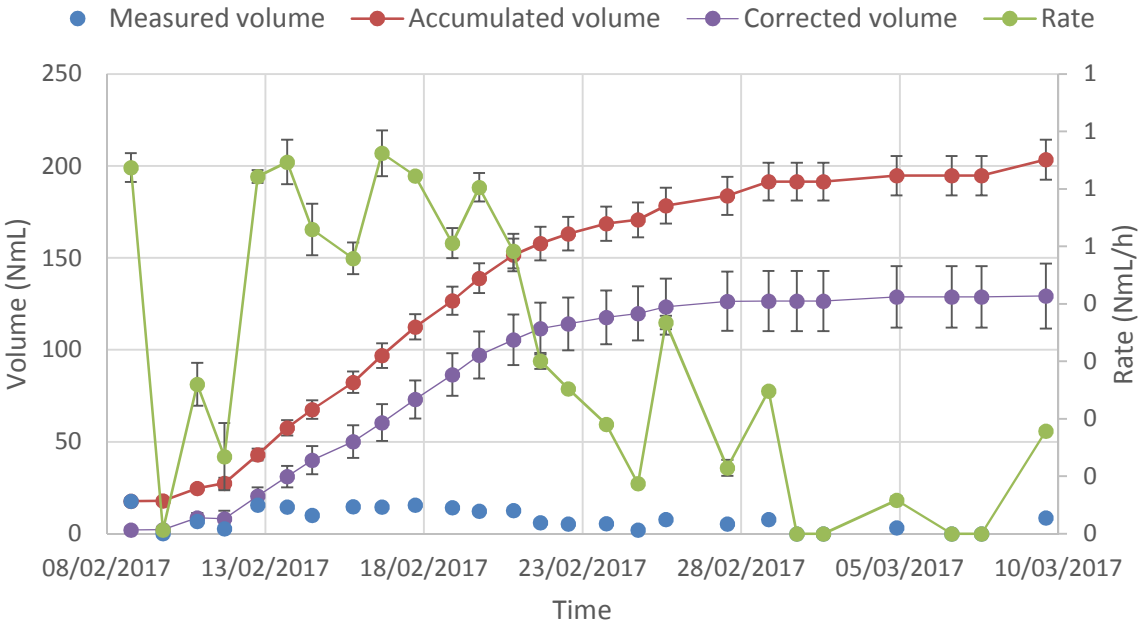
Moreover, once results of blank tests were available, it was possible to calculate a "corrected" accumulated volume of biogas which is obtained by subtracting the biogas production of the blank tests. The above average parameters were put into graphs for each of the 13 BMP tests. Error bars were taken as standard deviation (half of the value up and half down).

As an example the Positive Control test is presented in Graph 4-2. Four parameters are grouped in the same graph for comparison purposes. First each biogas measure is presented in blue (average values, in this example all between 0 and 20 NmL). Then two accumulated productions are shown: in red the measured one; in violet the corrected one (blank subtracted).

The final corrected accumulated volume gives the maximum biogas potential of the substrate (129.3 NmL for the positive control). By dividing it by the amount of grams of volatile solids added with the substrates (213 mgVS of cellulose in this example) you get the biogas yield (which for the positive control results in 0.241 NmL_{biogas}/mgVS). Ultimate BMP is then obtained by taking into account the CH_4 content of biogas, which can be measured or estimated to get a result in $NmL_{methane}$/mgVS.

Finally the production rate (in green) is included in a separate axis (vertical right). It can be seen that biggest values of production rate correspond to steeper slopes in the accumulated produced volumes.

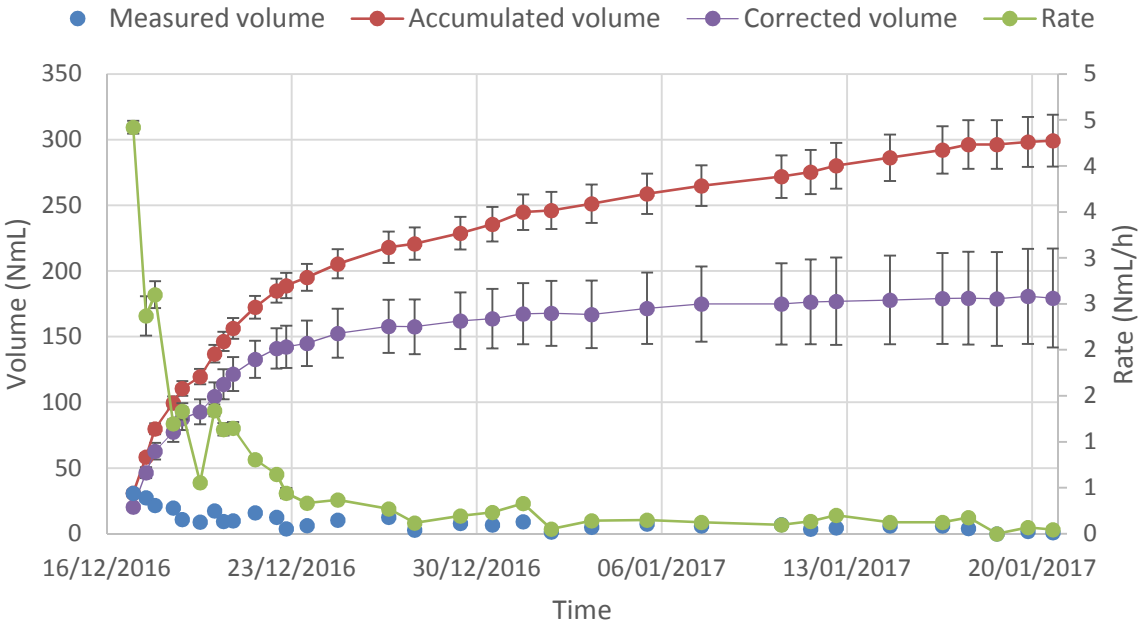
Graph 4-2 Average results for Positive Control tests



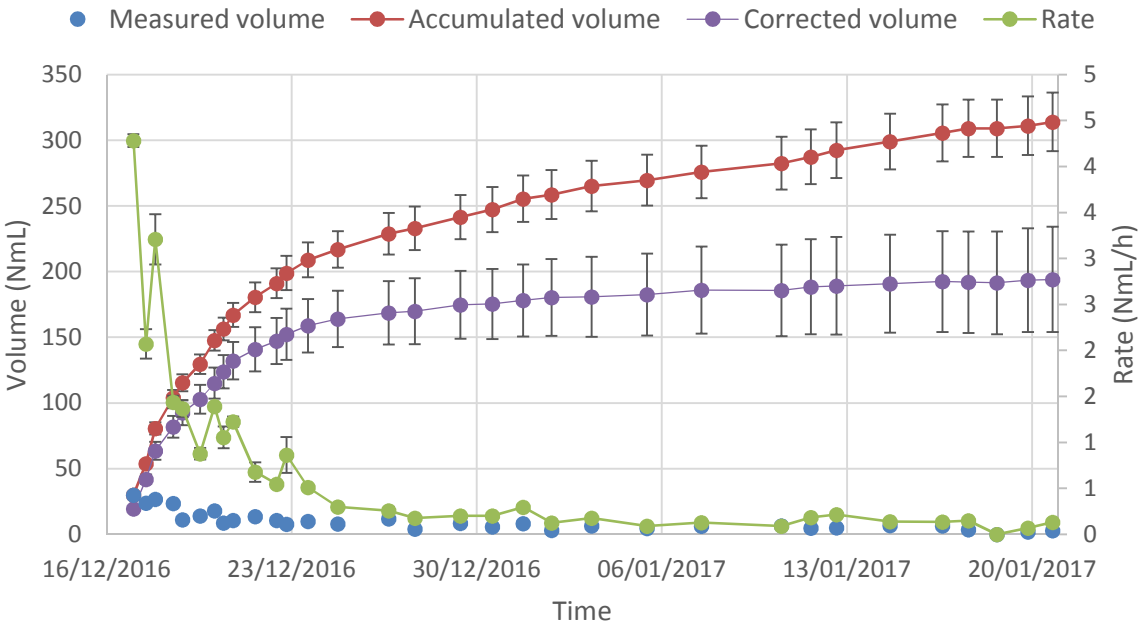
a) First round of BMP tests

Following Graphs 4-3 to 4-15 are presented. Each one shows the results of the six different BMP tests held in the first round, labelled respectively: 100% CB, 25% CB 75% WW, 50% CB-WW, 75%CB 25% WW, 100% WW. The first round went for 35 days from 16/12/2016 to 21/01/2017.

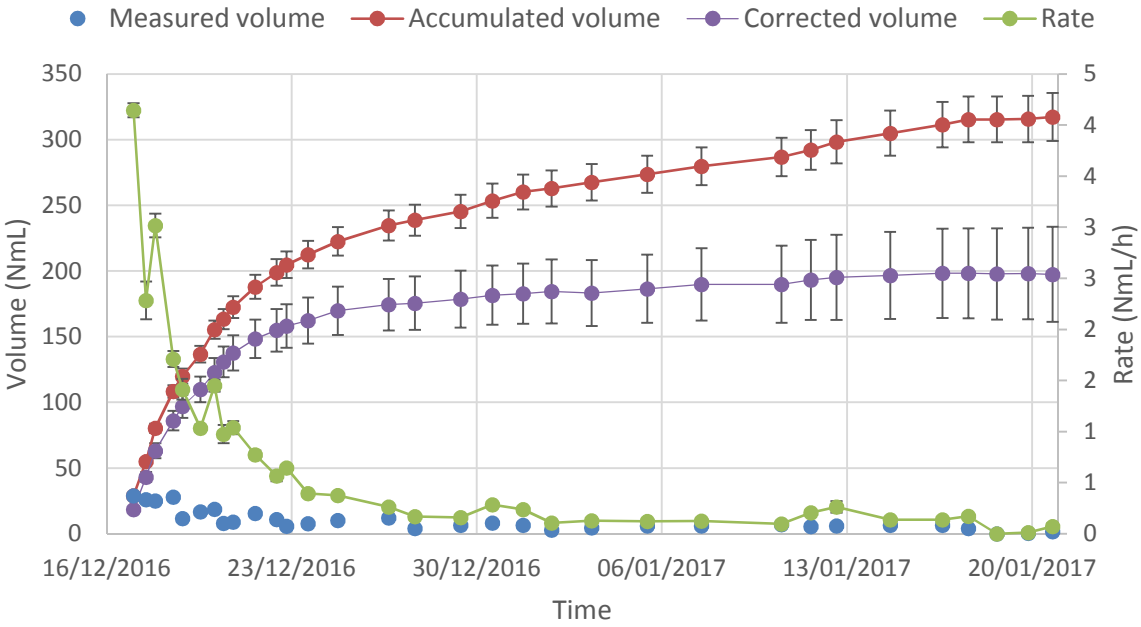
Graph 4-3 Average results for 100% CB tests



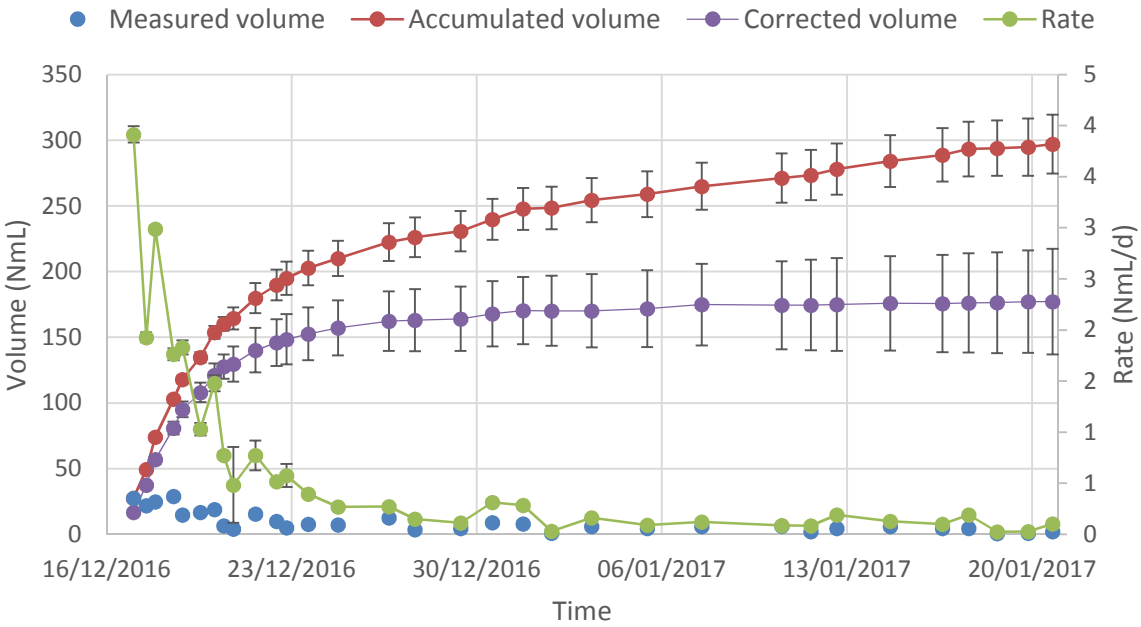
Graph 4-4 Average results for 25% WW 75% CB tests



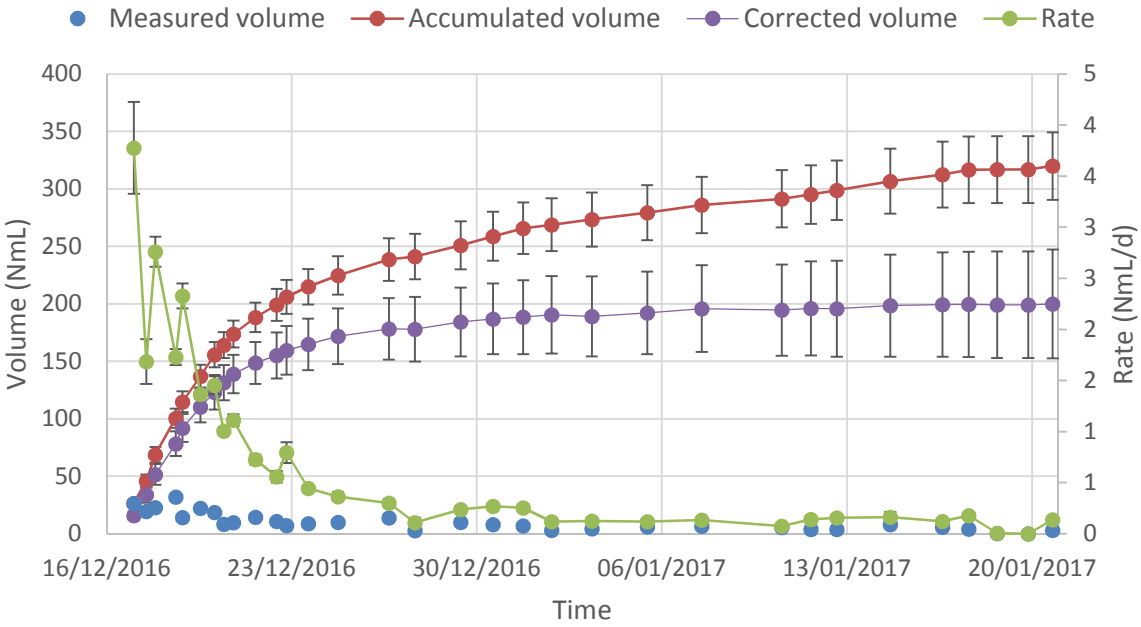
Graph 4-5 Average results for 50% WW 50% CB tests



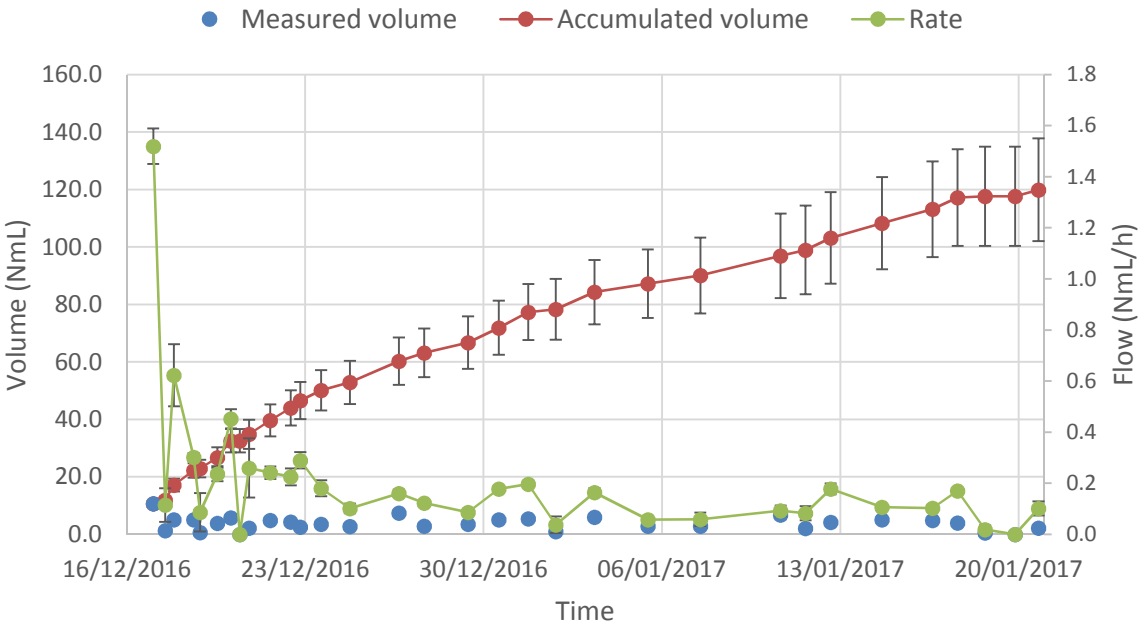
Graph 4-6 Average results for 75% WW 25% CB tests



Graph 4-7 Average results for 100% WW tests



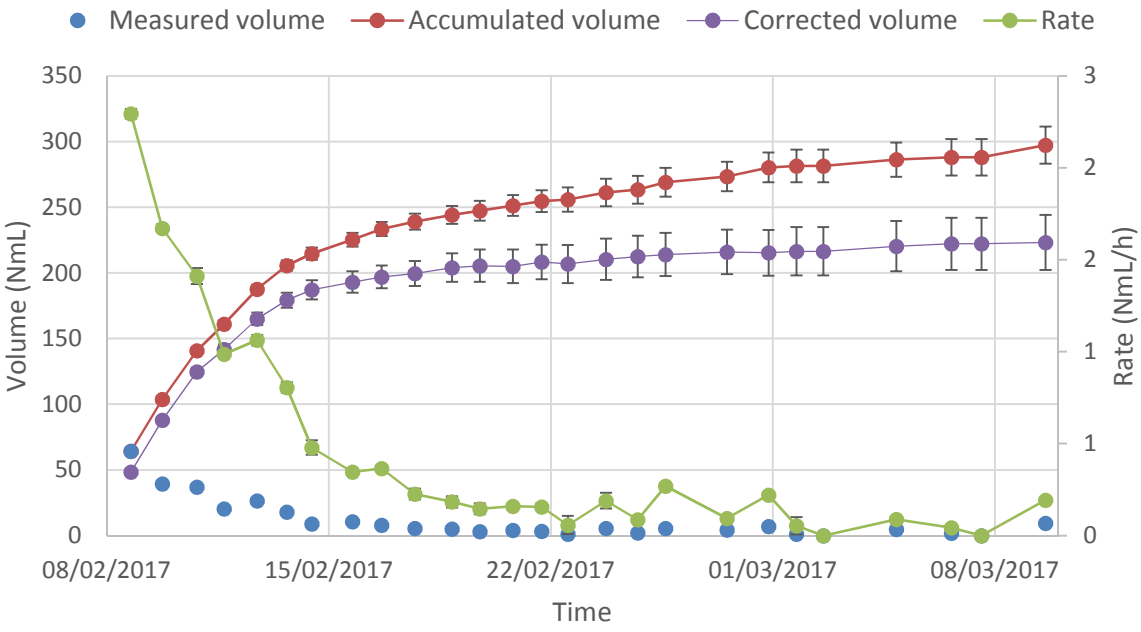
Graph 4-8 Average results for Blank 1



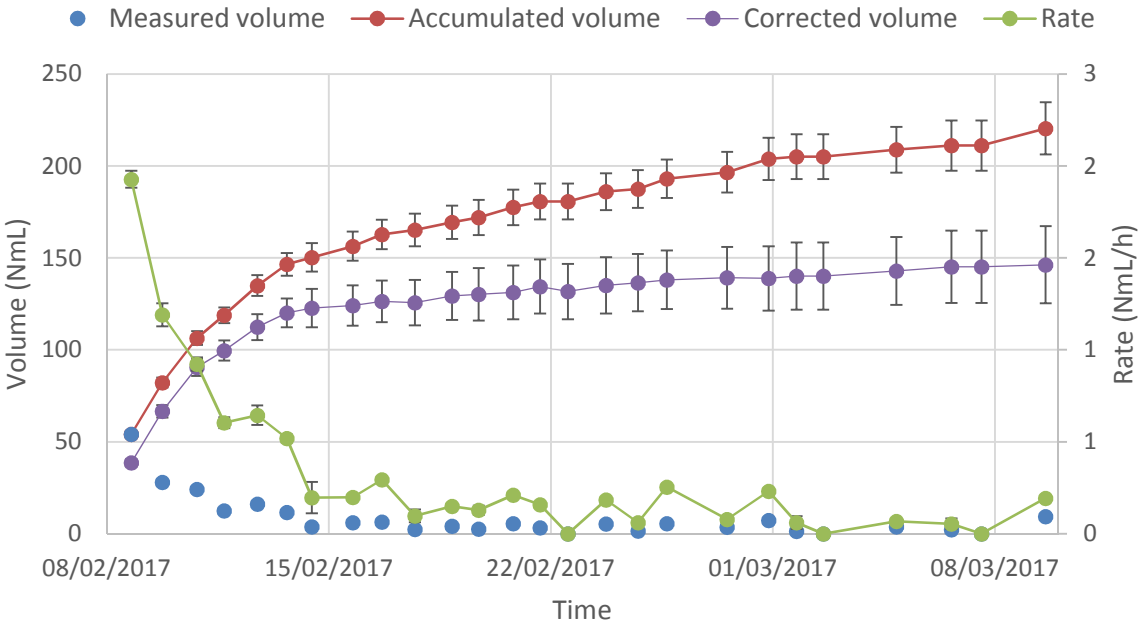
b) Second round of BMP tests

Graphs 4-9 to 4-15 presents the resultant biogas curves for the second round of BMP tests (16/12/2016 to 21/01/2017), for tests ISR2, ISR3, ISR4, CN5, CN10, Positive and Blank respectively.

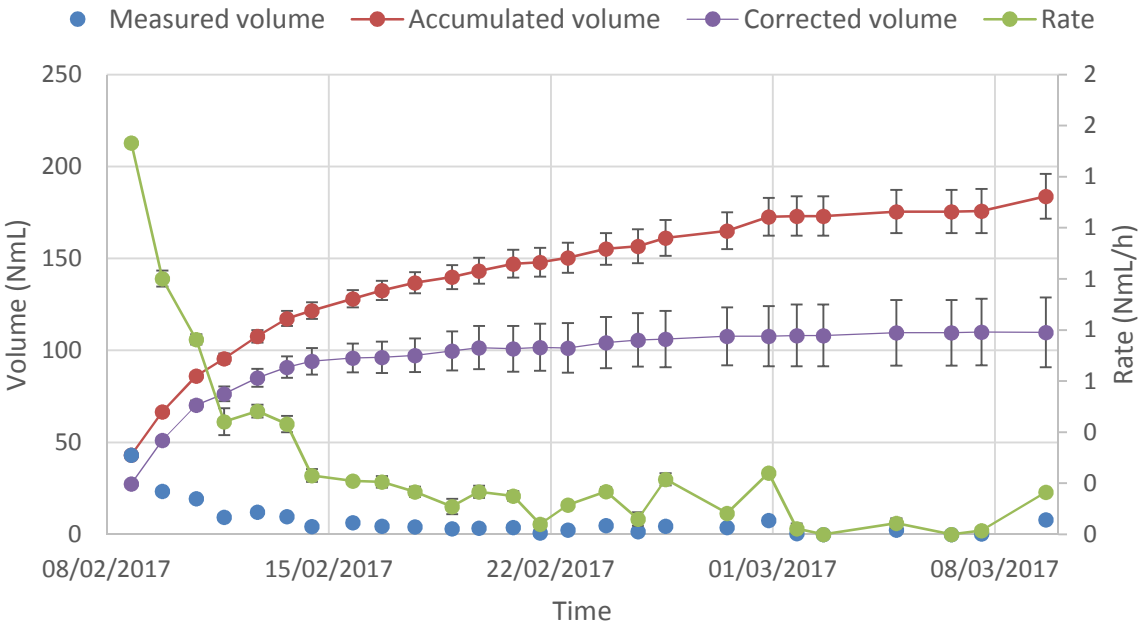
Graph 4-9 Average results for ISR2



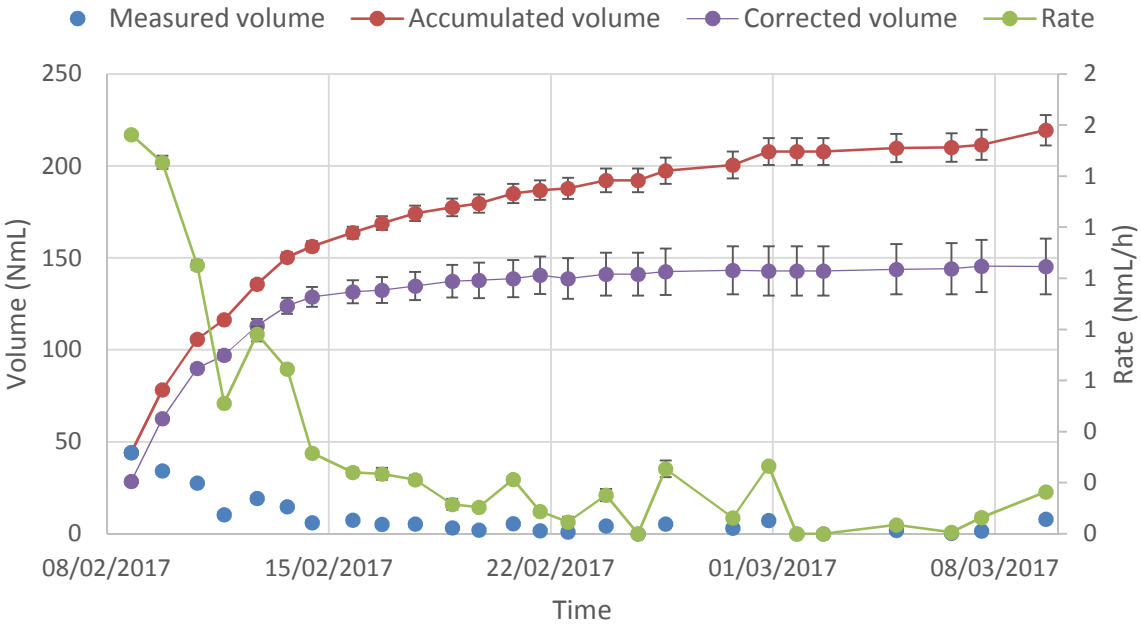
Graph 4-10 Average results for ISR3



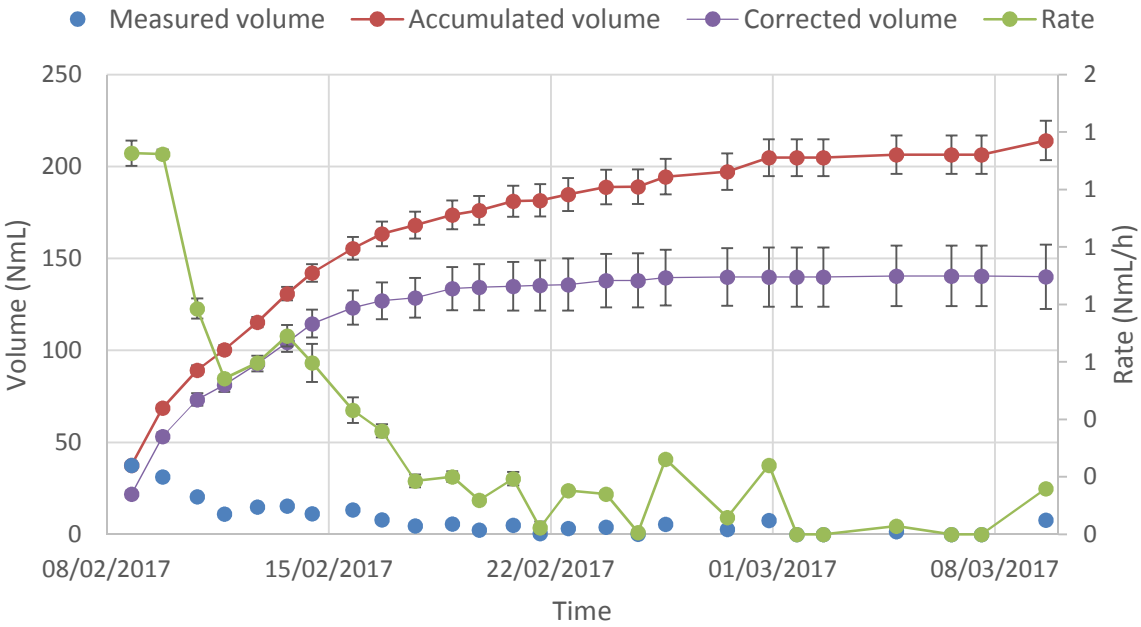
Graph 4-11 Average results for ISR4



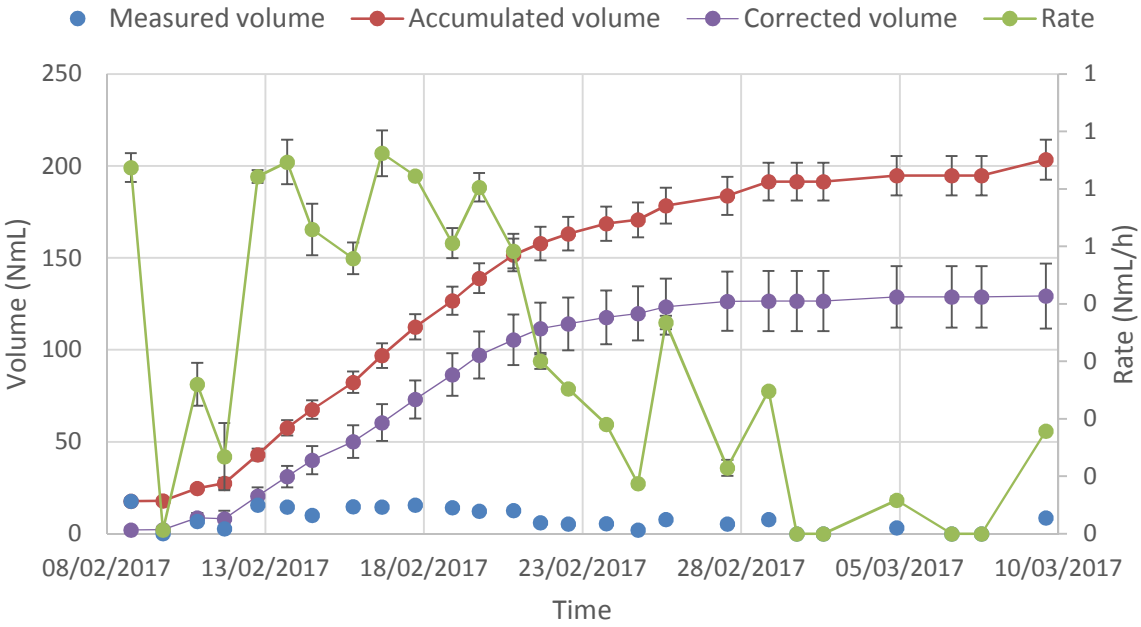
Graph 4-12 Average results for C/N5



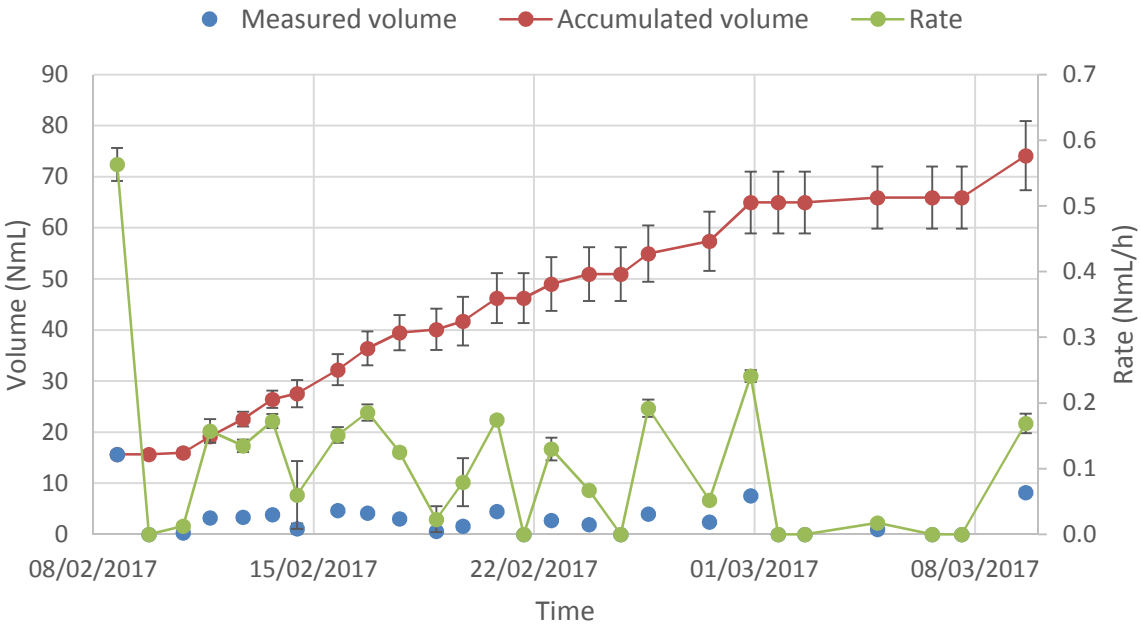
Graph 4-13 Average results for C/N10



Graph 4-14 Average results for positive control



Graph 4-15 Average results for Blank 2



4.2.5. Wet chemistry analysis results

Table 4-17 presents the results of the wet chemistry analysis technique performed in the first round (see section 3.3.3.6). The second technique consisted in bubbling biogas into a basic solution and measuring the increase in headspace. Those results are listed in Table 4-18.

Table 4-17 Results of biogas analysis by collecting a displaced basic solution

Configuration	%CH_4 in biogas
100% CB	(83.9±1.0)%
25% WW 75% CB	(81.1±1.0)%
50% WW CB	(81.7±1.7)%
75%WW 25% CB	(84.2±3.5)%
100% WW	(83.3±1.7)%
Blank	(93.3±1.7)%

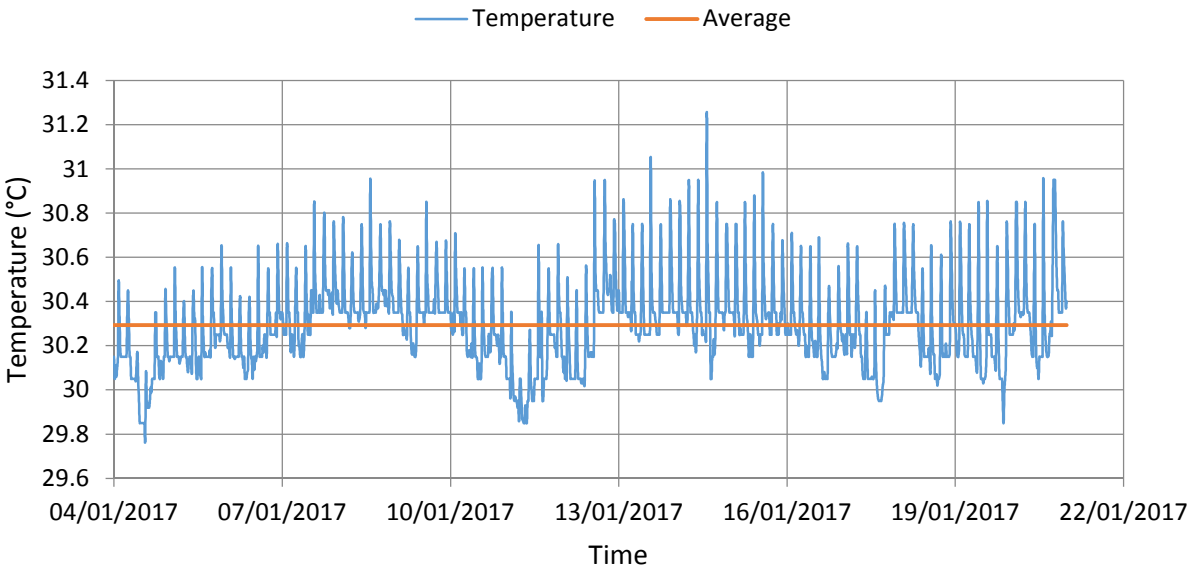
Table 4-18 Results of biogas analysis by bubbling in basic solution and measuring the headspace

Configuration	%CH_4 in biogas
ISR 2	(96.0±1.0)%
ISR 3	(97.7±1.5)%
ISR 4	(102.0±3.6)%
CN5	(99.0±1.7)%
CN10	(97.0±0.0)%

4.2.6. Temperature control

A PCIM temperature and humidity control system was installed on day 18 of the first round of BMP tests (04/01/2017) and temperature was registered until day 34 of experiment (21/01/2017). Results are shown in Graph 4-16. It can be seen that temperature ranged mostly from 30.0 °C to 30.8 °C with average value of (30.3±0.2) °C.

Graph 4-16 Temperature control of environmental chamber during round 1



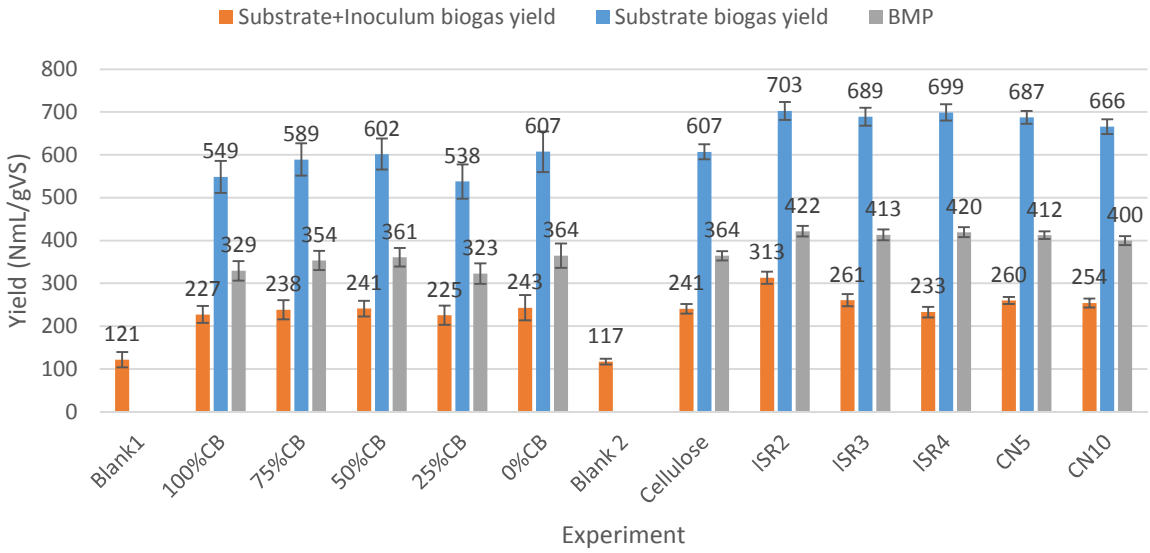
4.2.7. Production yield

With the results of biogas monitoring the net volume of biogas production is known. Therefore the biogas production yield (mL of biogas produced by one mg VS of substrate) can be calculated. Moreover, taking a reference value for CH_4 content in biogas (60%, see discussion in Section 5.3.2.5) BMP can be estimated. Results can be found in Table 4-19 and Graph 4-17.

Table 4-19 Summary: biogas production potential and BMP of the 13 configurations

Test	Inoculum (mgVS)	Substrate (mgVS)	Net biogas produced (NmL)	Corrected biogas produced (NmL)	Substrate + Inoculum biogas yield (NmL/gVS)	Substrate biogas yield (NmL/gVS)	BMP (NmL_{CH4}/gVS)
Blank1	988.0	0.0	119.9		121	**-**	**-**
100% CB	988.0	329.3	299.3	180.7	227	**549**	**329**
75%CB	988.0	329.3	314.0	194.0	238	**589**	**354**
50%CB	988.0	329.3	317.3	198.2	241	**602**	**361**
25%CB	988.0	329.3	297.0	177.1	225	**538**	**323**
0%CB	988.0	329.3	319.9	200.0	243	**607**	**364**
Blank 2	632.8	0.0	74.1		117	**-**	**-**
Cellulose	632.8	212.9	203.4	129.3	241	**607**	**364**
ISR2	632.8	317.6	297.4	223.2	313	**703**	**422**
ISR3	632.8	212.3	220.4	146.3	261	**689**	**413**
ISR4	632.8	157.2	183.9	109.9	233	**699**	**420**
CN5	632.8	211.7	219.4	145.5	260	**687**	**412**
CN10	632.8	211.0	214.2	140.5	254	**666**	**400**

Graph 4-17 Biogas production yield (NmL_{biogas}/gVS) and BMP (NmL_{CH4}/gVS) for the 13 experimented configurations



CHAPTER 5

Discussion

5.1. Introduction

In the wastewater treatment system assessment, the goal is to answer the following question: is that system performing as required? Why? The ultimate goal of a wastewater treatment system is to reduce loads and concentrations in order to comply with the law or other standards. In consequence apart from system performance (removal rates) the resultant water quality also determines the success of the system.

Moreover, besides taking the system as a whole it is interesting to study the performance of the individual elements of the wastewater treatment system. However, to be able to quantify the removal rates of individual treatment elements, monitoring of points within the wastewater treatment line needs to be carried out, which was only done in the second monitoring campaign of December 2016.

For the work performed in the laboratory first the methods used are discussed with a focus on what can be learned from this experience and what errors not to repeat. Then results are analysed and compared both between each other and with the literature. Finally, a theoretical biogas production estimation is presented, based on the COD measurements.

5.2. Wastewater treatment system assessment

5.2.1. Elements of the system

The following comments on what was concluded from visits to the dairy farm site are presented. A logic of following the water line through the whole system is used, starting with the source of water, followed by the production of wastewater, the treatment system, and the receiving body.

a) Water source

Water sources are groundwater and rainwater. In Uruguay, extracting water from a well inside your property is free of charge; groundwater quality was not analysed, but it was assumed good and plenty. The CRS dairy farm dwell is one of the 471 swells located in the Southern Cretaceous Aquifer (DINAMIGE-MIEM, 2009). The aquifer has an average depth of 62.2 m and an annual extraction of only 2.4% over total volume.

b) Wastewater generation

In Uruguay, the milking parlours consume an important amount of water; therefore, it is important to have a rational management over this resource (CONAPROLE, 2008). A way of decreasing water usage, and in consequence wastewater generation, is to perform a dry solids removal with rake in the waiting esplanade at the end of the milking operation and before washing down with water. Dry solids removal has the following benefits and it is recommended:

- Dung collection that can be composted or distributed on soil as soil improver.
- Making floor cleaning easier with an associated reduction of water needed and as wastewater generation.
- Increasing useful life of downstream treatment units by decreasing amounts of coarse solids in wastewater.

c) Solids chamber

After the wastewater is generated, it is conducted to a pre-treatment step with a rectangular solids chamber. During most of the visits the chamber was totally filled up with solids (Figures 5-1). When the chamber is so full there is pretty much no working volume for suspended solids to settle. In that situation solids retention is null.

Figures 5-1 Solids chamber on 14/12/2016 (left) and 21/09/2016 (right)

The amount of solids passing through the solids chamber was evident after conducting the wastewater sampling. In the wastewater sampling point, located in the gutter connecting the chamber with the anaerobic pond, the water level was not enough for the automatic sampler take to operate underwater. Therefore, a small dam had to be built in order to achieve a sufficient water depth in the wastewater stream (left in Figures 5-2). The dam acted like a settler and after it was dissembled lots of settled solids appeared (right in Figures 5-2).

Figures 5-2 Resultan settled solids after sampling dam is removed (14/12/2016)

It was said by a worker that solids were removed once a week every Tuesday or Thursday. On the first sampling campaign, on Monday, solids were removed just before sampling by the operator with a backhoe (Figures 5-3). Considering chamber depth is 2 m it could be observed that very little solids were actually removed. In that day wastewater production filled the empty volume with water before overflowing to the gutter, where sampling was made.

Figures 5-3 Left: operator with backhoe. Right: solids chamber after after solids remotion (24/10/2016)

d) Anaerobic pond

In the sampling campaign of October 2016 (Uruguayan middle spring) the anaerobic pond could not be sampled because it was filled until the top with sludge, no water phase could be sampled (Figures 5-1). Therefore only the "overall" removal rate could be estimated for that day (meaning the removal due to biological system which is the two stabilization ponds).

Figures 5-4 Dry anaerobic pond on October 2016

The lack of maintenance on the anaerobic pond, particularly sludge removal, was evident. When a pond is left like that, with very little water phase, hydraulic retention time (HRT) is very small and poor water treatment is expected to happen in the anaerobic pond.

In the sampling campaign of December 2016 the anaerobic pond was found with water phase and it could be sampled (Figures 5-2). Apparently a portion of the sludge had been removed from the anaerobic pond. This allows to calculate the individual performance (in terms of removal rates) of the two ponds, besides the overall removal.

Figure 5-5 Anaerobic pond on December 2016

e) Facultative pond

Apart from having the perimeter overgrown with plants the facultative pond seemed to be working properly. The pond could be sampled in both campaigns.

f) Polishing ponds

The last shallow plug-flow ponds (Figures 4-9) might have functioned some years ago but now it cannot be considered integral part of the treatment system. The state of abandonment, the fact that it is dry in some periods of the year, and the nature of the construction (excavated in the ground without cement coating) make these element to act more like a mere floodable channel than a stabilization or maturation pond.

g) Tajamar

The Organisation for Economic Co-operation and Development (OECD) has a classification of trophic level for inland waters based on concentration of phosphorus (TP), chlorophyll-a and turbidity with Secchi disk. For the OECD eutrophic level is achieved when TP concentration lie between 35-100 µg/L and hypertrophic category is defined for TP>100 µg/L.

Phosphorus in the tajamar was measured only in the second monitoring campaign and result of TP was 2.52 mg/L (2520 µg/L), two orders of magnitude above the eutrophication limit. Moreover, considering local law for surface water quality (Decree No. 253/79, see Section 2.3.4) from the regulated parameters that were measured (BOD_5, NH_3, NO_3^-, TP, pH and FC), the tajamar exceeds in everyone but FC (Table 5-1).

Table 5-1 Tajamar water quality compared to national standards (results from December 2016)

Parameter	Unit	*Tajamar*	Decree No. 253/79 Class 3 water quality
pH	-	9.68	6.5-8.5
BOD_5	mg/L	39 & 95	<10
Ammonium*	mgN/L	7.50	<0.02
Nitrates	mgN/L	0.35	<10
TP	mgP/L	2.52	<0.025
FC	FCU/100mL	200	<2000 in every of 5 samples (average <1000)

The measured parameter was dissolved ammonia, not free ammonium. But knowing pH and temperature (25 °C) free ammonia can be estimated from total ammonia value as they have a relation depending on equilibrium constants like shown in Figure 5-6. A simple on-line ammonia calculator was used to determine ammonium content of reported TN in 7.50 mgNH_3-N/L (Bührer, 2017).

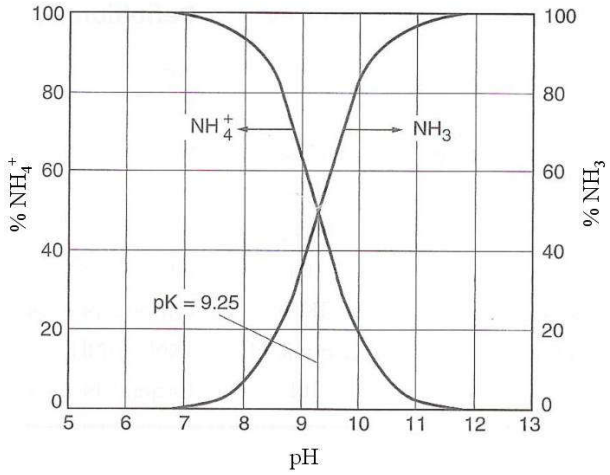


Figure 5-6 Ammonia/ammonium relation with pH at 25 °C (Kunz, 2016)

During the visits from 21/09/2016 to 03/02/2017, from early spring to mid-summer, it was observed a change in the colour of the water surface from blue to green illustrated with Figures 5-7. Phytoplankton analysis showed an increase in cell concentration from October to December to February from $1.1x10^6$ cell/mL to $8.6x10^6$ cell/mL and to $1.7\ x10^7$ (see Section 4.2.3).

Figures 5-7 Tajamar views from September '16 (left) and February '17 (right)

The phytoplankton community in the tajamar is totally dominated by Cyanobacteria (Class Cyanophyceae). Only in February eukaryotic microalgae (Class Chlorophyceae) appeared in the analysis representing only 0.1% of total cell count.

Moreover, it appears to be a swift in dominating cyanobacteria species from spring to summer. In spring genus *Planktothrix* dominated with 81% and 61% of cell count (October and December samples respectively), while in summer the phytoplankton community was largely dominated by *Raphidiopsis/Cuspidothrix* with 95% of total cells being identified as belonging to that genus.

Figure 5-8 presents illustrations of mentioned dominating cyanobacteria. All types are potentially producers of cyanotoxins. Table 5-2 presents cyanotoxins related to these genus. It is good to recall that presence of potential producers of cyanotoxins does not mean that cyanotoxins are always produced and/or liberated. In fact in both monitoring campaigns

microcystin-LR and saxitoxin were analysed from tajamar samples and results were below detection limit (<0.34 μg/L for microcystin-LR and <1.0 μg/L for saxitoxin).

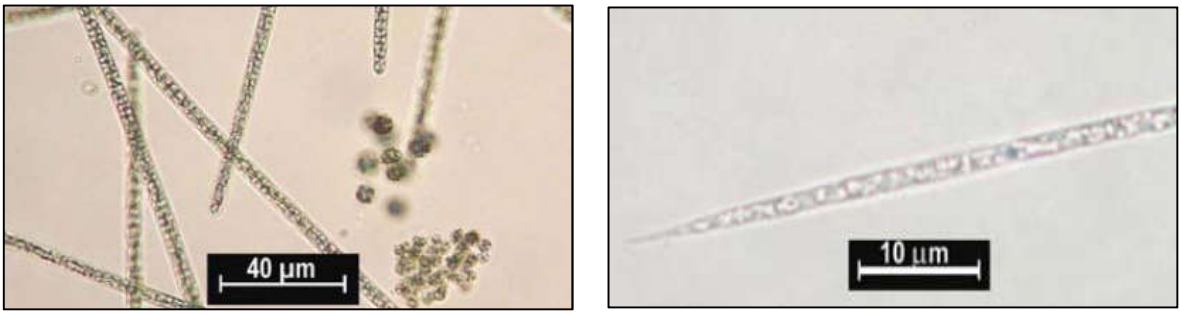


Figure 5-8 Planktothrix agardhii (left) and Raphidiopsis mediterranea (right) (Bonilla, 2009)

Table 5-2 Cyanotoxin potential of cyanobacteria found in the tajamar (Bonilla, 2009)

Genus or species	Cyanotoxin production
Raphidiopsis	Microcystin, lipopolysaccharides (LPS), homoanatoxin-a, anatoxin-a, 4-hydroxyhomoanatoxin-a
Planktothrix	Microcystins (RR, LR, Dirr), anatoxin-a, aplysiatoxin, saxitoxin and LPS.
Cuspidothrix issatschenkoi	Anatoxin-a, saxitoxin

5.2.2. Treated effluent quality

If the facultative pond is completely mixed, the sample from the pond also represents the final effluent (water exiting the pond). In reality that does not happen and there might be physicochemical differences between points in the pond (for completely mixed assumption at least some mechanical mixing should be present).

In this study only grab samples were taken in the facultative pond. Samples were taken considering the irrigation pump intake, which is closer to the influent than the effluent. In consequence actual effluent quality may deviate from the results obtained. The results obtained in this monitoring campaign should represent a primary approximation to effluent quality.

For a deeper understanding of the system performance and the effluent quality monitoring should be intensified not only in space (sampling in different points) but also in time sampling at different times of the day, also in winter and autumn, etc.

a) Standard selection

To compare the results with a standard first it has to be decided what standard to take as reference. The main one, the only legal-binding, is the Uruguayan law. In consequence, the national decree no. 253/79 is the most important to comply with (see chapter 2.3.4). However, the local regulations are often outdated or uncomplete.

This is the case for Uruguay where irrigation water quality is not regulated. Therefore, international standards need to be considered, as they have better reputation, are more complete and generally are taken as reference when formulating local laws. WHO and FAO standards are considered here (previously presented in Section 2.3.5).

b) Defining system's effluent

The studied system has characteristics that makes defining the system's effluent not a simple task. This is because the water that exits the facultative pond by overflow, travels along a permeable and floodable channel until reaching the *tajamar*. In consequence it is partially infiltrated into the ground and partially discharged into the *tajamar* which in that case would be the receiving water body. Moreover, the water from the facultative pond is pumped for fodder crop irrigation. That is, all uses are included: irrigation, infiltration and discharge.

Water exits the *tajamar* by infiltration, evaporation, human subtraction for irrigation and by a small creek that flows downhill. Therefore one could argue that the *tajamar* works as a last treatment step (polishing pond) before discharge, infiltration or irrigation and it should be considered as the system's effluent.

However, due to the fact that *tajamars* are generally built for water provision, the classic approach is to treat it as the receiving water body, classify it as "Class 3 surface waters", and consider the facultative pond's effluent as the "discharging system's effluent". What is sure is that in the current situation both the facultative pond and the tajamar should comply at least with the fodder crop irrigation standards and the infiltration standards, and one of the two with the discharge standards.

c) Standards compliance

Regarding faecal coliforms and irrigation standards for health protection, there is no health guideline set by WHO for category B (Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees). Following these guidelines it's acceptable to use water with any amount of FC for fodder crop irrigation which is the case at the CRS. There is however a guideline for intestinal nematodes which was not analysed.

In the case that a category A is sought (Irrigation of crops likely to be eaten uncooked, sports fields, public parks), results show the facultative pond does not comply with the standard of <1000 FCU/100 mL (920,000 and 24,000 FCU/100 mL in October and December respectively) but the *tajamar* does with 200 FCU/100 mL (December sampling campaign).

Considering the uncertainty about how the effluent's use would be classified by local authorities, it is compared with all the contemplated standards. Table 5-3 compare the results of the facultative pond samples with the national law and the FAO guidelines.

Table 5-3 Comparation of facultative pond's water quality and standards

Parameter	Unit	Facultative Pond	Decree No. 253/79 discharge	Decree No. 253/79 infiltration	FAO restricted irrigation
pH	-	**8.38**	6.0-9.0	5.5-9.0	6.5-8.0
EC	mS/cm	**4.7 &3.6**	-	-	<0.7 (none); 0,7-3,0 (slight to moderate); >3.0 (severe)
TS	mg/L	**3,040**	-	<700	-
TSS	mg/L	**236**	<150	-	-
BOD_5	mg/L	**192 & 445**	<60	-	-
Nitrates	mgN/L	**0.19**	-	-	<5 (none); 5-30 (slight to moderate); >30 (severe)
TP	mgP/L	**27.1**	<5	-	-

If the effluent from the facultative pond was to be subject of the Decree No. 253/79 for discharge or even irrigation, it does not comply with any of those standards neither for TS, TSS, BOD_5 or TP. Moreover electric conductivity results fit within severe restriction for irrigation according to FAO.

Table 5-4 with the results obtained in the *tajamar* is presented for comparison purposes and to work on the "what if *tajamar* is part of the wastewater treatment system" scenario. Water quality of this artificial shallow water body of 5 hectares of area barely comply with discharge standards: pH and TSS are out of range, TP is below the limit and BOD_5 complied in December but not in October.

The pH was high probably because of the effect of photosynthesis (increased pH with increased CO_2 intake due to cyanobacteria photosynthesis) and sampling was made in a sunny day. Besides pH, total solids content complies with infiltration standard. In addition, both EC and nitrates comply with non-restricted irrigation.

Table 5-4 Comparation of tajamar's water quality and standards

Parameter	Unit	*Tajamar*	Decree No. 253/79 discharge	Decree No. 253/79 infiltration	FAO non restricted irrigation
pH	-	**9.68**	6.0-9.0	5.5-9.0	6.5-8.0
EC	mS/cm	**0.38 &0.49**	-	-	<0.7
TS	mg/L	**455**	-	<700	-
TSS	mg/L	**159**	<150	-	-
BOD_5	mg/L	**95 & 39**	<60	-	-
Nitrates	mgN/L	**0.35**	-	-	<5
TP	mgP/L	**2.52**	<5	-	-

d) Impact in *tajamar*

The facultative pond's effluent is still concentrated and further treatment is needed to comply with regulations. Considering hypereutrophic state of the tajamar, it is very likely that the wastewater treatment system's effluent has significantly contributed to deterioration of its water quality.

In addition, it is good to recall the key role diffuse pollution has over surface waters eutrophication. The presence of other agricultural activities in the *tajamar*'s surroundings means organic matter-rich surface run-off reaching the water body. Therefore the milking parlour is not the only contributor to hyper-eutrophication levels on the artificial shallow water body.

5.2.3. System's performance

a) Removal rates

The most important parameter to assess the performance of a treatment system are the removal rates. In biological systems, the removal rates are affected by: temperature and weather factors, water characteristics (dissolved oxygen, pH, and presence of inhibitors), substrate (wastewater) composition and the degree of adaptation of microbial community to that substrate profile, the HRT which sets the contact time between substrates and biomass, the SRT which affects the growth and endogenous residue of biomass, among others.

Table 5-5 presents the overall removal rates (from influent to facultative pond) of the most relevant of the measured parameters in the two sampling campaigns: 24/10/2016 and 14/12/2016. In the December campaign it was possible to calculate the individual removal rates of the anaerobic pond (with respect to the influent) and the facultative pond (with respect to the anaerobic pond effluent).

Table 5-5 Removal percentages of overall biological system and individual elements of the biological wastewater treatment system (December campaign only)

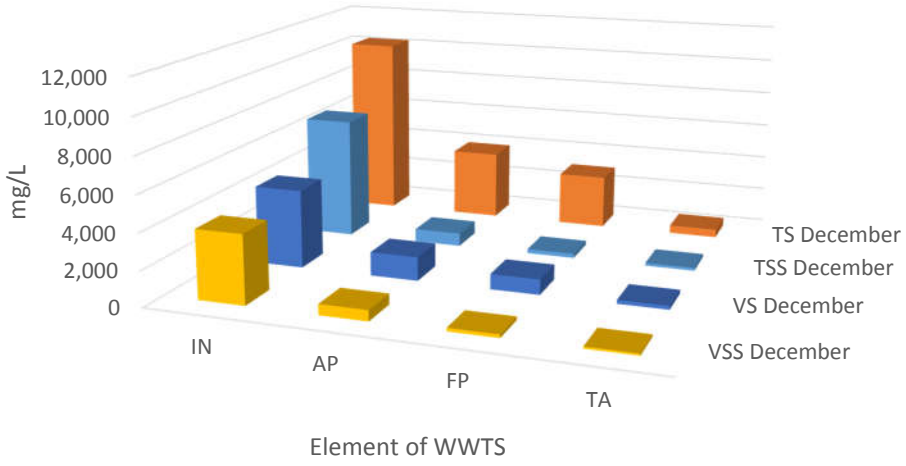
Unit	Overall removal	Anaerobic pond	Facultative pond
October – Middle spring			
COD	**92%**	-	-
BOD_5	**95%**	-	-
TN	**68%**	-	-
FC	**>43%**		
December – Final spring/early summer			
COD	**90%**	79%	53%
SCOD	**92%**	87%	36%
BOD_5	**96%**	90%	57%
TN	**81%**	48%	62%
TP	**48%**	6%	45%
TS	**70%**	62%	22%
VS	**81%**	70%	36%
TSS	**97%**	89%	68%
VSS	**95%**	84%	68%
FC	**>99%**	>43%	97%

b) Concentration evolution

The removal rates can be better visualized presenting the concentrations levels of the selected parameters in bar graphs. The fluctuation in concentrations through the different element of the system can be observed: from the influent (IN), to the anaerobic pond (AP), the facultative pond (FP) and the receiving water body the *tajamar* (TA).

First is interesting to analyse solids behaviour throughout the system (Graph 5-1). The solids concentration of the biological system influent are still high even after the solids removal chamber (around 10 gTS/L and 4 gVS/L), evidencing the poor performance of the solids chamber, which was totally filled in December and partially filled in October (see Section 5.2.1.3).

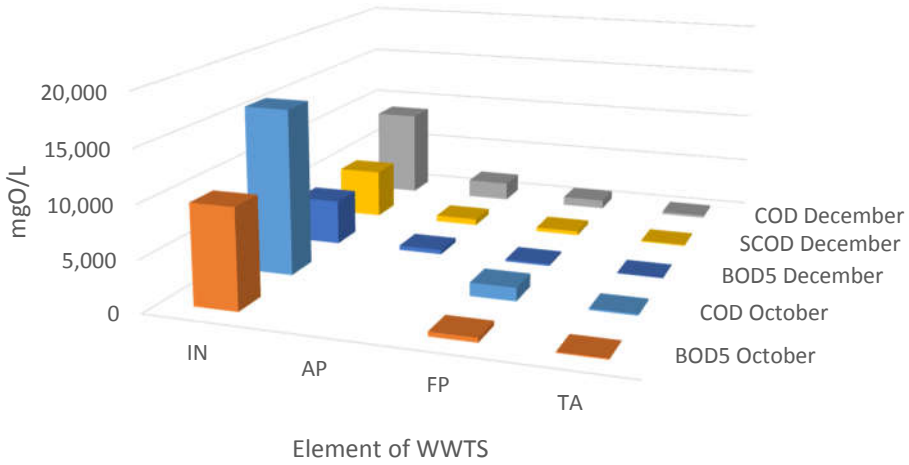
Graph 5-1 Evolution of solids concentration throughout the system



This has consequences because if not properly maintained (periodic sludge removal) the ponds fill up, as it was actually verified. Because the anaerobic pond comes first, it can be seen that most of solids are removed by the anaerobic pond (62% TS and 70% VS removal) compared to the facultative pond (22% TS and 36% VS removal). Overall volatile solids (representing organic solids) where removed more efficiently (81%) than total solids (70%) indicating some degree of biological degradation of the particulate matter on top of the physical settling.

Graph 5-2 presents the concentration of organic matter as oxygen demand, differentiated in chemical (COD), chemical soluble (SCOD) and 5-day biological (BOD_5). The anaerobic pond could not be sampled in the October campaign due to lack of a water phase in the pond. A first observation is the similarity between values of SCOD and BOD_5 which could be an indicator of the correlation between soluble organic matter and biodegradable organic matter.

Graph 5-2 Evolution of oxygen demand concentration throughout the system



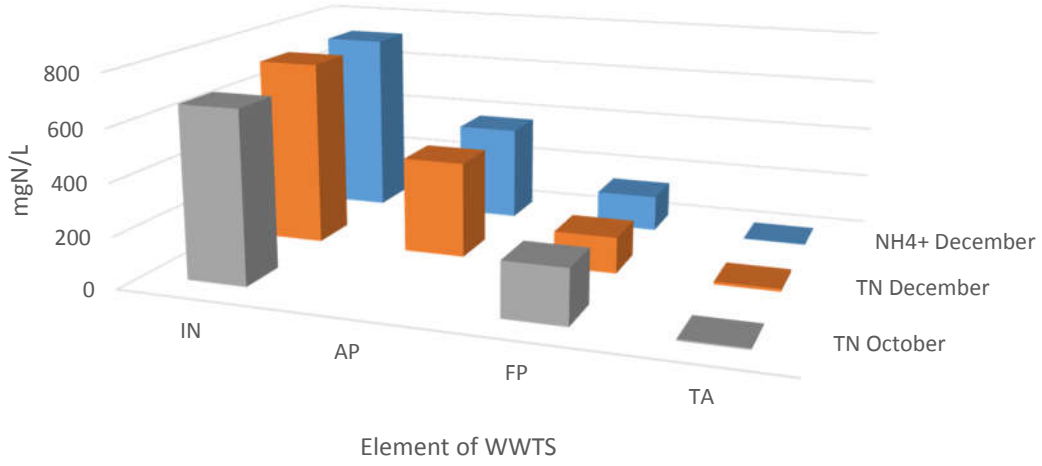
Again values of biological influent samples are very high, evidencing significant amount of fresh organic solids (dung) with high oxygen consumption potential. Interestingly, organic matter content in biological influent (pre-treatment effluent) were higher in October than in December.

In October solids from the pre-treatment chamber were removed by the operator a few hours before the milking parlour wastewater generation. This operation must have loosen solids from the bottom of the chamber that afterwards were carried with wastewater resulting in higher BOD_5 and COD in the analysed samples.

The chemical and biological oxygen demand were also removed with more intensity in the anaerobic pond than the facultative pond. An overall removal of COD, SCOD and BOD of 79%, 87% and 90%, respectively, was registered in the anaerobic pond; while a removal of 53%, 36% and 57%, respectively, in the facultative pond.

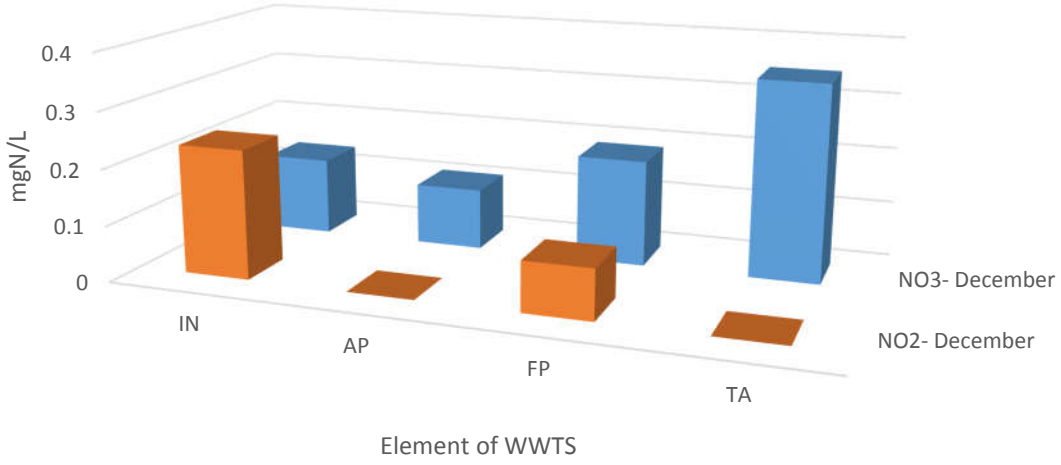
In relation to nitrogen, dissolved ammonia composed the big majority of total nitrogen in the influent and in the ponds (Graph 5-3). Overall TN removal was 81% and more removal (in percentage) was observed in the facultative pond than in the anaerobic pond (62% vs. 48%). This make sense because besides the loss of NH_3 volatilized in both ponds, in the facultative pond nitrification-denitrification processes could occur, that end up removing N from the system in form of gaseous N_2.

Graph 5-3 Evolution in concentrations of nitrogen (TN and ammonia) throughout the system



Nitrate and nitrite concentrations were very low (0.1-0.2 mgN/L approximately) although when including the *tajamar* in the comparison (Graph 5-4) nitrate concentration increases showing a correlation with the degree of oxygen in the matrix: lower concentration in the influent and the anaerobic pond (where dissolved oxygen is very low), increased in the facultative pond where some photosynthesis occurs and maximum (0.35 $mgNO_3^-$-N/L) in the *tajamar* where high photosynthesis activity results in big amounts of O_2 liberated.

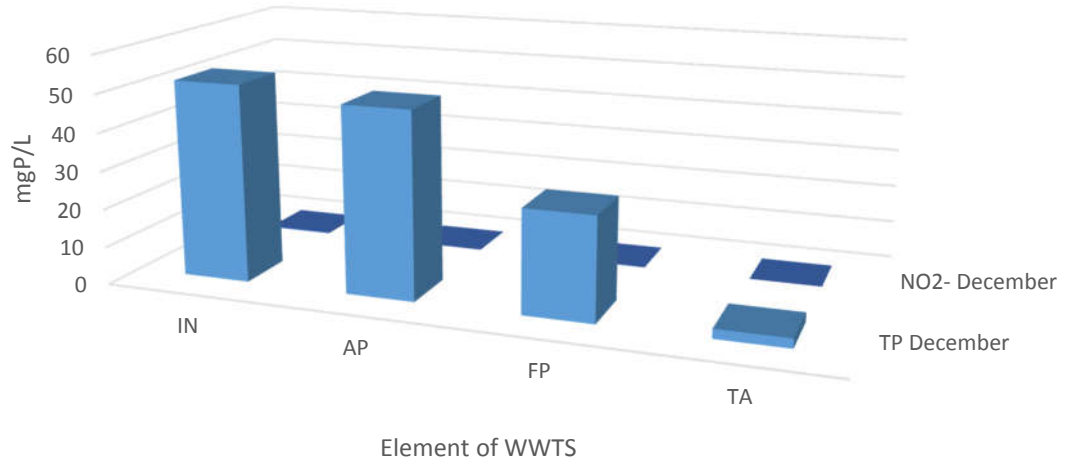
Graph 5-4 Evolution of nitrate throughout the system



Nitrate is the product of complete nitrification of ammonia held by a consortium of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). AOB utilize O_2 for starting the process and generate intermediate nitrite. Therefore nitrite or nitrate is unlikely to be formed in anaerobic environments.

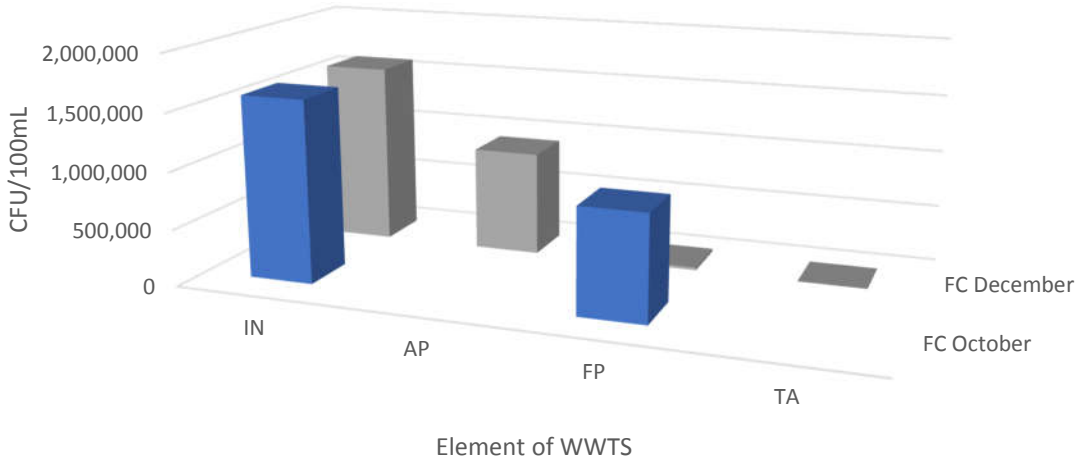
Phosphorus results are presented in Graph 5-5. Total phosphorus (TP) was barely removed in the anaerobic pond (6%), but soluble phosphorus (SP) increased 35%. When the organic matter is anaerobically degraded, organic P is liberated as soluble PO_4^{3-}. The facultative pond removed more TP (45%) and even more SP (67%). The main mechanism for P removal in water of facultative ponds is settling as unbiodegradable particulate P. This involves soluble P uptake for biological growth of microalgae and bacteria and later decay.

Graph 5-5 Evolution of phosphorus concentration (total and soluble) throughout the wastewater teatment system



Finally, a decrease in faecal coliforms concentration can be seen throughout the wastewater treatment system in Graph 5-6. Because in both campaigns influent values of faecal coliforms could not be determined (both resulted above quantification limit of $1{,}6x10^5$ FCU/100mL), the removal percentages cannot be calculated. Nevertheless it is possible to know that overall removal was at least 43% in October and at least 99% in December.

Graph 5-6 Evolution of faecal coliform concentration thourghout the system (IN concentration expressed as maximum quantification limit)



c) Comparison with literature references

For reference values on removal percentages the Uruguayan guide for the integral management of waters in dairy establishments (CONAPROLE, 2008) was consulted. According to this source expected BOD_5 removal of anaerobic pond is between 50-70% and in facultative ponds is found between 60-80%, resulting in overall removals of 80-96%.

This is concordant to the results of the monitoring campaigns where BOD_5 overall removals of 95% and 96% for October and December respectively were reached. However, individual removals did not behave like reference values. In the December campaign BOD_5 was removed more efficiently in the anaerobic pond than in the facultative pond (90% vs. 57%).

5.2.4. Maintenance

In any constructed wastewater treatment system the process of preserving is vital. This includes at least cutting weeds and grass from pond's banks, paths and working area in general, and having a solids removal plan for pre-treatment chamber and stabilization ponds.

The plan should improve also the solids disposal that can have a better use than pilling them up next to the milking parlour. This is an area where there is much to improve and which could rapidly lead to the system perform better.

5.2.5. Design assessment

Another way to assess the treatment system is to check with design parameters if the size of the pond make sense according to the influent loads. There are many approaches and references for designing wastewater stabilization ponds; particularly, considering the wide range of wastewaters treated with ponds, from domestic origin to chemical-based industries or an agro-industry as is this case.

Taking that into account it was decided to use the Uruguayan milking parlour wastewater treatment guidelines to estimate de size of treatment ponds in a way it is comparable to local reality. The Uruguayan guidelines rely on reference values of organic loading rates (OLR) as BOD_5. In the anaerobic pond a volumetric OLR of 33 $g_{BOD}/d/m^3$ is taken and in the facultative pond a surface OLR in the range of 8-9 $g_{BOD}/d/m^2$ is proposed (CONAPROLE, 2008).

Depths used were the same as current ones (nominal): 3 m for anaerobic pond and 1.5 m for facultative pond. To be on the very safe side a surface OLR is taken as 9.0 $g_{BOD}/d/m^2$ and a wastewater flow of 18.5 m3/d is chosen which is low (see Section 4.1.2.3).

The influent BOD_5 monitoring gave considerable different values between campaigns: in October BOD5 was as twice as much as in December. Average value was taken (7.0 g/L) resulting in an influent load of 129.5 kg_{BOD}/d. The concentration of BOD_5 in facultative pond's influent (anaerobic pond's effluent) was taken from the December monitoring campaign.

Table 5-6 present the results of the design assessment which clearly show that, based on the local guidelines, the current treatment ponds are significantly under-designed and they should be or bigger or more units working in parallel or series.

Table 5-6 Designed dimentions compared with current dimensions of treatment ponds

Parameter	Unit	Current value	Designed value
Anaerobic pond			
HRT	d	78	212
Volume	m^3	1440	3924
Surface	m^2	480	1308
Surface	ha	0.048	0.13
Length (width = 20 m)	m	24	65
Facultative pond			
HRT	d	39	74
Volume	m^3	720	1369
Surface	m^2	480	913
Surface	ha	0.048	0.091
Length (width = 20 m)	m	24	46

As suggested in the guidelines (CONAPROLE, 2008) a water mass balance for the worst month was done in order to check that the flow does not stop between ponds and exiting facultative pond. The mass balance is presented in equation 5-1. The run-off component was not included as the treatment ponds are constructed in an elevated place.

Equation 5-1 Water mass balance over treatment ponds

$$Effluent = Influent + Rain + Runoff - Evaporation$$

$$Q_{effluent}\left(\frac{m^3}{month}\right) = Q_{influent}\left(\frac{m^3}{month}\right) + (Rain - Evap)(\frac{mm}{month}) \times A_{pond}(m^2)/1000$$

A yearly average rainfall in the studied area is 90 mm/month while yearly average evaporation is 157 mm/month. Following the guidelines the "critical month" has to be determined which was December with a Rain-Evaporation balance of -201 mm/month. In conclusion if the anaerobic pond influent flow is 18.5 m^3/d in the driest month the anaerobic effluent would be 10 m^3/d. And if the facultat ive pond influent is 10 m^3/d, its effluent is 4 m^3/d. That is, even in the driest month of the year the designed treatment ponds would have water flowing in and out.

5.3. Batch experiments

5.3.1. Methods

a) Material collection

A first cyanobacteria harvesting attempt was made on the 30th of November, 2016. At that time, it was not clear which harvesting method was the most efficient; therefore it was decided to try first with an electric (12 V) submersible pump that operated at 900 L/h flow (Figures 5-9). It was soon clear that using a pump was not the best option.

First, the flow was too low; thus, the filtering process was very slow. Second, entering the *tajamar* with the pump caused a lot of sediment re-suspension which was suctioned by the pump and ended up in the filtrate. A good cyanobacteria harvesting method for anaerobic digestion should not collect the sediments, which are composed by a larger fraction of unbiodegradable organic matter.

Figures 5-9 Harvesting cyanobacteria with submergible pump. Left: holding the pump take just below the surface. Right: water outlet was put into the 0.20 µm pore-opening phytoplankton net

The second harvesting attempt was succesfull1y accomplished two weeks later, on 14/12/2016. It was decided to harvest cyanobacteria by directly throwing the phytoplankton net into the tajamar from the shore (Figures 5-10). The net had to be dragged quickly in order not to sink and collect material only from the subsurface and not from the bottom (sediments).

The net was later dewatered by swinging the net as shown in Figures 5-11. Once most of water left the net, the "filtrate" (that is, the fraction remaining inside the net) was transferred to a plastic container which was later stored at low temperatures.


Figures 5-10 Harvesting cyanobacteria with rope. Left: throwing the net. Right: transfering the filtrate to a bucket.


Figures 5-11 Draining water from the phytoplankton net after dragging it with the rope from inside the water

The "manual" method (without using the pump) proved to be much more efficient. By directly submerging the net a few meters inside the lagoon, a considerable bigger flowss could be handled. That is, the time needed for the overall harvesting operation was much shorter. Moreover, the quality of the filtrate improved considerable by leaving the sediments out. This was possible because the phytoplankton net is a lightweight item which takes time to sink, so by dragging it is possible to keep it near the surface without disturbing the sediments.

b) Set-up

The materials utilized in this research proved to be suitable for performing the BMP tests (see section 3.3.2). The system was composed of glass opaque amber colour 500 mL bottles, butyl rubber seals and aluminium caps (mechanically sealed with a crimper machine). The sealed bottle proved to be good enough for withstanding the internal elevated pressure build up due to biogas production. From the 39 test bottles only one bottle failed (CN 5-1). It was broken when being manually mixed. It was suspected that it contained a little stone that hit the glass and favoured the glass to break.

There was no pressure transmitter available in order to monitor the pressure inside the vessels, although it can be calculated from the biogas production. Applying the ideal gas law, knowing the volume of biogas measured in the inverted graduated cylinder at 1 atm of pressure and 303 K of temperature, the number of moles of an ideal gas that came out of the bottle can be calculated. Then, considering that those moles of gas were inside the bottle before puncturing the seal (compressed in 250 mL of headspace) the pressure can be calculated. The highest pressures calculated with the ideal gas law in the two BMP rounds are presented in Table 5-7.

Table 5-7 Maximum biogas measurements of round 1 and 2 converted to headspace overpressures

Replicate	Day and hour of measurement	Measured biogas volume at 30 °C (mL)	Moles of ideal gas	Headspace overpressure (mbar)
ISR 2-3	08-Feb 18:22	68.0	$2.7x10^{-3}$	276
100% WW-2	18-Dec 14:47	30.0	$1.21x10^{-3}$	122

Valero, et al. (2016) reported that the methane production from dairy manure was not affected by headspace overpressures in the range of 600-1000 mbar (Valero, et al., 2016). Therefore, from the pressure point of view, daily biogas monitoring (meaning daily pressure release) did not affect the methane production.

c) Measurement of biogas

On Friday 16th of December, 2016, biogas production batch tests were first launched. The inverted graduated cylinder method was used to measure the volume of biogas that exited the vessel when punctured. The method looks simple but many important aspects were considered to get reliable readings. Some of these aspects are described below.

The first aspect to be considered is the position of the tube in relation with the water inside the graduated cylinder (Figure 5-12). The tube occupies volume so it has not to interfere with the headspace of the inverted graduated cylinder. Otherwise, the tube will add volume to the amount of gas in the headspace and the measure will be distorted.

The needle at the end of the connecting plastic tube was allowed to interfere with the headspace. This was because the needle adds up an insignificant amount of volume which is within the error of the method.

Figures 5-12 Details on the position of the tube inside the inverted graduated graduated cylinder

Because of the above the lower the end of the tube the more practical the method turns. That s, more headspace is available allowing the execution of several readings before resetting the graduated cylinder (that s, taking the volume of water out and refilling it). For example in a 50 mL graduated cylinder it was possible to execute three readings of approximately 15 mL biogas production each, making the completion of all readings much faster (not forgetting there were 18 to 21 bottles to be read daily).

On the other hand, if the end of the tube is low or if the water level is high, the end of the tube gets under water and water enters forming a significant "hydraulic seal" (left in Figures 5-13). This situation was observed to set a minimal pressure in the headspace of the test bottle in order for the biogas to flow into the inverted graduated cylinder.

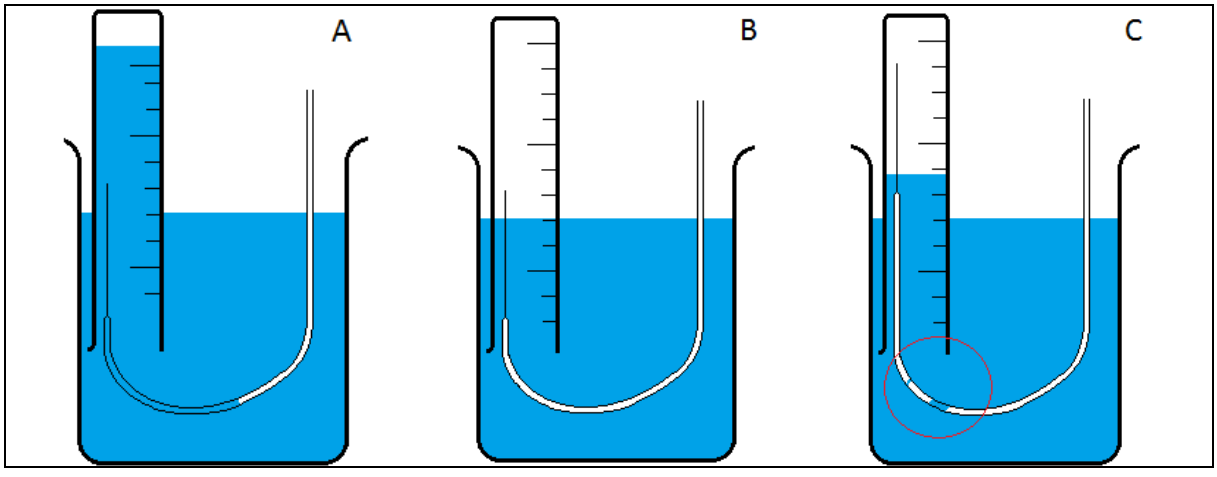


Figure 5-13 Three situations regarding the hydralic seal explained. Left (A): tube has a significant amount of water in it; high pressures are needed for biogas to flow into the invrerted graduated cylinder. Centre (B): tube has no water; biogas can freely move in. Right (C): tube has an aliquot of water; biogas needs some pressure to flow break the "hydraulic seal" and flow into the inverted graduated cylinder.

Tests were done with a basic set-up, presented in Figure 3-12 on page 60, and it was observed that, when the tube had a significant amount of water (A in Figures 5-13), a biogas production

of 5 mL (which represents 50 mbar of headspace overpressure) was the lowest measurable biogas production. Injecting lower volumes of air into the test set-up would result in no gas flowing into the inverted graduated cylinder.

Therefore, when biogas production rates started to be lower, situation A was avoided and it was worked with less amount of water inside the tube like shown in picture C, right of Figures 5-13.

If the tube was dry inside (no hydraulic seal, as shown in picture B, Figures 5-13, centre) the gas could freely come in or out the inverted graduated cylinder's headspace. In this optimum situation accuracy is maximum; therefore, very little (<1 mL) volumes of biogas production can be read. However, keeping the tube dry inside was nearly impossible and most of the time it was it was worked in the situation C (right in Figures 5-13).

A final aspect to mention is the effect of the inverted graduated cylinder height (altitude). In situation A or C of Figures 5-13 water won't flow down if the inverted graduated cylinder is elevated. However, it was noticed that when the graduated cylinder is moved up, water tends to go down and headspace volume is slightly increased. Although variation of volume was less than 1 mL, care was taken to maintain water meniscus at the same height (same head) when doing the readings in order to neutralize this effect.

d) Wet chemistry analysis

In general wet chemistry analysis did not return satisfactory results. The first attempt (see Section 3.3.3.6) was performed as in Pham, et al (2013) and consisted in changing the measuring liquid pH after having captured biogas in the inverted graduated cylinder headspace. Thymol blue indicator was used to confirm a water pH greater than 9.6 by changing the colour from yellow to blue. At the beginning problems come up because water colour inside the graduated cylinder would not change. After adjusting the cylinder's height and adding magnetic stirring the colour finally changed (Figures 5-14).

Figure 5-14 Water with thymol blue after being added a few NaOH pellets

After checking water had turned completely basic, 5-10 minutes were waited without seeing any change in the concave meniscus' height. Because digestion biogas with no CO_2 portion pretty much does not exists, results were unsatisfactory. Three attempt with the replicates of 100% CB (first round of experiments) were made and the same result was obtained.

The attempt may have failed due to presence of air. After this experience, the volume of the plastic tube was measured and it resulted in 13 mL. The average biogas measured in 100% CB on that day was 6.3 NmL. Apparently, what entered at the inverted graduated cylinder is air displaced by biogas, or a much diluted mix of both. Pham, et al (2013) did not mention what volume of tube was to be used to connect the vessel with the graduated cylinder, but is evident that the smaller the better.

Following the reasoning that no connecting tube was better, it was decided to re-try the analysis with a syringe, thus eliminating the plastic tube. This worked better and values different than zero were got although still not reliable. When a closed inverted bottle containing basic solution was used and, after bubbling biogas from a syringe into it, a displaced liquid was collected and measured results were from 81 to 93%.

These values are higher than the 50 to 70% expected (Parajuli, 2011), which generates distrust in the method. Particularly, because the chance of CO_2 not being completely removed is high, considering that the system was rudimentary (see Figures 3-16 in Chapter 3).

Moreover when there was no liquid displacement, just headspace measure, the results obtained were between 96 and 102%. This simply makes no sense. Therefore it is recommended to not take this values for calculating BMP, choosing instead values from literature. And it was learnt that, for making true BMP tests, availability of biogas composition analysis with gas chromatography is pretty much essential, when using manual biogas monitoring like in this work.

5.3.2. Results

a) Comparison of production curves

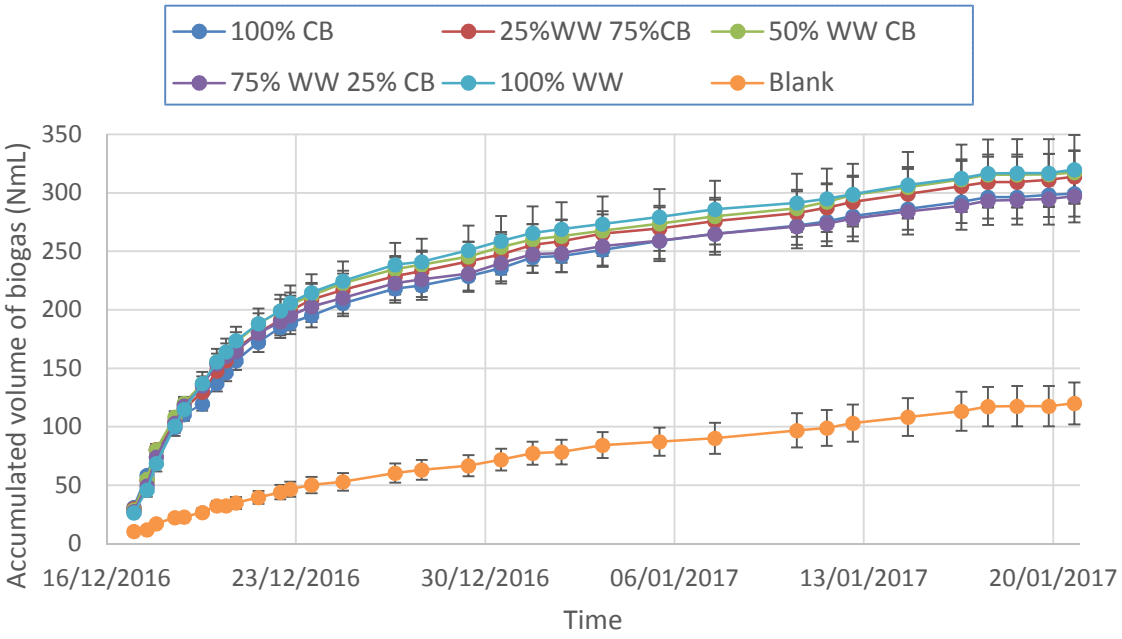
Daily measurement allowed to confection of time graphs presented in Section 4.2.4 of Results chapter. In addition Graphs 5-7 and 5-8 are presented showing all the accumulated biogas production curves (average values of triplicates, standard deviation represented as error bars) for the six tests of the first round of experiments, and the seven evaluated configurations of the second set of experiments, respectively.

As expected all the tests (100% CB, 25% CB, 50% CB, 75% CB and 100% WW for the first round of experiments and ISR2, ISR3, ISR4, CN5 and CN10 for the second round of experiments) presented the characteristic shape of growth curves with an exponential phase followed by a stationary phase. There was no lag phases in the tests indicating inoculum suitability. This was not the case for the control tests (blanks and positive).

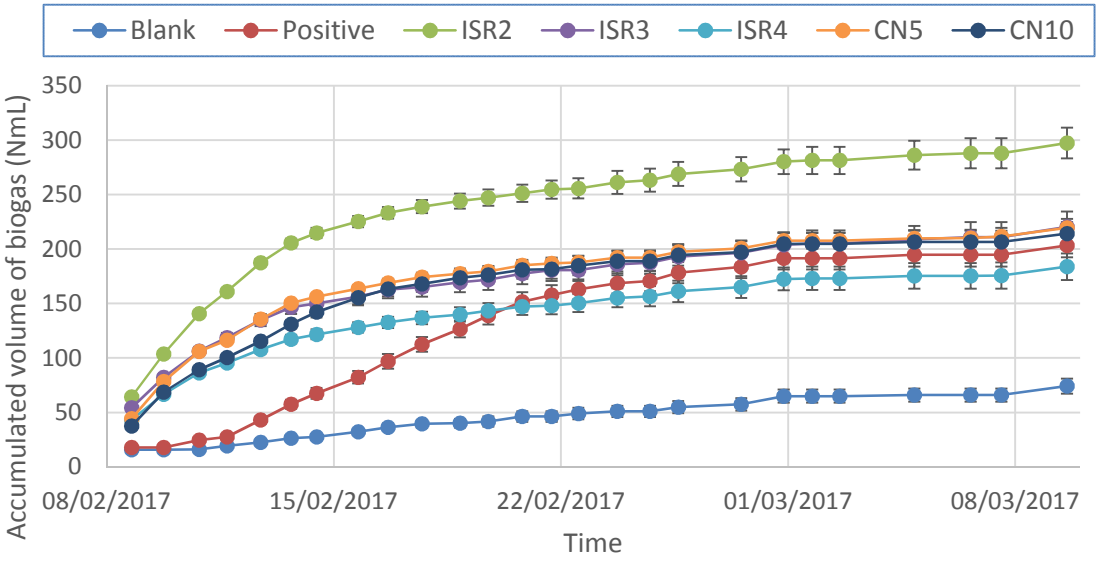
The two blank curves were linear (Graphs 4-8 and 4-15), suggesting a steady biogas production due to endogenous digestion that could have continued for many days. On the other hand, the

positive control showed a different biogas production curve (Graph 4-14) with a four-day lag phase followed by an exponential phase of 10 days and then the stationary phase. This was reasonable considering that the inoculum from a wastewater treatment anaerobic pond is not used to digest microcrystalline cellulose and some time for adaptation is needed (four days in this case).

Graph 5-7 Comparation of biogas production for the six configurations of the first round of experiments

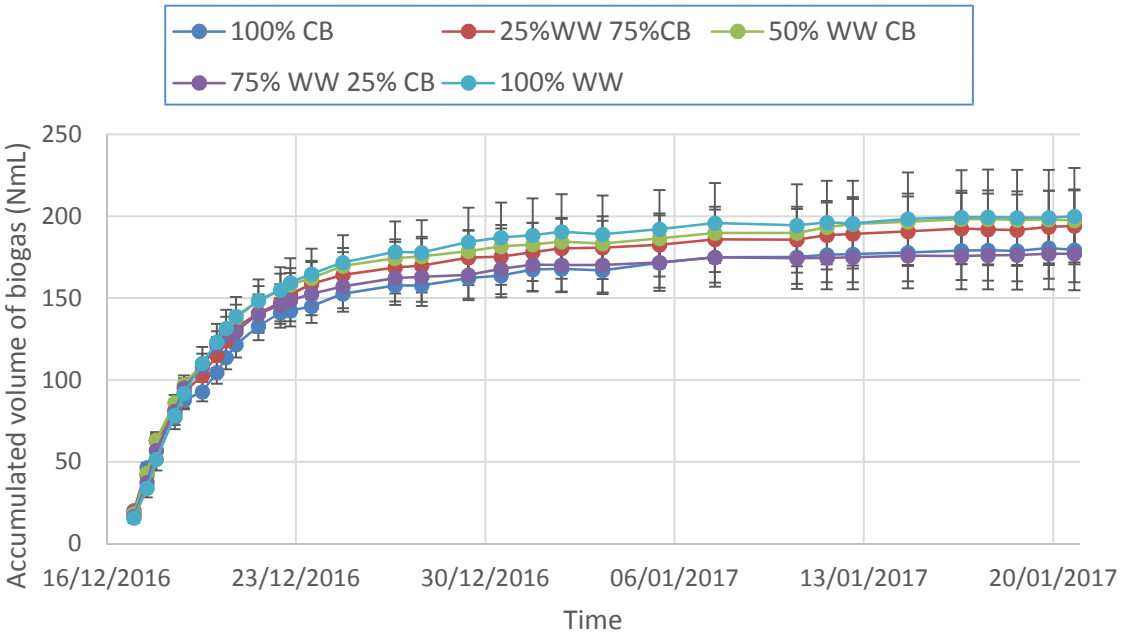


Graph 5-8 Comparation of biogas production for the seven configurations of the second round of experiments

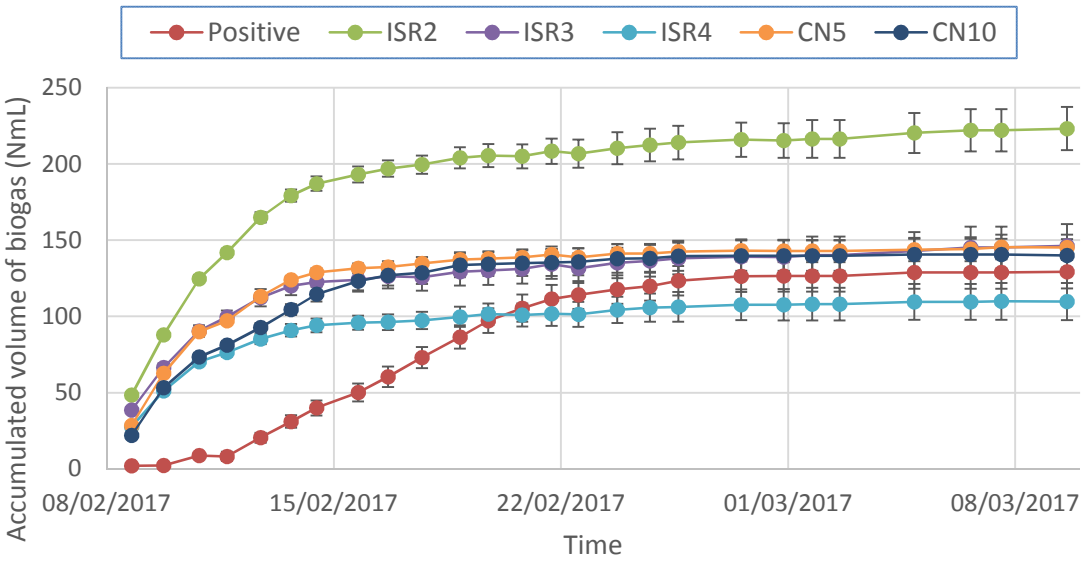


The curve changes significantly when the biogas production of the blank is subtracted from the accumulated volume of the other tests. In this corrected biogas production curves (Graph 5-9 for the first round of experiments and Graph 5-10 for the second round of experiments) the division between the exponential phase and the stationary phase is accentuated with the stationary phase curves approximating to a horizontal asymptote. This means that substrate-based biogas production lasted only one week in both BMP rounds.

Graph 5-9 Comparation of corrected biogas production for the otherfive tests of the first round (blank subtracted)



Graph 5-10 Comparation of corrected biogas production for the other six tests of the second round (blank subtracted)



The first round of BMP tests was continued for 34 days and the second round for 29 days. Tests were run in stationary phase for another 23-24 days for the first round of experiments and 14-15 days for the second round. This was done because the amount of measured biogas was still significant, due to inoculum endogenous biogas production, and because the BMP reference of consideration (Holliger, 2016) states the following:

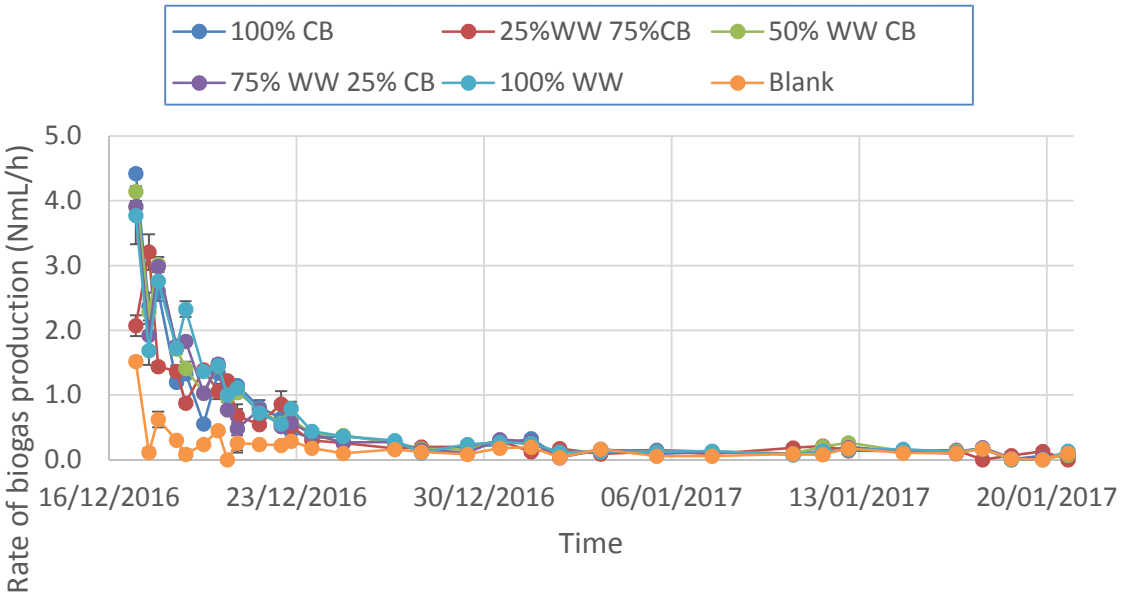
"The duration of the BMP tests should not be fixed in advance, and tests should only be terminated when daily methane production during three consecutive days is <1%of the accumulated volume of methane (i.e. $BMP_{1\%}$)."

b) Comparison of rate curves

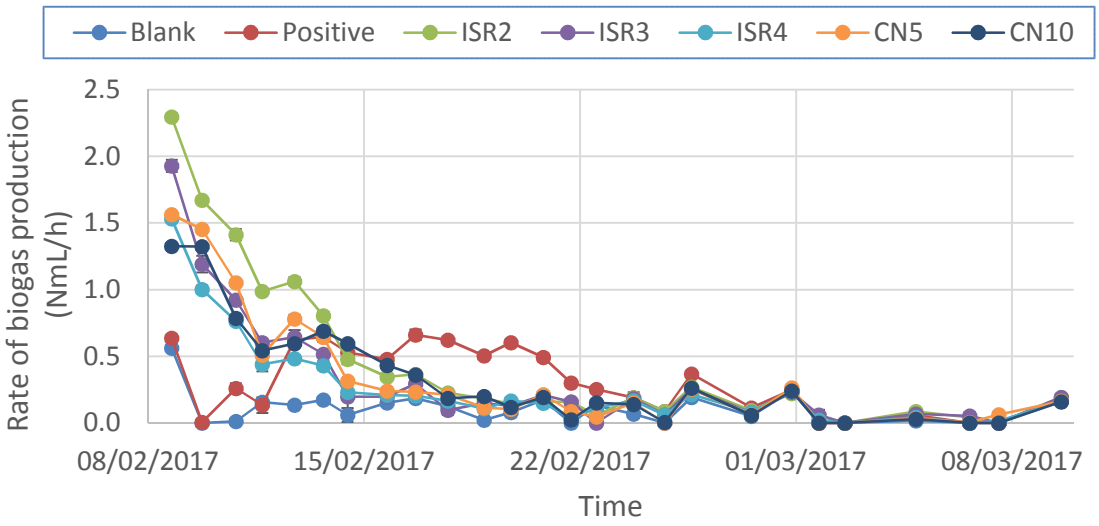
Knowing the exact time every biogas measure was executed, the biogas production rate curves can be compared. First round rates are grouped in Graph 5-11 while Graph 5-12 includes the rates for the second round tests. The rate curve shows clearly the inverse exponential nature of biogas production (starts very high and decreases rapidly). It can be seen that in both rounds rates over 0.5 NmL/h were obtained during first 4-6 days (same period than exponential phase in production curves).

This is associated with consumption of readily biodegradable organic matter first (dissolved volatile fatty acids, sugars, nucleic acids, etc.) and after degradation of slowly biodegradable organic matter (non-dissolved lipids, carbohydrates polymers, proteins, etc.).

Graph 5-11 Comparation of biogas production rate for the six tests of the first round of experiments



Graph 5-12 Comparation of biogas production rate for the seven tests of the second round of experiments



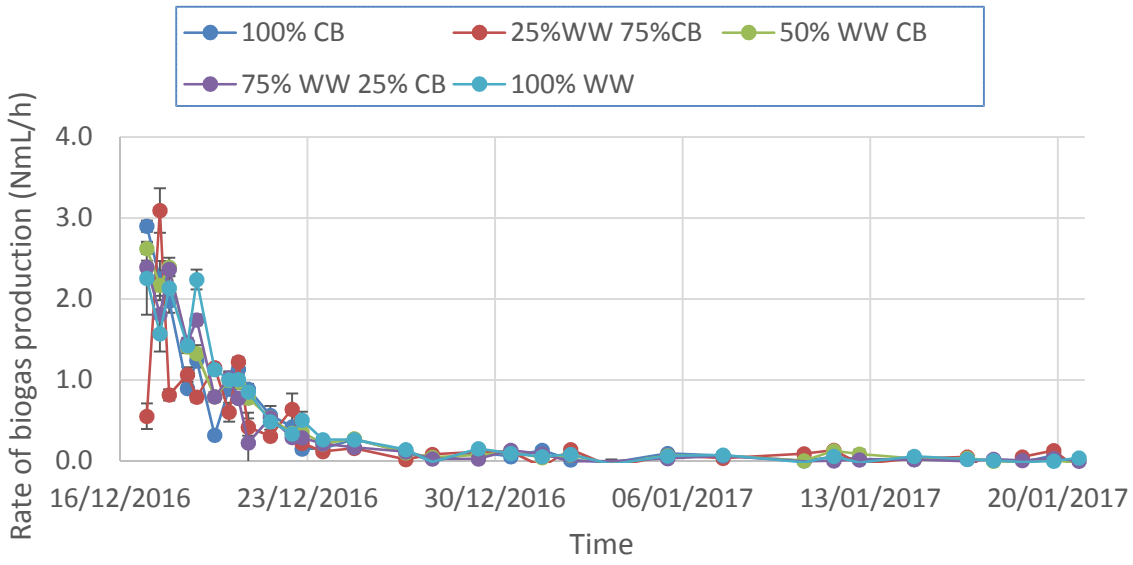
In general first round experiments shown higher production rates than second round experiments (as well as higher biogas production). This make sense because first round experiments contained more amount of volatile solids than second round experiments. The reasons for different amount of VS between rounds and tests are: changes in inoculum-to-substrate ratio, changes in concentration of materials and a limit of 250 mL of liquid volume (in order to leave the same 250 mL of headspace in every bottle).

A variable that could also explain the difference in biogas production between the two rounds of experiments is the measuring liquid: tap water was used in the first round while in the second round a saturated NaCl solution prepared with distilled water was employed due to its advantages discussed in Section 2.4.5.a.

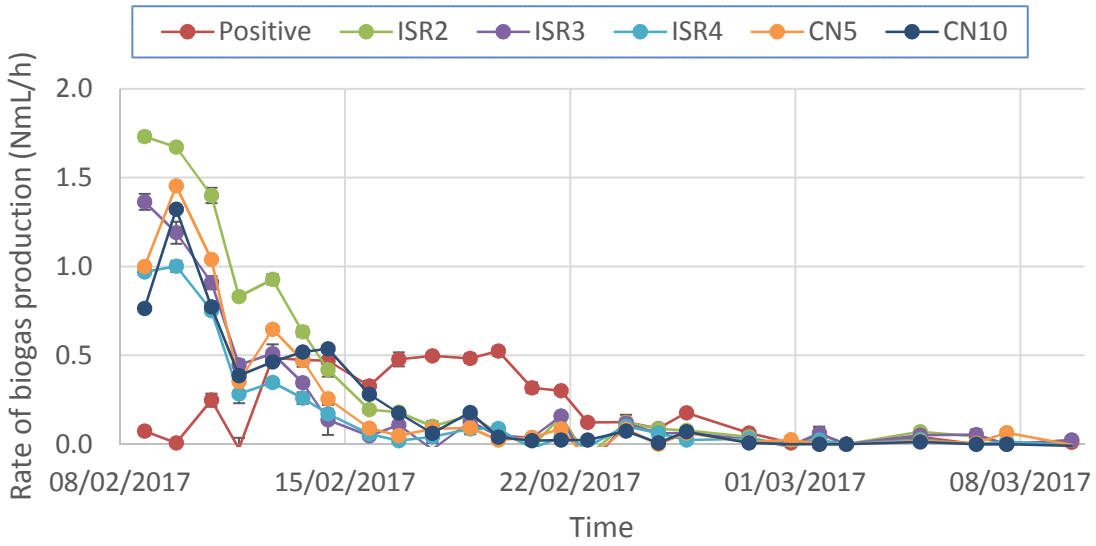
For this biogas production rate, when subtracting blank value the shape of the curve is not significantly changed (Graph 5-13 for first round and Graph 5-14 for second round). However some unexplainable fluctuations in the second round (on days 25/02, 28/02 and 09/03) disappear.

On the other hand, the corrected biogas production rate during the stationary phase becomes practically 0 NmL/h, evidencing substrate depletion. Due to a lag phase that lasted for four days, the positive control experiment is the only one out of the 13 experiments returning a different rate curve (red in Graph 5-12 and 5-14).

Graph 5-13 Corrected biogas production rates (blank rate subtracted) for tests of round 1



Graph 5-14 Corrected biogas production rates (blank rate subtracted) for round 2 tests



c) COD removal

Analysis made on the biological material during the first days of the second round of experiments are presented in Table 4-12, page 82. The initial CODs for tests ISR2, ISR3 and ISR4 (all containing as substrate a 50:50 (mgVS) wastewater:cyanobacteria solution) were calculated. After the experiments were complete, the COD of the resultant liquor was measured; and the COD removal was calculated (Table 5-8)

Table 5-8 COD removal in tests ISR2, ISR3 and ISR4 of the second round of experiments

Parameter	ISR2	ISR3	ISR4
Initial amounts			
CB (mgCOD and % of initial COD)	255 (20%)	171 (15%)	128 (13%)
WW (mgCOD and % of initial COD)	312 (24%)	208 (19%)	153 (15%)
AI (mgCOD and % of initial COD)	740 (56%)	740 (66%)	740 (72%)
Initial COD			
Initial COD (mg/L)	5,229	4,477	4,083
Initial substrate-only COD (mg/L)	2,268	1,515	1,122
Final COD			
Final COD (mg/L)	3,270	3,470	2,570
%COD removed	37%	22%	37%
Removed COD (mg)	490	252	378

First thing to remark is the significant uncertainty in the COD result for the anaerobic inoculum (15% RSD). This is because it was measured only by duplicate and a dilution of 1:100 was needed in order to reach the measurement range, which was 25 to 1,500 mgCOD/L. Such kind of dilutions applied on heterogeneous materials like an anaerobic inoculum (semi-solid containing stones and granules) add significant uncertainty.

Considering that the anaerobic inoculum is the main COD contributor (57-73% in those tests), big uncertainty on the COD values means large uncertainties to all the calculations, including the removed COD. This situation is aggravated by the final measures which gave results which are difficult to explain as follows: ISR 3 gave highest final COD value when it should have been between ISR2 and ISR4, considering the amount of substrate.

When pipetting from the test vessels sludge particles could be observed, as shown in Figure 5-15. Therefore heterogeneity of the materials may be the reason for this contradictory COD result.

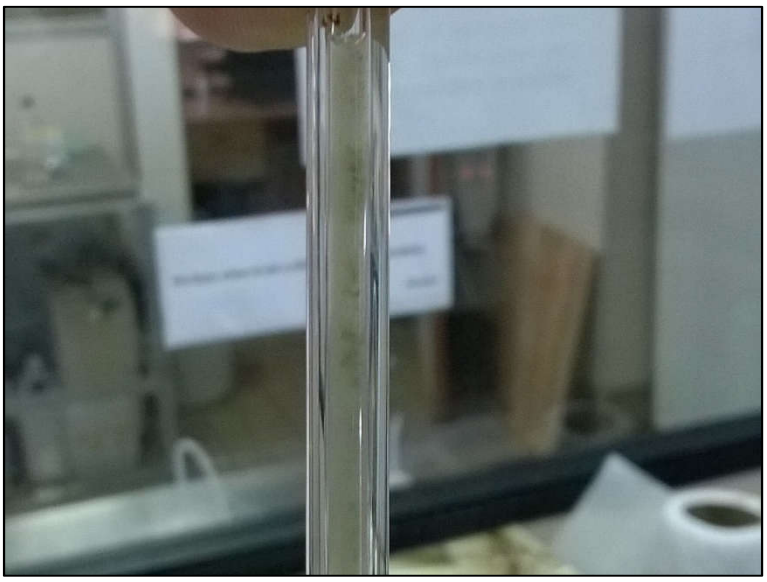


Figure 5-15 Heterogenous anaerobic inoculum dilution in the pipette

Taking into account that the other two tests (ISR 2 and ISR 4) gave concordant COD removals (both 37%) it is reasonable to assume an error happened in final COD measure for ISR 3. Therefore for future anaerobic co-digestion of 1:1 (mgVS) milking parlour wastewater and native harvested cyanobacteria an expectable COD removal percentage would be closer to 37%.

d) Sludge and CH_4 production

As described in chapter Fundamental Concepts (Chapter 2) the generated CH_4 and the removed COD are linked. Assumptions for COD mass balance are presented in Table 5-9. First, the sludge production is estimated. Afterwards, the methane COD production can be calculated by subtracting the effluent and sludge COD to the influent COD. As explained in Section 2.4.1.6, the methane COD can be converted to the volumetric production. The results of the calculation are presented in Table 5-10.

Table 5-9 Assumptions for COD mass balance

Item	Value	Comment
Sludge biological growth fraction ($kgVSS/kgCOD_{removed}$)	0.05	(Lier, 2016)
Sludge unbiodegradable COD settling fraction ($kgTSS/kgCOD_{removed}$)	0.05	(Lier, 2016)
Methane solubility in water at 30 °C (mL/L)	27.6	Known physicochemical property (see Section 2.4.1.4)
COD content of sludge ($gCOD/gVSS_{sludge}$)	1.42	Based on stoichiometric conversion ($C_5H_7O_2N$) (Lier, 2016)
Organic fraction in sludge (VSS/TSS)	0.7	(Lier, 2016)
Sludge concentration (mgTSS/mL)	80.0	(Lier, 2016)

Table 5-10 Sludge production calculation

Parameter	ISR2	ISR3	ISR4
Wastewater			
Initial COD (mg/L)	5,229	4,477	4,083
Final COD (mg/L)	3,270	3,470	2,570
Sludge			
Volatile sludge production (mgVSS)	24.5	12.6	18.9
Settled sludge production (mgTSS)	24.5	12.6	18.9
Sludge production (mgCOD)	59.1	30.4	45.6
Sludge production (mL)	0.31	0.16	0.24
Methane			
CH_4 COD production (mg)	430.6	221.3	332.6
CH_4 volume production at STP (mL)	150.7	77.4	116.4
CH_4 dissolved in water at 30 °C (mL) =	6.9	6.9	6.9
CH_4 liberated (mL) =	143.8	70.5	109.5
Normalized CH_4 liberated (NmL) =	**159.6**	**78.3**	**121.5**

e) CH_4 content in biogas

When comparing the measured values of produced CH_4, with the calculated values upon measurements of COD removal, knowing the precise concentration of CH_4 on the biogas is crucial. The methane content of the biogas was not possible to be analytical determined in this work as explained in Section 5.3.1.d. Therefore, one option to estimate the CH4 content can be by dividing the calculated methane production with the produced biogas. Results are presented in Table 5-11.

Table 5-11 Calculating CH_4 content based on COD measures

Parameter	ISR2	ISR3	ISR4
Calculated CH_4 liberated (NmL)	159.6	78.3	121.5
Measured biogas liberated (NmL)	297.4	220.4	183.9
Calculated CH_4 content in biogas (%)	54%	36%	66%

Results for the three replicates studied vary significantly (36-66%) but are useful to give a rough idea of the CH_4 content in the measured biogas. Considering the uncertainty around the ISR3 results because the final COD value were outside the expectable range, it is more likely that the CH_4 content in the produced biogas is closer to 60%. These results reinforces the position of not considering the wet chemistry results as valid, because all analysis gave results larger than 80%.

The proportion of materials (CB:WW) in the three tests were the same (1:1 in mgVS); in consequence, it can be assumed that the biogas composition is similar. A way to test which value (%CH_4) fits bests with the three COD results is to analyse the error between the calculated CH_4 and "measured" CH_4 (that is the measured biogas multiplied by the %CH_4).

The percent error is calculated using Equation 5-2. It was found that 54% of CH_4 content returns the smaller total error (Table 5-12 and Graph 5-15). If ISR3 result is not considered then CH4 content that best fit is any in the range 54-66%.

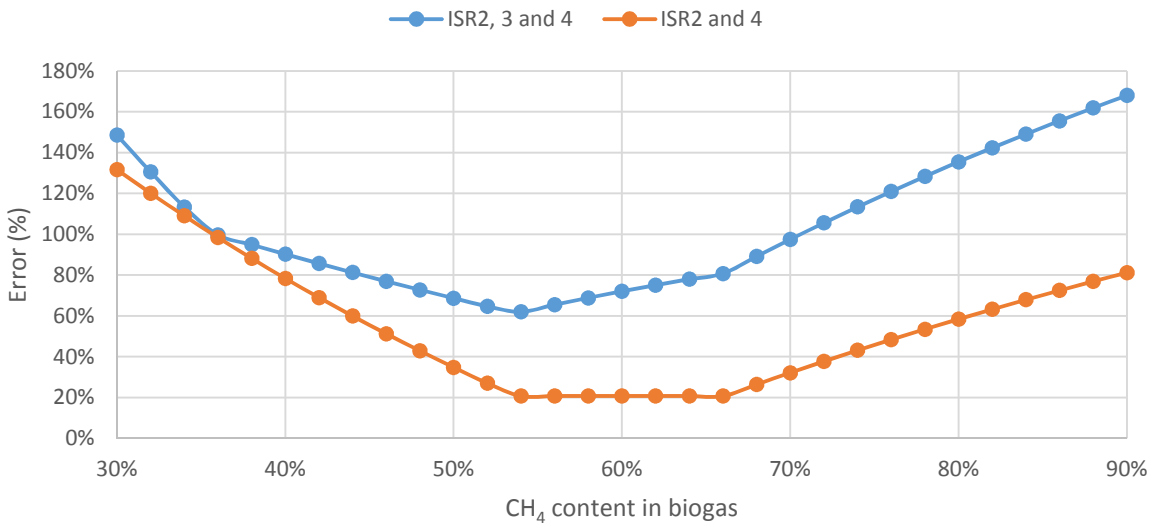
Equation 5-2 Percent error

$$Error\ \% = \frac{Absoulte\ difference}{Average} \times 100$$

Table 5-12 Calculating CH_4 content assuming it is the same for the three tests

Parameter	ISR2	ISR3	ISR4
Calculated CH_4 liberated (NmL)	159.6	78.3	121.5
Measured biogas liberated (NmL)	297.4	220.4	183.9
Assumed CH_4 content in biogas (%)	54%	54%	54%
Estimated CH4 measured (NmL)	160.6	119.0	99.3
Error (%), total = 62%	1%	41%	20%

Graph 5-15 Percent error of calculated and measured vs. CH4 content, assuming biogas is the same for the three tests



A third methodology to estimate the CH_4 content in the biogas is with using the positive control. The theoretical BMP for cellulose is 414 mL/gVS (Holliger, 2016). The positive control gave a biogas production of 607 mL/gVS. That is, the CH_4 content of biogas was 68%. However, this does not help to estimate the methane content of wastewater and cyanobacteria co-digestion as it is reasonable that pure microcrystalline cellulose gives a different biogas production and composition.

As introduced in Section 2.4.3 CH_4 content can be calculated from the TOC/COD ratio through stoichiometric ratios. However maximum TOC/COD ratio (for a 100% CH_4 content in biogas) is 5.33 whereas measured TOC/COD ratios for wastewater and cyanobacteria were 6.14 and 5.94 respectively (Table 4-11 in page 81). This suggests an analysis interference (due to e.g. chloride, ferrous iron or sulphides) that overestimated COD.

Comparing with the scientific literature, Panpong et al. (2015) reported a CH_4 composition in the range of 35-60% when doing anaerobic co-digestion of cannery seafood wastewater and pure Microcystis sp., with and without glycerol. Furthermore Cantu (2014) on his master's

thesis “Improving the methane production in the co-digestion of microalgae and cattle manure” obtained very broad results for CH_4 content in biogas ranging from 5% to 83%.

f) Biogas yield

The first round of experiments produced methane yields from 329 to 364 NmL_{CH4}/gVS (average value (346±19) NmL_{CH4}/gVS). Second round of experiments results were within 364 and 422 NmLCH4/gVS with an average value of (406±24) NmL_{CH4}/gVS.

In theory, the experiment carried out at 50%CB on the first round of experiments is similar in terms of proportion to the experiment ISR3 carried out on the second round. However this is not in terms of total amount of volatile solids because of differences between material’s concentrations from one campaign to the other (for example anaerobic inoculum was 83% water in the first round whereas in the second round it had a water content of 59%).

It was expected that biogas (or methane) yield to be similar. They were not the same but they were close, with only 13% error between the first round of experiments (361 NmL_{CH4}/gVS) and the second round (413 NmL_{CH4}/gVS).

Panpong et al. (2015) reported 192, 111 and 81 mL_{CH4}/gVS for the co-digestion of cannery seafood wastewater and pure Microcystis sp. with 3%, 6% and 10% ($mgVS_{CB}/mgVS_{WW}$) respectively. Moreover, Miao et al. (2014) obtained a yield of 213 mL_{CH4}/gVS when digesting cyanobacteria with swine manure as inoculum, at an ISR of 2. Regarding the reported milking parlour wastewater digestion yields, theoretical methane yield from dairy cattle manure was estimated as 469 mL_{CH4}/gVS but reported results are about half ofthat value (240 mL_{CH4}/gVS_{added}) (Labatut, Angenent and Scott, 2011).

The BMP experiments carried out in this research containing only wastewater resulted in a yield of 607 NmL_{biogas}/gVS, representing 364 mL_{CH4}/gVS (100% WW 0% CB experiment of the first round). However, it must be pointed out again that the CH_4 content was assumed; that is, despite the high biogas production observed on this research (considering the broad possible range of CH_4 content in biogas discussed in previous section), the methane yield may not be that high.

On the contrary, when comparing the measured biogas yields with values reported in literature for cyanobacteria digestion, the results reported in this research are within the reported range of values in the literature. Dębowski, et al. (2013) reported yields of 287-587 mL_{biogas}/gVS and 210-350 mL_{CH4}/gVS. The BMP experiment conducted only with cyanobacteria as substrate (labelled 100% CB and executed in the first round of experiments) produced a biogas yield of 549 NmL_{biogas}/gVS and, considering a 60% of CH_4 content, a methane yield of 329 NmL_{CH4}/gVS.

In conclusion, the obtained biogas yield for all the BMP experiments carried out exhibited relatively large values that can be associated with analytical errors that may eventually overestimated the biogas production; however, these values were not that large to conclude that are totally out of range. This experience can be taken as a first approximation in the field and further research with advanced equipment (automatic biogas monitoring, gas chromatography) should be carried out to obtain confirmatory results.

CHAPTER 6

Conclusions

6.1. Wastewater treatment system assessment

Monitoring campaigns were executed correctly and data obtained allowed a complete assessment of the wastewater treatment system at the CRS dairy farm. It was concluded that the treatment ponds work well in terms of removal rates, despite being overloaded (or undersized) and that current performance is not enough to produce a water quality effluent that complies with the standards.

The maintenance of the treatment system should definitively be improved; particularly, the solids removal. Finally, the construction of new treatment units should be studied if compliance with standards is needed.

6.2. Batch experiments

The first round of experiments provided statistically supported results that confirmed that the native cyanobacteria community is as much anaerobically digestible as the milking parlour wastewater in terms of biogas potential. All five mixes of varying proportions of these two biological materials (including pure tests) gave statistically undifferentiated biogas production yields within the range of (538 ± 122) NmL_{biogas}/gVS to (607 ± 144) NmL_{biogas}/gVS.

From the second round of experiments the following conclusions can be made:

- Varying levels of ISR (2, 3 and 4) did not affect the biogas yield (average values of (697±7) NmL_{biogas}/gVS) indicating that the substrate (a 1:1 cyanobacteria:wastewater (gVS) mix) has low inhibition potential due to VFA accumulation.
- Increasing C/N ratio does not improve biogas yields: natural N content of the mix is low enough to not cause inhibition by NH_3 release.
- Digestion of the 1:1 cyanobacteria:wastewater (gVS) mix gave similar values than observed during the first round of experiments with an overall value of (673 ± 48) NmL_{biogas}/gVS.

In addition, considering that the biogas yields were high compared to the reported values in the literature, the studied biological materials seem not to need pH buffer and/or nutrient solution to assure correct anaerobic digestion. All the experiments (including blank controls and positive control) provided curves with the expected form and behaviour, indicating good experiment design, execution and monitoring.

Wet chemistry analysis of biogas did not return reliable results; therefore, the CH_4 content in biogas could not be determined. Nevertheless, it was estimated in 60%, and therefore, the following BMP values could be calculated:

- Native harvested cyanobacteria: (329 ± 69) NmL_{CH4}/gVS.
- Milking parlour wastewater: (364 ± 86) NmL_{CH4}/gVS.
- 1:1 cyanobacteria:wastewater (gVS) mix: (404 ± 29) NmL_{CH4}/gVS.
- 1:4 cyanobacteria:wastewater (gVS) mix: (323 ± 73) NmL_{CH4}/gVS
- 4:1cyanobacteria:wastewater (gVS) mix: (354 ± 69) NmL_{CH4}/gVS

This work achieved a collateral objective without being posed as such; this research showed that it was possible to achieve relevant results using low cost analytical technology. This has the disadvantage of precision and accuracy. Because materials and methods were simple and rudimentary, results are approximate with high relative standard deviation. On the hand, it has the advantage of universality; that is, expensive and sophisticated equipment were not used in the laboratory work, therefore it can be replicated in any low-tech laboratory.

6.3. General conclusion

The anaerobic co-digestion of milking parlour wastewater with natural-occurring cyanobacteria was successfully and thoroughly evaluated and thus the general objective was accomplished.

6.4. Prospects

This work can be taken as a first approximation to the real BMP value of native cyanobacteria, wastewater and its co-digestion. For better results and inter-laboratory comparison, it is recommended to repeat the BMP experiments held in this research, but using modern equipment such as automated anaerobic respirometer systems and GC monitoring of the CH_4 content in biogas.

To further study the laboratory mesophilic anaerobic co-digestion of native cyanobacteria with dairy farm wastewater, the following variables could be tested in BMP experiments:

- ISR of 1, 0.5, and 0.1,
- C/N ratios of 20 and 30,
- cyanobacteria pre-treatment (by heating, blending or by natural fermentation) to evaluate its impact in biogas production yield,
- cyanotoxin spiking to evaluate possible inhibition of the anaerobic digestion process and to measure the extent of cyanotoxin degradation,
- no anaerobic inoculum (milking parlour wastewater acting as inoculum and substrate).

Finally, future research opportunities that were identified along the performance of this thesis include the following:

- pilot-scale anaerobic digester for milking parlour wastewater and harvested cyanobacteria,
- automated and more efficient mechanical cyanobacteria harvesting,
- digestion of single species or different communities,
- anaerobic bacteria community analysis and search for most suitable inoculum,
- biogas/biomethane yield in continuous reactor, and
- native cyanobacteria as inoculum for photobioreactors for wastewater treatment.

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CHAPTER 7

References

All figures based on aerial views were prepared with software Google Earth Pro 7.1.5.1557. Data processing and graphs were made in Microsoft Excel® 2013. Document was written in Microsoft Word® 2013. References were managed with EndNote® X7.7.1.

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