UNIVERSITÀ DEGLI STUDI DI PADOVA

MSc in Environmental Engineering

ICEA Department

Master Thesis

Francesca Anastasia

TREATMENT OF CONCENTRATED BLACK WASTEWATER USING AN EXPERIMENTAL UPFLOW-IMHOFF TANK

Supervisor

Prof. Ing. Maria Cristina Lavagnolo

A.Y. 2014-2015

Ad Andreina, la mia nonna

“Noi dobbiamo osare inventare l’avvenire: tutto quello che viene dall’immaginazione dell’uomo è per l’uomo realizzabile”.

Thomas Sankara

CONTENTS

PART 1 - MY THESIS AND ME

Looking for a thesis

In December 2013, I decided to make me a Christmas present choosing the subject of my Master Thesis. I was particularly interested in wastewater treatment and problems of water management in developing countries. I contacted Professor Lavagnolo because I knew her interest for African countries and I thought she could help me. She proposed to me to work on a new research field dealing with the upgrade of septic tanks (or Imhoff tanks) into UASB (i.e. upflow anaerobic sludge blanket) reactors. This particular reactor allows physical sedimentation and biological degradation in anaerobic conditions, in order to increase the removal efficiency of solids and organics, which is very poor in real Imhoff tanks. There was no previous experience at the LISA lab on this topic, so I had to start from the very basic aspects: first of all, the experimental reactor had to be designed and constructed. Then, the start-up of the reactor had to be carried out: through lab analysis on influent and effluent wastewater the process had to be monitored in order to understand the reactor's behaviour under different operational conditions. I thought it was a very challenging experience and extremely educational, so I decided to accept.

The bibliographic research

First, knowledge on the process and on previous researches had to be acquired. It was important to know and understand anaerobic digestion process in all its aspects, starting from the different phases (i.e. hydrolysis, acidogenesis and methanogenesis) and the optimal operative conditions. It suddenly emerged that there were only few articles in literature, which dealt with this topic, in particular from Palestine, Iran, The Netherlands and Sweden. Previous works underlined the need to gain data on the process: the most important parameters and the reactor performance under different operative conditions and with different wastewaters had to be assessed.
In order to start the lab test, we had to design the reactor: knowledge on UASB reactors and Imhoff tanks layout was required. The aim of this work was to upgrade Imhoff tanks into UASB-septic tanks reactors, thus two main aspects had to be taken into consideration: firstly, water flow is no more horizontal, but vertical (from the bottom to the top) to facilitate

sedimentation, favour the contact between inoculum sludge and wastewater and improve the biological removal of dissolved solids; secondly, the presence of a Gas-Solids-Liquid Separator (i.e. GLSS) allows biogas collection, settling of solids particles and water leaving.

Pilot plant set-up and management

In June, the designed reactor (Figure 1) was constructed by Officine Parisi SRL company. We decided to keep its height lower than typical UASB reactor in order to better simulate an Imhoff tank (compatibly with construction possibilities, especially in terms of materials, and operational needs). We adopted a cylinder-shape reactor, thus it did not have corners in which solids may accumulate. It was made of Plexiglass, with manholes along its height to allow samples collection. The total volume was about 9,5 litres.

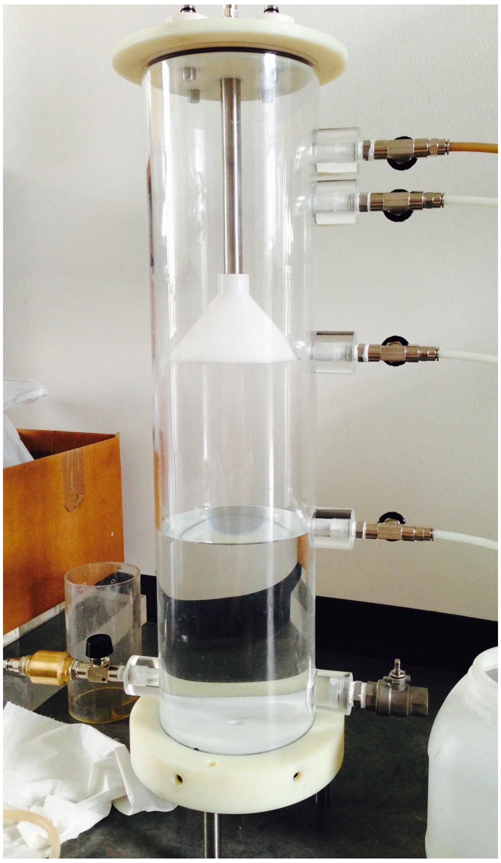


Figure 1 - Upflow-Imhoff tank prototype.

We chose to feed the reactor with black water coming from a private Imhoff tank, as it resulted to be highly contaminated in terms of organics, solids and nutrients. The influent wastewater was collected manually every 20 days. It appeared to be a quite realistic situation for which the upflow-Imhoff tank solution could be applied. Its composition varied with time, as different samples were taken during the research period.

At the end of June, we set up the pilot plant and the start-up could take place. For the first two weeks, it was operated in fed-batch mode as the only pump available at the lab provided a too high flowrate not compatible with the reactor operation. A timer was used to switch on the pump three times a day for ten minutes each time. In this condition, the contact time between inoculum sludge and wastewater was very low (i.e. 90 minutes). When a smaller pump became available, we changed the feeding mode from batch to continuous, thus, as expected, the performance of the reactor was enhanced. For both peristaltic pumps I built a flowrate scale in order to use them consciously (Figure 2).

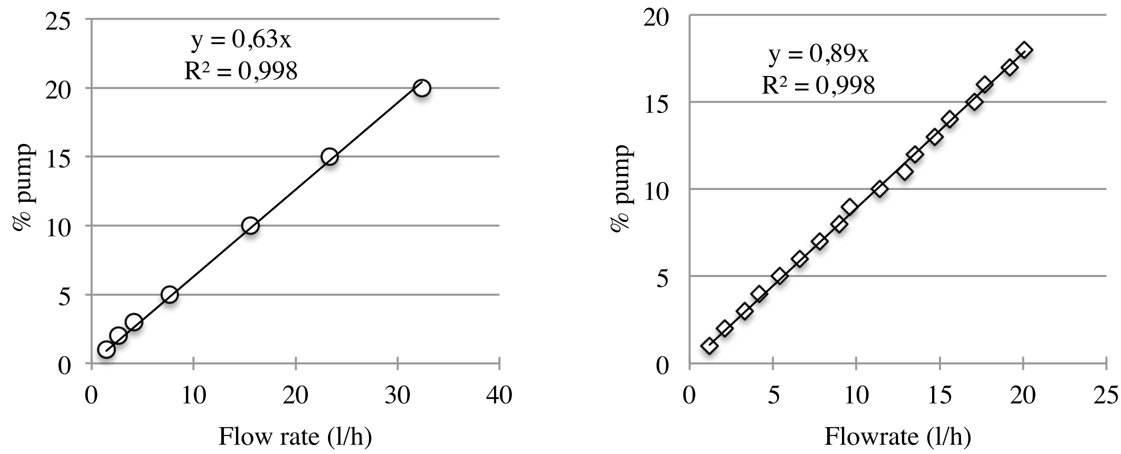


Figure 2 – a) IP55 Washdown Cellai pump flowrate scale; b) Watson Marlow Q400 pump flowrate scale.

During the experimental period some operational problems emerged and we had to manage them in order to let the reactor work. Some of these problems were:

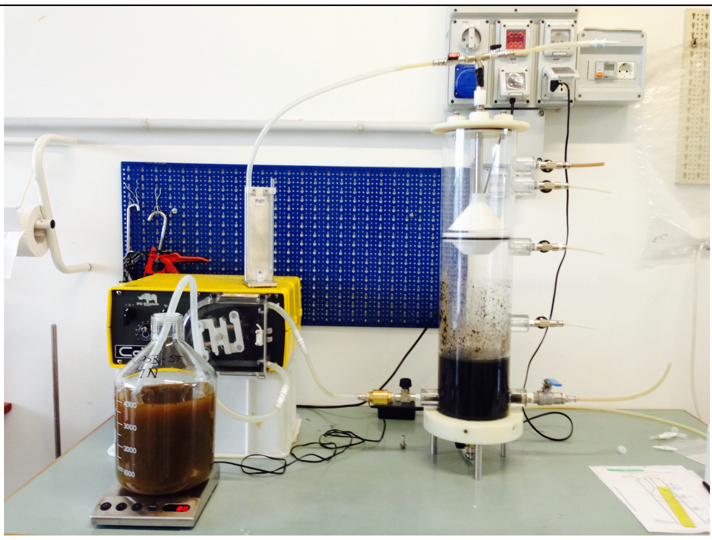
- Clogging of the non-return valve at the influent manhole: I removed it and, in order to avoid syphoning, I located the influent tank up above the effluent's height;
- Clogging of the effluent manhole due to bacterial film on the surface: I introduced plastic-biomass-growth devices;

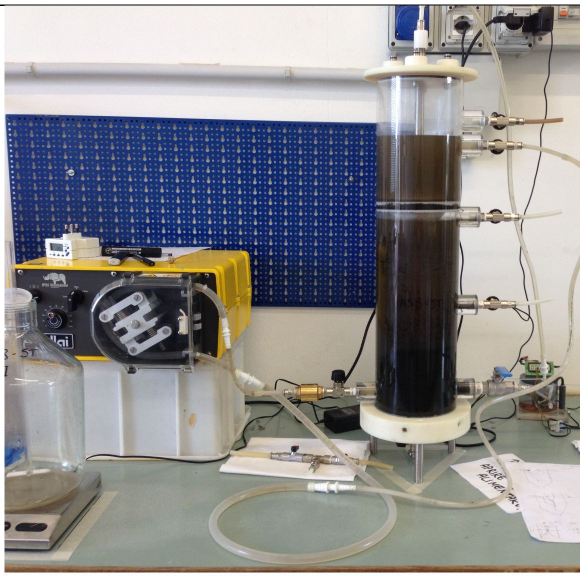
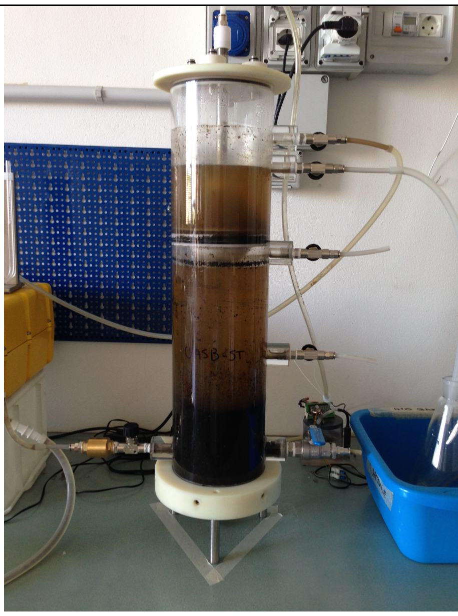
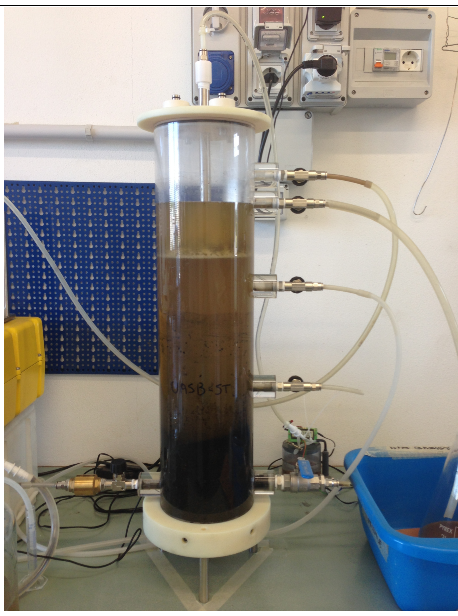
- Pump's velocity was lower than sedimentation velocity of the particulate matter in wastewater, thus only part of it was fed to the reactor: I changed the influent tank and the configuration of the pilot plant;
- No biogas monitoring during the first weeks and with recycling, probably due to a malfunctioning of the gas counter: a solution of acetic acid was injected to understand were the problem was (inactive biomass, no methanogenic bacteria, gas meter malfunctioning, etc…).

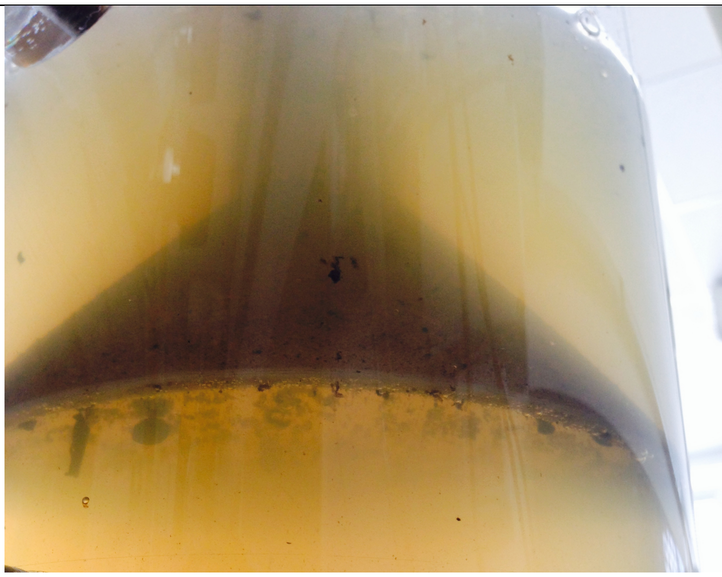
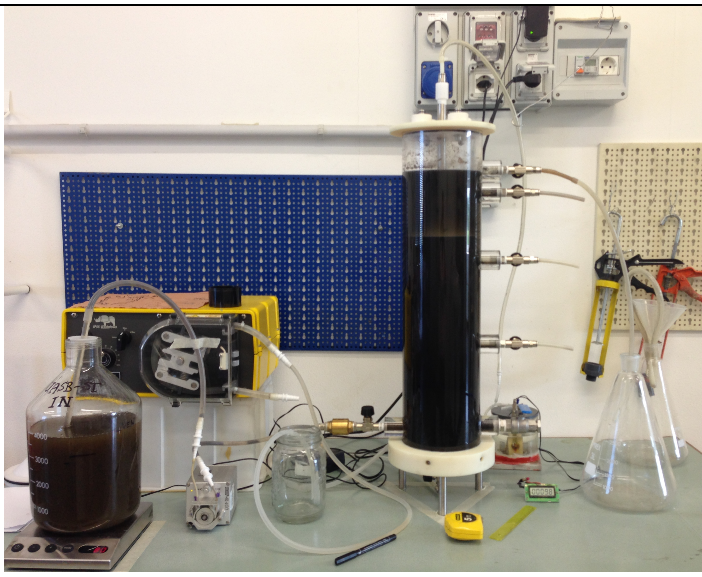
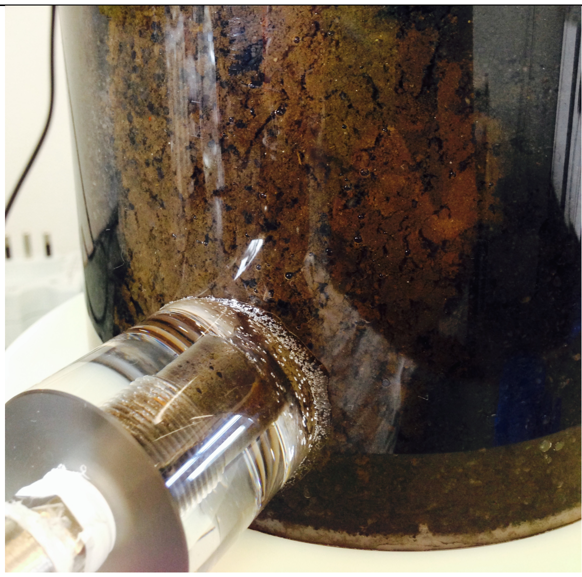
Working at the lab

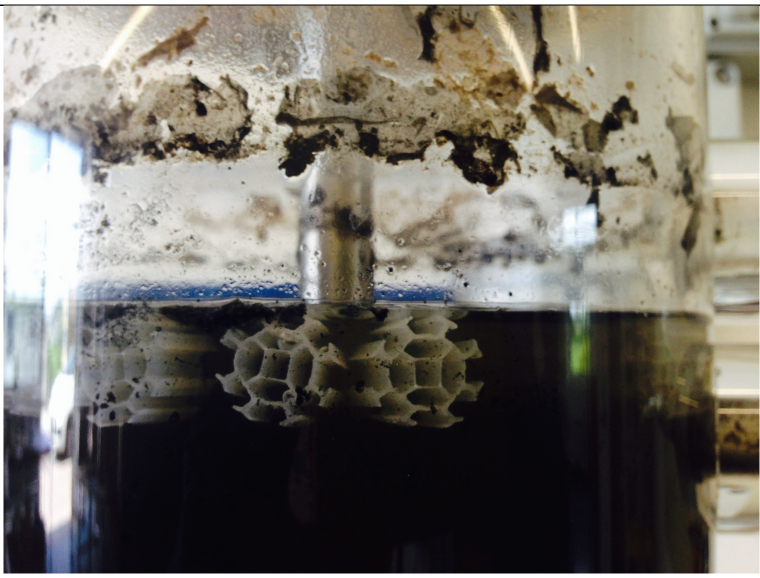
From July to November, I carried out different chemical analysis in order to monitor the reactor. First, I had to learn how to make the analysis and how to be confident with laboratory instruments. It was extremely educational to learn how the different parameters are measured according to Standard Methods: it allows you to be careful when collecting the representative samples, to gain sensitivity in managing the results obtained, to understand how "numbers" can be affected by different errors and when they are significant or not.
I analysed the influent once every time it was collected (about 20 litres per time), I had to filter it with a 2 mm grid in order to avoid clogging of the influent pipe. Then it was stored at 4°C for a maximum of one month. I carried the analysis on the effluent twice a week: I collected 500 ml of effluent every Monday and Thursday. In addition, samples from the inside of the reactor were collected once/twice a week. Moreover, thanks to Luca Alibardi, we carried out biochemical methane potential tests in order to evaluate the maximum methane production of wastewater used as influent.
My special thank you to Annalisa and Luca for their patience, helpfulness and competence.

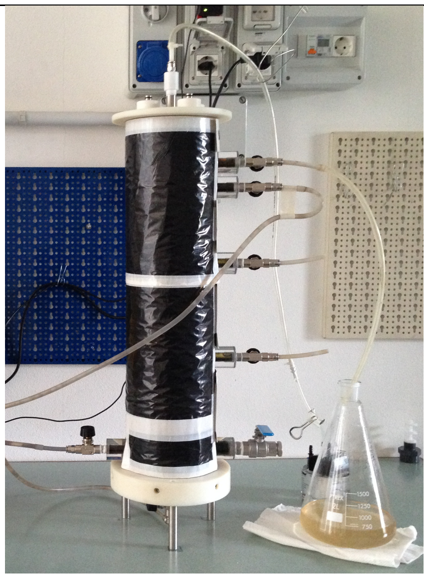
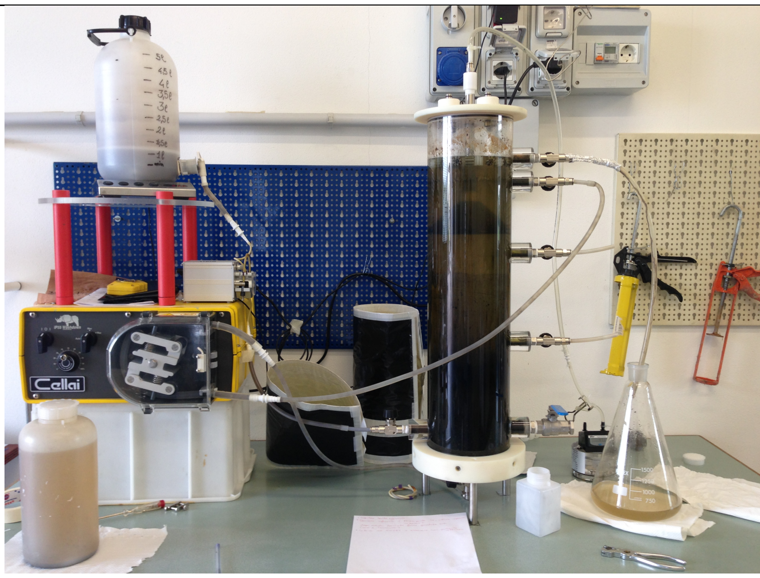
Photo story

Data		*Description*
24/06/2014		Influent wastewater in Imhoff cones
25/06/2014		Filling of the reactor with black water
25/06/2014		Sludge granules.

02/07/2014		Reactor completely filled.
15/07/2014		Gas bubble under the intermediate separator: it was not possible to avoid it. Even though some biogas would be lost, it was decided to keep out the separator.
17/07/2014		Reactor without the intermediate separator.

22/07/2014		Biogas was not measured from the beginning, but gas level was visible under the GLS.
25/07/2014		Changing colour inside the reactor: it was dark as the sludge.
28/07/2014		Clear colour distinction between sludge and influent wastewater.

29/07/2014		Biomass film on the free surface: plastic-biomass devices were added to avoid clogging of the effluent tube.
30/07/2014		Changing of the influent tank to allow better feeding of solids.
28/08/2014		Gas bubble inside the sludge.

01/09/2014		Covered reactor due to algae formation within the sludge.
08/09/2014		Preferential paths in the sludge.
15/09/2014		Recycle pump was activated.

17/09/2014		Depression measured inside the reactor because of the liquid inside the tube linking GLS separator and gas counter.
09/10/2014		Sludge granules and influent settled solids.
30/10/2014		Enhanced mixing of the influent in the sludge bed thanks to recycle.

PART 2 - SCIENTIFIC PAPER

1. Introduction

The driving principle of this research was the necessity to look for alternatives in the management of existing water resources. At the moment, centralised wastewater collection and treatment plants, mainly in Europe and USA, are inadequate because of high management costs, technological requirements, skilled manpower and complexity, especially where population density is low and dispersed houses are present (Ali *et al.*, 2007; Al-Jamal and Mahmoud, 2009; Elmitwalli *et al.*, 2003; Lohani *et al.*, 2013). This conventional sanitation system, commonly implemented in richer countries, is based on the collection and transport of wastewater, often together with rainwater, via extended sewer systems. Drinking water is illogically wasted to transport the highest load of pollution, originally produced in small quantities such as faeces and urines (Kujawa and Zeeman, 2005). On the other hand, developing countries suffer from the lack of proper wastewater collection and treatment facilities, especially in rural areas (Elmitwalli *et al.*, 2003). When existent, these wastewater systems are usually unreliable and deficiencies are quite noticeable (Da Silva *et al.*, 2012). In addition, funding, resources and skilled personnel are insufficient to implement, operate and maintain these plants (Lohani *et al.*, 2013). In the worst conditions, people living in less developed areas have no access to wastewater collection, therefore they often dispose their wastewaters improperly on-site (Elmitwalli *et al.*, 2003; Moussavi *et al.*, 2010). For example, in Palestine only 6% of the population is served with functioning treatment facilities (Al-Jamal and Mahmoud, 2009). No sanitation at all results in serious public health risks and adverse environmental impacts: cesspits are left without lining thus sewage infiltrates into the earth layers and eventually to groundwater, wastewater is directly discharged into rivers and streams and greenhouse and toxic gases are released into the atmosphere (Al-Shayah and Mahmoud, 2008; Lohani *et al.*, 2013). Such negative impacts must be mitigated finding affordable sanitation options in order to improve human health and preserve the environment (Da Silva *et al.*, 2012; Lohani *et al.*, 2013; Moussavi *et al.*, 2010).

As a consequence, decentralized wastewater management appears to be an attractive and sustainable alternative, dealing with the protection of the environment and public health worldwide (Al-Jamal and Mahmoud, 2009; Al-Shayah and Mahmoud, 2008; Kujawa and Zeeman, 2006; Lohani *et al.*, 2013). Decentralized systems are simpler and lower-cost operations at or near the point of waste generation (Moussavi *et al.*, 2010). In less developed countries the DESAR sanitation concept (Decentralised Sanitation and Reuse) may help solving the problem of a lack of any sanitation system. In richer countries it may be an answer when existing old infrastructure needs to be replaced or when wastewater management services have to be provided for dispersed human settlements, where the connection to sewage system is too expensive (Kujawa and Zeeman, 2005; Luostarinen and Rintala, 2005). DESAR concept focuses on reducing drinking water use and energy consumption, producing energy (biogas) and recycling of resources present in domestic wastewater (e.g. plant nutrients and water). Any kind of application can be considered: small communities, large buildings or residential areas (Kujawa and Zeeman, 2005-2006).

Furthermore, the separation of domestic wastewater at the source could have several benefits in managing decentralized sanitation systems. This separation results in black water from the toilet (faeces and urine diluted with clean water used for their transport) and grey water from showers, laundry and kitchen. These streams differ both in quantity and quality and should be treated separately according to their concentrations and composition. Faeces and urine, the lowest fraction produced, are the most concentrated in terms of organics and nutrients, but also contain most of the pathogens and micro pollutants, like pharmaceuticals and hormones (Kujawa and Zeeman, 2006). The nutrient content varies according to the food intake. Grey water usually contains a higher amount of heavy metals and a lower amount of nutrients, mainly inorganics (De Graff *et al.*, 2010; Kujawa and Zeeman, 2005). Grey water volume cannot be reduced, while black water can be concentrated with the use of low flush or water free toilets. For this reason, the collection, transport and treatment of concentrated wastewater streams can be realised within a restricted area, while diluted streams can be transported to a semi-centralised location in the neighbourhood for their purification (Kujawa and Zeeman, 2005).

The high concentration of organics in black water makes it particularly suitable to be separately collected and anaerobically treated (Kujawa and Zeeman, 2006). Anaerobic on-site treatment is a sustainable option due to its low energy demand, small space requirement, simple reactor design, lower sludge production than aerobic treatment and feasibility at low temperatures (even though the process efficiency decreases) (Ali *et al.*, 2007; Al-Jamal and Mahmoud, 2009; Loustarinen and Rintala, 2005; Moussavi *et al.*, 2010). In anaerobic digestion organic matter is converted into methane, which can be used to produce electricity and heat, while nutrients contained in digested medium are preserved for reuse in agriculture (Kujawa and Zeeman, 2005; Loustarinen *et al.*, 2007).

Among anaerobic reactors, the septic tank (or Imhoff tank – Figure 1.1) is probably the most used. It was developed during the last quarter of the 19th century, but its diffusion took place only in 1930s (Da Silva *et al.*, 2012). This kind of reactor, consisting in two communicating chambers, is designed for solid-liquid separation and digestion of the settled sludge. It is usually located underground, to let the influent flow by gravity. Water flow is horizontal, thus no contact between influent wastewater and sludge is established resulting in low conversion of dissolved components (Kujawa and Zeeman, 2006). A suspended solids reduction of 50 to 70% and a COD reduction of 25 to 50% could be achieved. Biogas produced in the digestion chamber rises into the gas vents at the edge of the reactor, without being collected. The sludge accumulates at the bottom and partially stabilizes through anaerobic digestion. Hydraulic retention time is usually not more than 2 to 4 hours to preserve an aerobic effluent for further treatment or discharge. The digestion chamber is usually designed for 4 to 12 months sludge storage capacity to allow for sufficient anaerobic digestion (Tilley *et al.*, 2014). Due to anaerobic conditions in the septic tank, green house gases, such as methane, can be emitted to the atmosphere instead of being collected (Zamalloa *et al.*, 2013).

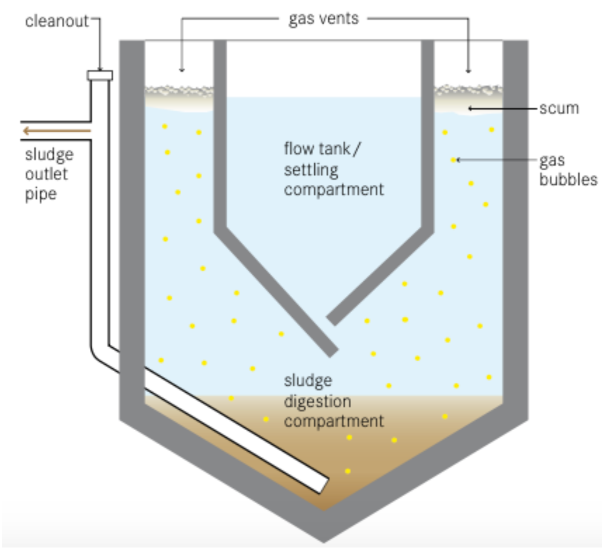


Figure 1.1 - Typical Imhoff tank (Tilley *et al.*, 2014).

According to ISTAT (2014), in Italy there are 18'786 urban wastewater treatment plants, of which 44,7% are Imhoff tanks, 11,6% and 43,7% are primary and secondary treatment plants respectively. Similarly, about 20% of the United States population (more than 20 millions homes) is served with onsite wastewater treatment facilities, consisting mainly of septic tanks (Al-Shayah and Mahmoud, 2008).

A significant improvement of the Imhoff tank can be achieved by applying upward flow and adding a gas-solid-liquid separator at the top. This upgrade results in the so-called UASB-septic tank, which combines features of UASB reactors (Figure 1.2) and conventional septic tanks. It was firstly investigated for onsite sewage treatment at Dutch and Indonesian ambient conditions by Lettinga and his co-workers (Al-Jamal and Mahmoud, 2009). The upward flow allows increased physical removal of solids and improved biological degradation of dissolved components. The sludge bed, differently from UASB reactors, lies on the bottom because the upflow velocity is limited. In UASB reactors the upward velocity ranges between 0,5 and 1 m/h, while in UASB-septic tanks it is about 0,01-0,2 m/h (Henze *et al.*, 2008).

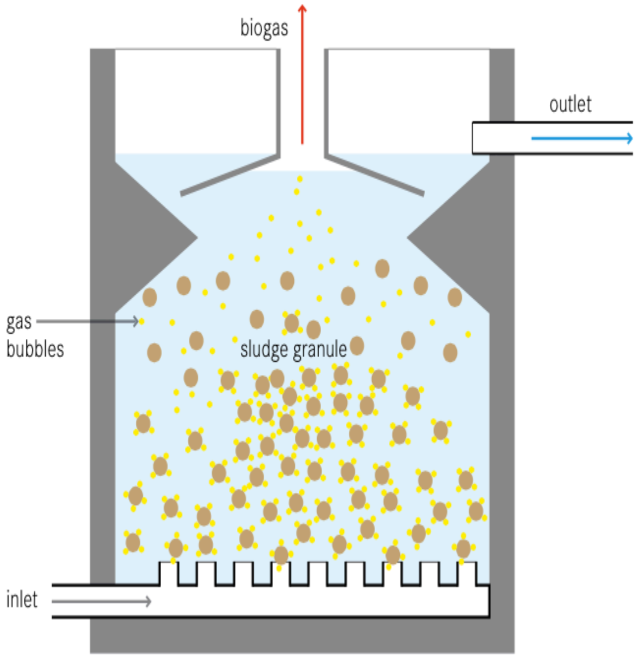


Figure 1.2 - UASB reactor configuration (Tilley *et al.*, 2014).

The aim of this experimental research was to study the feasibility of upgrading existing Imhoff tanks into upflow-Imhoff tanks, in order to improve the quality of the effluent wastewater. In recent years few studies were conducted, especially in The Netherlands, Palestine and Sweden. Promising results were obtained, in terms of COD and solids removal efficiencies. Different authors underlined the need of further researches, because the process strictly depends on the kind of wastewater, on inoculum sludge and climatic conditions. This investigation is necessary to determine design parameters for the upgrade of current conventional septic tanks to advanced ones, based on local conditions and wastewater characteristics (Moussavi *et al.*, 2010).

First, the lab-scale upflow-Imhoff tank was designed and constructed. Concentrated black water coming from a private Imhoff tank, with average COD concentration of 3285 mg L^{-1}, was selected as influent feed. The reactor was operated for four months (from July to October) at LISA lab in Padova. The HRT varied from 12 days (during the first three months) to 6 days (during the last month). High removal efficiencies were obtained for both suspended solids and organics (TSS 95%, VSS 96%, COD_{tot} 89-96%, COD_{susp} 92-99%, COD_{diss} 23-44%). Average biogas production was 72 Nm^3CH_4 $t^{-1}VS$, but decreased to 33 Nm^3CH_4 $t^{-1}VS$ when recycle was applied, probably because of gas counter malfunctioning or interferences between the measurement device and the recycle. The mass balance on total input COD was carried out, taking into consideration the amount of COD accumulated inside the reactor (50,7%), methane production (9,4%), effluent COD (6,5%) and sludge withdrawn (7%). A missing fraction of 26,4% was attributed to under measurement of biogas produced and sulphate reduction.

2. Materials and methods

2.1. Design of the reactor

The reactor had to be designed and constructed, combining literature data and knowledge on existing Imhoff tanks. First, literature data on UASB-septic tank dimensions were collected (Table 2.1).

Table 2.1 - Literature data on experimental UASB-septic tanks

	Volume (m^3)	Height (m)	Diameter (m)
Ali *et al.*, 2007	0,4	1,88	0,265
Al-Shayah and Mahmoud, 2008	0,8	2,5	0,638
Al-Jamal and Mahmoud, 2009	0,8	2,5	0,638
De Graaff *et al.*, 2010	0,05	1,3	0,2
Kujawa and Zeeman, 2005	0,2	1,65	0,4
Luostarinen and Rintala, 2005	0,012 ; 0,003	0,7 ; 0,5	0,15 ; 0,09
Moussavi *et al.*, 2010	0,25	1,20	0,5 x 0,5

In addition, web sites of companies selling Imhoff tanks were consulted. It resulted that the inner diameter varied between 1 and 2 m, with a height of 1,5-4 m.

Comparing literature data, it emerged that, in UASB-septic tank reactors, the height was the dominant dimension being at least one order of magnitude bigger than the diameter. On the contrary, as regards Imhoff tanks (and septic tanks), the dimensions of diameter and height were nearly the same. For this reason, as the aim was to better simulate real Imhoff tanks, it was decided to design a compact reactor keeping the ratio height/diameter as low as possible (compatibly with construction possibilities, especially in terms of materials, and operational needs). It seemed that a cylinder-shape reactor was the best solution, avoiding the presence of corners in which solids might accumulate.

Guidelines from Metcalf and Eddy (2004) for the design of gas-solids-liquid separator (i.e. GLSS) on UASB reactor were taken into account:

- The slope of the GLS separator should be between 45-60°;
- The surface area of the apertures between the gas collectors should be 15-20% of the reactor surface area;
- The height of the gas collector should be between 1.5-2 m at reactor heights of 5-6 m.

According to these considerations, it was decided to design a reactor with a diameter and a height of 15 and 54 cm (maximum water level) respectively. The volume resulted to be 8,8 liters (9,5 liters from 22/07, as the effluent was discharged from the uppest manhole located at 54 cm). Five ports were located on the reactor wall at 3, 20, 37, 50 and 54 cm to allow sampling collection at different heights. The GLS separator, as shown in Figure 2.1, had a diameter of 13 cm, covering 80% of total reactor area.

The upflow-Imhoff tank was made of Plexiglass (by the company Officine Parisi s.r.l., Riviera del Garda – Trento), in order to visually monitor the situation inside.

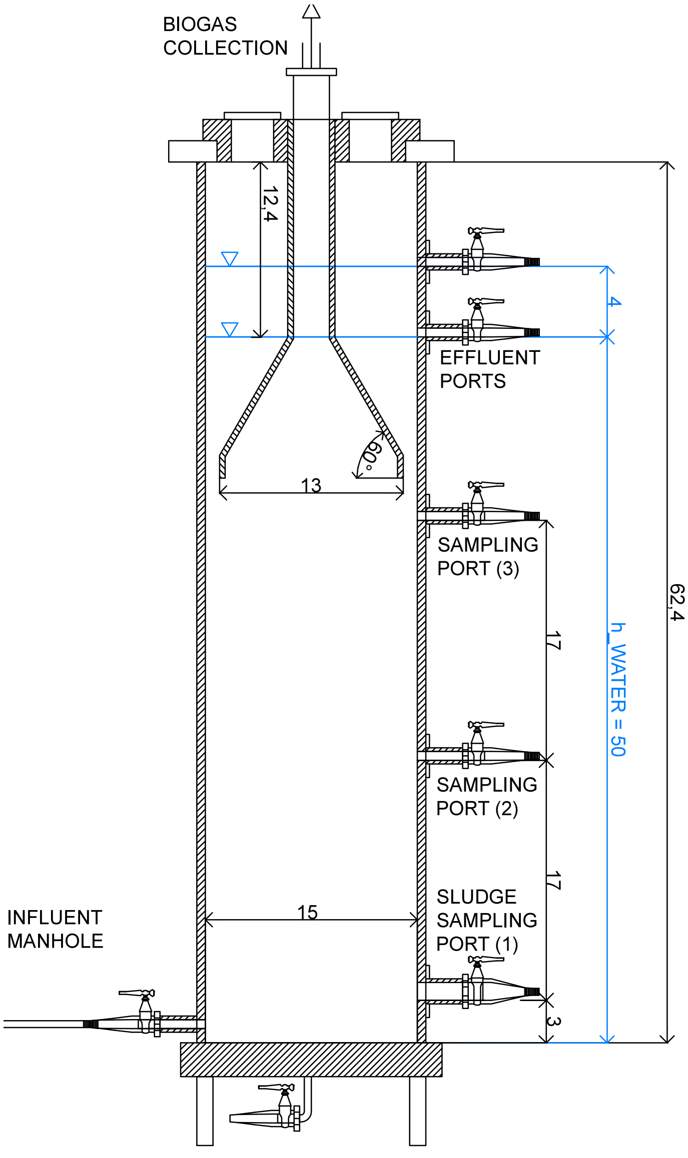


Figure 2.1 - Upflow-Imhoff tank design.

2.2. Reactor feeding

In previous works dealing with this topic, real wastewater was used as influent for the UASB-septic tank reactors: domestic sewage (Ali *et al*., 2007; Al-Shayah and Mahmoud, 2008; Al Jamal and Mahmoud, 2009; Elmitwalli *et al*., 2003; Moussavi *et al*., 2010), concentrated black water from experimental pilot plants (Kuiawa and Zeeman, 2005; Luostarinen *et al*., 2006), grey water highly contaminated due to low water consumption (Halalsheh *et al*., 2008) and synthetic wastewater made of tap water, toilet paper and primary sludge from a municipal wastewater treatment plant (Luostarinen and Rintala, 2005-2007).

Focusing on the concept of decentralized wastewater treatment, it was decided to analyse different wastewaters coming from small treatment plants and remote housing. Those of low strength with no significant biogas potential production, as wastewater from Montà treatment plant (Padova), were rejected.

Particular attention was posed on concentrated black water, collected in existing Imhoff tanks, because of its highly contamination in terms of organics, solids and nutrients. In addition, it appeared to be a quite realistic situation for which the upflow-Imhoff tank solution could be applied. Typical values of high strength wastewater and septic sludge are reported in Table 2.2, while the composition of wastewaters used in previous research is shown in Table 2.3.

It was decided to feed the reactor with wastewater coming from a single private Imhoff tank serving 3 to 4 people, which collected only black water. The concentrated black water was collected periodically in cans of about 30 liters, transported to the lab and stored at 4°C. In order to prevent clogging of the influent pipe, screening at 2 mm was carried out before feeding. The reactor was fed when wastewater reached ambient temperature. A magnetic stirrer was installed in the influent tank to allow good mixing and prevent sedimentation.

The composition of the influent black water (reported in Table 2.4) varied with time, as different samples were taken during the research period. It can be noticed that COD values are similar to those of septic sludge, with a prevalence of suspended COD. However, it is evident that the wastewater used is stronger than the one used in previous research, with the exception of De Graaff *et al.* (2010) and Kujawa and Zeeman (2005) that used low flushing toilets making black water highly concentrated.

On June 25th, the reactor was firstly fed. Once the influent completely filled the reactor, 100 litres of nitrogen gas were flushed in order to assure anaerobic conditions and to speed up the start-up phase.

Table 2.2 - Typical composition of high strength municipal wastewater, septic sludge (* Henze *et al*., 2008), black water and domestic wastewater from conventional flushing toilets (mg L^{-1}) (** Henze and Ledin, 2001).

Parameter	High strength wastewater (*)	Septic sludge (*)	Black water (**)	Domestic wastewater (**)
BOD_5	560	2000	300-600	115-400
COD total	1200	6000	900-1500	210-740
COD dissolved	480	200		
TKN	100	200	100-300	20-80
P	25	40	40-90	6-23
TSS	600	7000		
VSS	480	4000		

Table 2.3 - Wastewater composition (mg L^{-1}, with the exception of pH) in previous research (DS = domestic sewage; CS = concentrated sewage; (C)BW = (concentrated) black water).

	Al-Jamal and Mahmoud, 2009	Al-Shayah and Mahmoud, 2008	Ali *et al.*, 2007	Elmitwalli *et al.*, 2003	De Graaff *et al.*, 2010	Luostarinen and Rintala, 2005	Luostarinen and Rintala, 2007	Moussavi *et al.*, 2010	Kujawa and Zeeman, 2005
Feeding	DS	DS	DS	CS	CBW	BW	BW	DS	CBW
pH	7,6	7,4	7,4-8,1		8,7			6,2-8	8,8
TSS	371	623	330-1160					148-629	
VSS	313	526	240-450						
COD tot	905	1267	267-888	3600	8750	926	1104	154-395	9500
COD diss	350	439	22-260	410	2850	118	87		1400
BOD_5	502	641	116-333					118-268	
TKN	70	76	n.d.		1550	39	31		1000
$N\text{-}NH_4$	39	58	20-57		1125		6		710
P tot	10	14	4-15		185	17	13		110

Table 2.4 - Composition of the influent black water

	Unit	Average	Min	Max
pH	-	7,7	7,5	7,9
BOD_5	mg L^{-1}	306	172	426
COD total	mg L^{-1}	3285	1157	5350
COD dissolved	mg L^{-1}	177	119	244
COD suspended	mg L^{-1}	2976	913	5225
TS	mg L^{-1}	2412	1088	4120
VS	mg L^{-1}	1619	498	2843
TSS	mg L^{-1}	1581	490	3448
VSS	mg L^{-1}	1228	360	2812
TKN	mgN L^{-1}	237	188	272
$N\text{-}NH_4^+$	mgN L^{-1}	165	106	194
N org	mgN L^{-1}	72	16	155
NO_3^-	mgN L^{-1}	1	1,3	1,3
P tot	mgP L^{-1}	41	31	52
SO_4^{2-}	$mgSO_4^{2-}$ L^{-1}	<250	<250	<250

Metals were once measured in the influent. As their concentration had non-concerning values, they were not monitored during the experimental period (Cd <10μg L^{-1}; Cr 70μg L^{-1}; Cu 423μg L^{-1}; Fe 3496μg L^{-1}; Mn 198μg L^{-1}; Ni <10μg L^{-1}; Pb 47μg L^{-1}; Zn 1602μg L^{-1}).

2.3. Inoculum

The reactor was inoculated with a mixture of three different sludges, in order to have a wider variety of microrganisms. In addition, the use of granular seed sludge is proved to enhance the methanogenic capacity of the reactor (Kujawa and Zeeman, 2006) and to overcome the obstacle of possible low temperatures (Lettinga *et al.*, 1993). Granular sludge was taken from a UASB reactor treating brewery wastewater, while mesophilic sludge from anaerobic digestion and activated sludge from return sludge line were taken from a civil wastewater treatment plant (Ca' Nordio, Padova).

As expected, it resulted that granular sludge had a higher TS and VS concentration than mesophilic and activated sludges. Therefore, it was decided to take a lower volume of granular sludge.

The characteristics of the different sludges and the inoculum are reported in Table 2.5. The total volume of the inoculum resulted to be 2,3 litres (26% of reactor volume), with a height of 13 cm.

Table 2.5 - Sludges and inoculum characteristics

Type of sludge	TS (mgL^{-1}; % w/w)	VS (mgL^{-1}; % w/w)	Volume (L)	Density (kgL^{-1})
Activated sludge	19636	9469	1	1
Mesophilic anaerobic sludge	14948	7388	1	1
Granular sludge	10,8%	8,5%	0,3	1
Inoculum	61993	44285	2,3	

2.4. Biochemical Methane Potentials tests

Lab-scale tests were performed to evaluate the Biochemical Methane Potential (BMP) of substrates examined by anaerobic digestion process. Tests were carried out in 1-liter batch reactors under mesophilic condition (35±1 °C). Reactors were hermetically closed by a silicon plug, which enabled the sampling of the biogas produced during the test. The liquid volume in each reactor, consisting of the substrate and deionized water, was 500 mL (i.e. 250

mL substrate, 250 mL deionized water). Tests were performed at a substrate concentration of 2,8 gVS L^{-1}. The ratio between the volatile solids of the substrate to be degraded and the volatile solids of the inoculum biomass (Food/Microorganisms – F/M) used in each test was 0,2 gVS/gVS, corresponding to 50 g of granular sludge added in each reactor. To provide sufficient buffer capacity and to set the pH at 7,5 sodium bicarbonate (0,2 g) was added in each reactor. Micro and macro nutrients were added to assure the necessary conditions for biomass activity. When the reactors were filled, nitrogen gas was flushed for three minutes to create anaerobic conditions. After that, reactors were incubated without mixing in a thermostatic chamber.

Blank tests using the inoculum alone were also prepared to measure the quantity of methane produced only by the biomass. All tests were performed in triplicate. Periodically, the volume of biogas produced was volumetrically quantified and its quality, in terms of CH_4 and CO_2 percentages, was measured with LFG 20 (Telepan).

At the end of the tests, liquid samples from the reactors were collected. Samples were filtered on a 2 mm pore size plastic mesh to remove granular sludge and filtrate liquids were analysed for TKN and $N\text{-}NH_4^+$ concentrations. Filtered samples were further centrifuged at 5000 rpm for 15 minutes. Supernatants obtained from centrifugation were filtered at 0,45 μm and analysed for dissolved COD concentration.

2.5. Experimental set-up of the pilot plant

A schematic diagram of the experimental set-up is shown in Figure 2.2. The reactor was run at ambient temperature (20-28°C), in order to maintain operative conditions similar to those of real Imhoff tanks. Influent wastewater was manually spilled every few days in a 5 liters storage tank and it was fed thanks to a peristaltic pump (during the first phase IP55 Washdown, Cellai – Figure 2.3a; after, Watson Marlow Q400 – Figure 2.3b) through an HDPE tube. The GLS separator was connected to a gas counter (MilliGascounter® Type MGC-1 V3.0, Ritter – Figure 2.4) used to measure the amount of biogas produced. The gas to be measured flows in via the gas inlet nozzle (3), through the micro capillary tube (9) located at the base of the gas counter and up into the liquid casing which is filled with a packing liquid (12). The gas rises as small gas bubbles through the packing liquid, up and into the measurement cell (13). The measurement cell consists of two measuring chambers, which are filled alternatingly by the rising gas bubbles. When a measuring chamber is filled, the buoyancy of the filled chamber causes the measurement cell to abruptly tip over into such a position that the second measuring chamber begins to fill and the first empties. The measurement of gas volume therefore occurs in discrete steps by counting the tilts of the measurement cell (13) with a resolution of approximately 3 mL (= content of one measuring chamber). The tilting procedure of the measurement cell creates by the permanent magnet (11) on top of the cell and one of the two magnetic sensors (reed contacts) (11) a pulse, which is registered by the counter unit (1). The measured gas escapes through the gas outlet nozzle (4) (http://www.ritter.de).

The effluent was collected in a 3 liters storage tank, which was emptied manually every day.

When recycle was applied, a PVC tube from the manhole at 50 cm was connected to a peristaltic pump (IP55 Washdown, Cellai – Figure 2.3a), which enabled wastewater to re-enter from the bottom port.

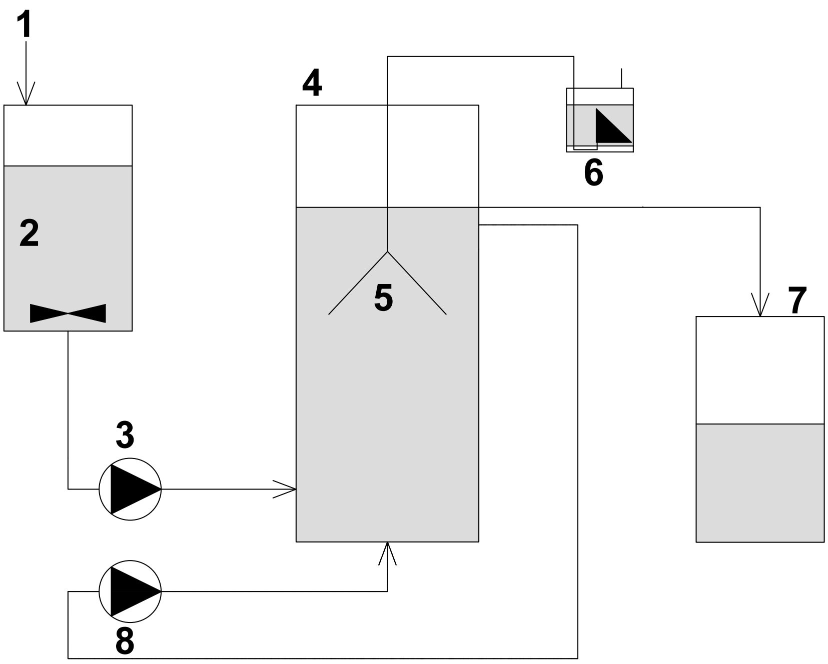


Figure 2.2 - Schematic diagram of the experimental set-up. 1- influent wastewater; 2- influent tank with magnetic stirrer; 3- peristaltic pump; 4- upflow-Imhoff tank; 5- GLS separator; 6- gas counter; 7- effluent tank; 8- peristaltic pump for recycle.

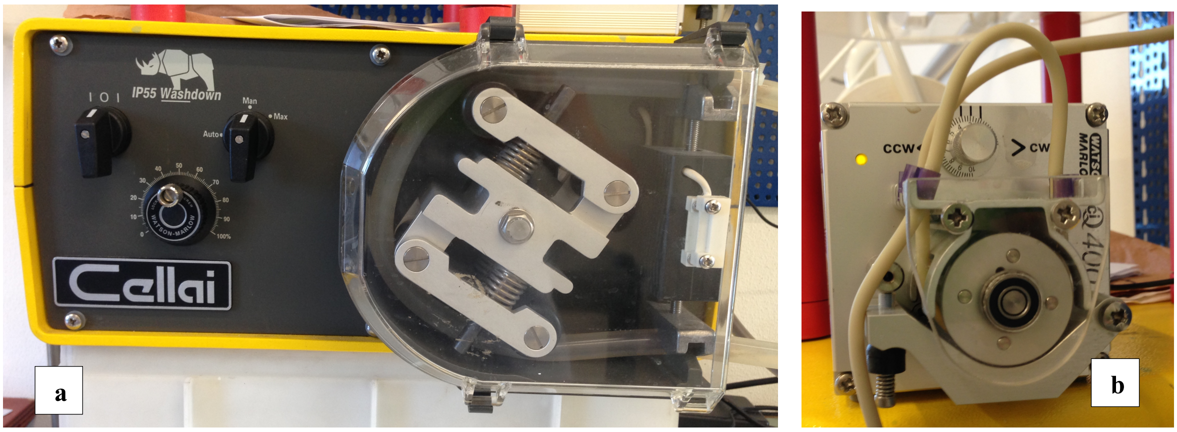


Figure 2.3 - Pumps used: a) IP55 Washdown, Cellai; b) Watson Marlow Q400.

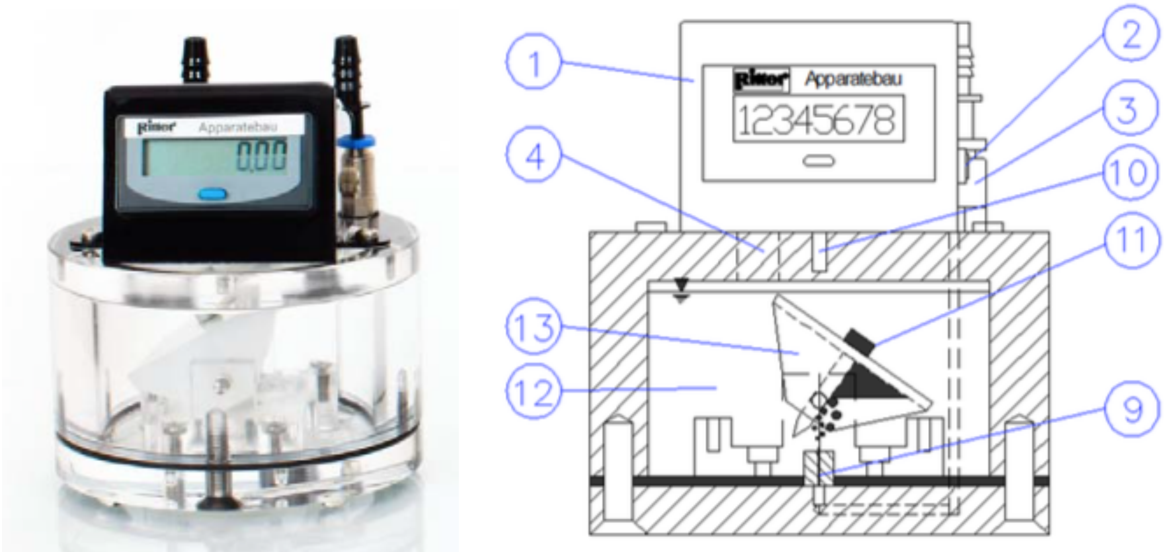


Figure 2.4 - MilliGascounter® (1- Counter unit including LCD display; 2- Signal output socket of reed contact; 3- Gas inlet connector; 4- Gas outlet connector; 9- Micro capillary tube; 10- Two Reed Contacts; 11- Permanent magnet; 12- Packing liquid; 13- Measurement cell (tilting body) with twin-chambers) (http://www.ritter.de).

2.6. Operational conditions and monitoring

The upflow-Imhoff tank was started up during Summer (between June and July) and operated for 4 months, until the end of October.

The research study was divided into different run phases:

- *First phase (25/06/2014 - 15/07/2014):* the reactor was operated as a fed-batch with a daily load of 750 mL (i.e. HRT 12 days). The pump worked three times per day for 10 minutes each. The contact time between biomass and wastewater was too low (only 90 minutes). The upflow velocity during feeding time was 0,085 m h^{-1}.

- *Second phase (17/07/2014 - 14/09/2014):* the reactor was operated in continuous flow with a daily load of 780 mL (i.e. HRT 12 days). This choice allowed to increase the contact time between biomass and wastewater to an average value of 76 hours. However, the upflow velocity was lower than literature values (0,002 m h^{-1}).
- *Third phase (15/09/2014 - 12/10/2014):* The reactor was operated in continuous flow with a daily load of 780 mL (i.e. HRT 12 days). The recycle line was installed in order to assess if a better mixing of the sludge bed and a higher value of the upflow velocity lead to a better performance. The volume of wastewater was recycled 4 times per day, with an average upflow velocity of 0,08 m h^{-1}.
- *Fourth phase (13/10/2014 - 31/10/2014):* The reactor was operated in continuous flow with a daily load of 1600 mL (i.e. HRT 6 days). It was decided to decrease the HRT in order to assess if the efficiency of the process changed, keeping in mind that, at real scale, lower HRT means lower volumes and so lower costs and space involved. The recycle line, as in the previous phase lead to problems in biogas measurement, was not active.

Table 2.6 - Operational conditions of different phases

	1^{st} phase	2^{nd} phase	3^{rd} phase	4^{th} phase
Duration (d)	15	60	28	19
Feeding	Batch	Continuous	Continuous (Recycle)	Continuous (Recycle)
HRT (d)	11,8	12,2	12,2	6
Flow rate (L d^{-1})	0,75	0,78	0,78	1,6
Recycle flow rate (L h^{-1})	-	-	(1,2)	(1,2)
OLR (kgCOD m^{-3} d^{-1})	0,14	0,31	0,31	0,48
Upflow velocity (m h^{-1})	0,085	0,002	0,068 $^{(*)}$	0,068 $^{(*)}$

(* due to recycle)

2.7. Sampling and analysis

Analysis on the influent were carried out once for each sampling (nearly every 3 weeks), while analysis on the effluent were done twice a week immediately after sample collection (Table 2.7).

The parameters monitored for influent and effluent during this study were:

- pH (IRSA-CNR 29/2003 vol. 1 n. 2060);
- Temperature;
- Total Solids, TS (IRSA-CNR 29/2003 vol. 1 n. 2090 A);
- Volatile Solids, VS (IRSA-CNR 29/2003 vol. 1 n. 2090 D);
- Total Suspended Solids, TSS;
- Volatile Suspended Solids, VSS (IRSA-CNR 29/2003 vol. 1 n. 2090 B) - using cellulose filter 0,45 μm;
- Chemical Oxygen Demand, COD_{tot} (IRSA-CNR 29/2003 vol. 2 n. 5130) was determined from unfiltered samples, while dissolved COD (i.e. COD_{diss}), was determined from cellulose filtered samples (0,45 μm);
- Total Kjeldahl Nitrogen, TKN (IRSA-CNR 29/2003 vol. 2 n. 5030);
- Ammonium nitrogen, NH_4^+ (IRSA-CNR 29/2003 vol. 2 n. 4030 A2, C);
- Total phosphorous, P_{tot} (IRSA-CNR 29/2003 vol. 2 n. 4110 A2).

Samples from the second manhole (at 20 cm) were collected once a week to monitor the situation inside the reactor. The parameters monitored inside the reactor were:

- pH (IRSA-CNR 29/2003 vol. 1 n. 2060);
- Alkalinity (IRSA-CNR 29/2003 vol. 1 n. 2010 B);
- Volatile Fatty Acids (VFA) (titration with sulfuric acid until pH 4,4 is reached).

Metals (IRSA-CNR 29/2003 vol. 1 n. 3020) (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) were evaluated only once in the influent, while BOD_5 (IRSA-CNR 29/2003 vol. 2 n. 5120 A, B, B2) was measured for the influent and the effluent three times and once respectively.

Sludge samples were collected 6 times during the study, with a total volume of 360 mL. The characteristics of the inoculum were evaluated in terms of TS, VS, TSS and VSS. COD total was measured at the beginning and at the end of the experimentation.

The quality of the biogas collected was monthly analyzed through gas chromatography (Varian 490-GC Micro GC).

Liquid substrate from BMP test was analyzed in terms of TKN, N-NH4+ and TOC (IRSA-CNR 29/2003 vol.2 n. 5040).

Table 2.7 - Types and frequencies of analysis

Parameter	Influent	Effluent	Inside	Sludge
pH	Every 2 weeks	Twice a week	Once a week	-
TS, VS	Every 3 weeks	Twice a week	Four times	Six times
TSS, VSS	Every 3 weeks	Twice a week	Four times	Six times
COD	Every 3 weeks	Twice a week	Twice	Six times
N	Every 3 weeks	Twice a week	Twice	Twice
P	Every 3 weeks	Once a week	Twice	Twice
VFA	-	Twice	Once a week	-
Alkalinity	-	Twice	Once a week	-
BOD_5	Three times	Once	-	-
Metals	Once	-	-	-

2.8. Calculations

2.8.1. Hydrolysis and methanogenesis

Percentages of hydrolysis and methanogenesis were calculated according to the following equations, as suggested by Al-Jamal and Mahmoud (2009).

$$\%Hydrolysis = \frac{COD_{CH_4} + COD_{diss,eff} - COD_{diss,\inf}}{COD_{tot,\inf} - COD_{tot,eff}} \bullet 100$$

$$\%Methanogenesis = \frac{COD_{CH_4}}{COD_{tot,\inf}} \bullet 100$$

2.8.2. Biogas production

The area of the GLSS separator was 80% of total reactor area, thus it was supposed that biogas collected was 80% of biogas produced. The percentage of methane was determined gas cromatographically.

2.8.3. COD mass balance

The COD mass balance was carried for the entire period of experimentation, being:

$$\Delta COD = COD_{INFLUENT} - COD_{EFFLUENT} - COD_{CH_4} - COD_{ACCUMULATED} - COD_{ANALYSIS}$$

Where (i = days):

- $COD_{INFLUENT}[mg] = \sum_{i=1}^{n} COD_{INF,i}[mg/L] \bullet Q_i[L/d]$, mass of COD entering the system;

- $COD_{EFFLUENT}[mg] = \sum_{i=1}^{n} COD_{EFF,i}[mg/L] \bullet Q_i[L/d]$, mass of COD leaving the system through the effluent;
- $COD_{CH_4}[mg] = \sum_{i=1}^{n} COD_{CH_4-gas,i}[mg/d] + \sum_{i=1}^{n} COD_{CH_4-aq,i}[mg/d]$, mass of COD in the biogas (both gaseous and aqueous forms);
- $COD_{ACCUMULATED}[mg] = [COD_{SLUDGE} + COD_{LIQUID}]_{t=n} - COD_{SLUDGE,t=0}[mg]$, mass of COD accumulated inside the reactor, with:
  - $COD_{SLUDGE,t}[mg] = (COD_{SLUDGE}[mg/kgTS] \bullet TS_{SLUDGE}[kgTS/L] \bullet V_{SLUDGE}[L]$, mass of COD accumulated in the sludge;
  - $COD_{LIQUID,t=n}[mg] = COD_{EFFLUENT}[mg/L] \bullet (V_{TOTAL} - V_{SLUDGE})[L]$, mass of COD accumulated in the liquid inside the reactor;
- $COD_{ANALYSIS}[mg] = \sum_{i=1}^{6} (COD_{SLUDGE}[mg/kgTS] \bullet TS_{SLUDGE}[kgTS/L] \bullet Q_{SLUDGE}[L/d])_i$, mass of COD removed from the system to analyse the sludge.

<u>Determination of the correspondence mL CH_4 / mg COD removed</u>

As the methane produced was measured in mL of CH_4, it was necessary to make a correspondence between mL of CH_4 and mg of COD removed in order to make the mass balance. Reminding that, as shown in the following equation (Metcalf and Eddy, 2004), 2 moles of O_2 (i.e. 64 g COD) are needed to oxidize 1 mole of CH_4.

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

At standard conditions (T=273K, P=1atm), 1 mol of any gas occupies a volume of 22,414 L mol^{-1}. Applying perfect gases law at constant pressure, it results that:

$$Ts/Vs = Tav/V$$

where: Ts =273K; Vs= 22,414 L; Tav = average temperature inside the reactor.

So, the molar volume of CH_4 occupied at operative conditions can be calculated as:

$$V\ (L\ mol^{-1}) = Tav/Ts * Vs$$

Determination of dissolved CH_4 in the effluent

Ambient temperature decreased during the experimentation from 30°C to 20°C. It appeared possible that a fraction of CH_4 produced was dissolved in the liquid phase and left the system through the effluent (Giménez *et al.*, 2012; Singh and Viraraghavan, 1998). In order to estimate that mass of CH_4 in the liquid phase, the expression (1) suggested by Giménez *et al.* (2012), based on Henry's law, was followed.

$$m_L^{CH4} = \frac{M^W \cdot P \cdot y^{CH4} \cdot \overline{M}^{CH4} \cdot V_L}{H^{CH4}(T) - P \cdot y^{CH4}} \quad (\text{mg } CH_4 \text{ d}^{-1}) \qquad (1)$$

Where:

- T: temperature inside the reactor (= T effluent), to simplify an average temperature for the different phases was taken;
- Henry's constant: $H^{CH4}(T)$ = 10^ (-675,74/T(K) + 6,88);
- M^W : molarity of pure water (55,56 mol L^{-1});
- P: pressure (1atm);
- $\overline{M}^{CH4}$: molar weight of CH_4 (16 g mol^{-1});
- y^{CH4} : fraction of CH_4 in the biogas;
- V_L : daily wastewater volume (mL d^{-1}).

The daily mass of CH_4 produced as COD removed (mg COD d^{-1}) is calculated as reported in equation (2).

$$m_L^{CH4}(mgCOD/d) = m_L^{CH4}(mgCH_4/d) \cdot 1gCOD/0{,}25gCH_4 \qquad (2)$$

2.8.4. COD fractioning

COD can be characterized as shown in Figure 2.5, thanks to BMP test results.

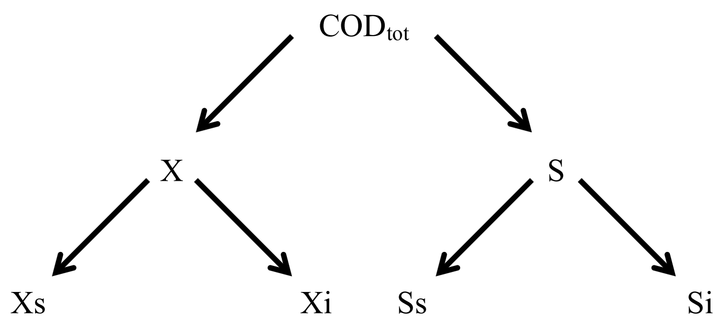


Figure 2.5 - COD fractioning: X = particulate; S = soluble; s = biodegradable; i = inert.

COD_{tot}, X and S were known values. In order to determine the four different fractions, it was possible to hypothesize that:

- Soluble inert COD is the remaining COD after BMP tests (analysis was carried on 0,45 μm filtered samples), thus $S_i = S - S_S$;
- COD transformed into methane is $COD_{CH4} = X_S + S_S$ (mg L^{-1}). The specific maximum methane production (L CH_4 m^{-3}) is used to determine COD_{CH4} (3)

$$\frac{lCH_4 / m^3}{22,414 l / molCH_4} \bullet 64 gCOD / molCH_4 = gCOD_{CH4} / m^3 \qquad (3)$$

It results that $X_S = COD_{CH4} - S_S$, thus $X_i = X - X_S$.

2.8.5. Solids retention time

Solid retention was estimated for the overall experimental period as:

$$SRT[d] = \frac{V \bullet X_{VSS}}{Q \bullet VSS + Q_{sludge} \bullet X_{VSS}}$$

Where:

- V (L) is the sludge volume;
- X_{VSS} (mg L^{-1}) is the concentration of VSS in the sludge;
- Q (L d^{-1}) is an average flow rate;
- VSS (mg L) is the concentration of VSS in the effluent;
- Q_{SLUDGE} (L d^{-1}) is the amount of sludge withdrawn from the system.

3. Results and discussion

3.1. Operational problems

Some operational problems emerged, such as:

- Clogging of the non-return valve at the influent manhole: it was removed and, in order to avoid syphoning, the influent tank was located up above the effluent height;
- Gas bubble under the intermediate separator: it was removed to avoid biogas staying inside the reactor, even though a part of it would have escaped from the GLS separator;
- Clogging of the effluent manhole due to bacterial film on the surface: plastic-biomass-growth devices were introduced (Figure 3.1);
- Pump's velocity was lower than sedimentation velocity of the particulate matter in the influent wastewater, thus only part of it was fed to the reactor: the influent tank and the configuration of the pilot plant was changed (Figure 3.2);
- Algal growth was noticed in the sludge bed, therefore the reactor was covered as in real plants it would not be subjected to sun light;
- The gas counter did not work during the first weeks of operation. In order to understand if it was a device problem or if the biomass did not produce biogas at all, a 1-liter solution containing 5 g of acetic acid, i.e. CH_3COOH, was fed. Gas bubbles were visible and the liquid surface under the GLSS was clearly lower than the one of the free surface (Figure 3.3). For this reason, the gas counter was changed and the biogas was finally measured from 22/07/2014. During the third phase, problems in biogas measurement arose: it was not clear if this had to deal with the recycle line or if the biogas produced was very low thus making it difficult for the gas counter to measure it. Again, a 2-liters solution containing 3 g of acetic acid was injected (maintaining the HRT constant) to make sure that the gas meter worked properly and that biomass inside the reactor was still active. As biogas was produced and measured, it was decided to stop the recycling as it appeared to be the only variable that changed from phase 2 to phase 3;
- The gas counter measured a depression inside the reactor when recycle was applied. Liquid from the measurement device was sucked inside the tube that linked it with the GLS separator. Again, recycle in some way caused interference which the gas measurement.

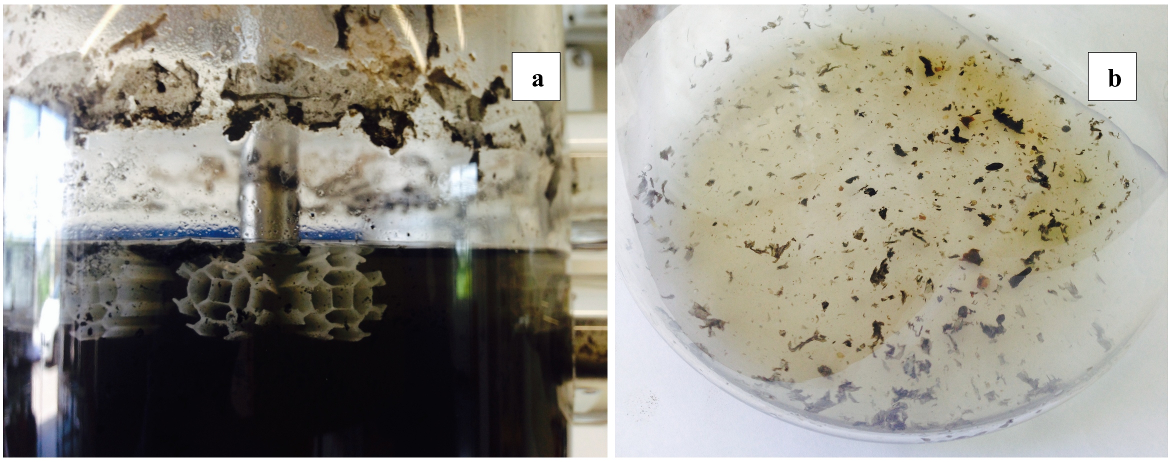


Figure 3.1 - Bacterial film: a) plastic-biomass-growth devices; b) effluent.

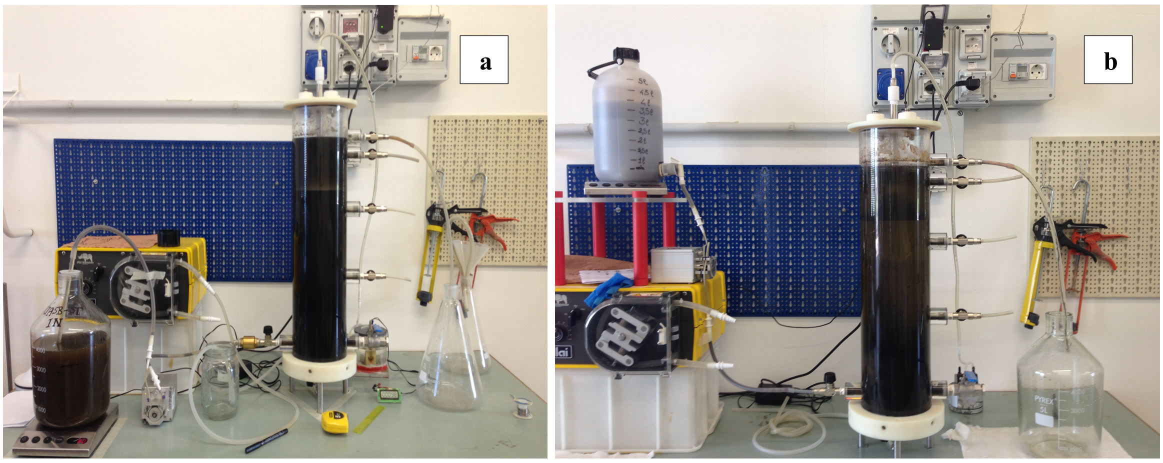


Figure 3.2 - Pilot plant configuration: a) first weeks; b) final optimal configuration.

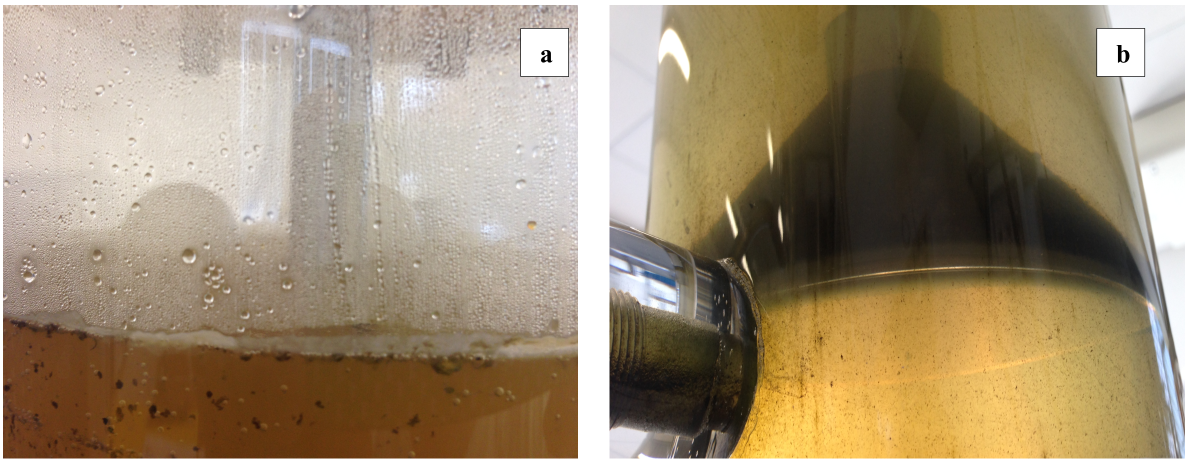


Figure 3.3 - a) Gas bubbles on the free surface inside the reactor; b) gas level under the GLS separator.

3.2. Effluent characteristics

The average effluent composition during the different run phases is reported in Table 3.1. It can be noticed that, after the first phase, effluent quality remained constant under different operative conditions. Therefore, it seemed that, compared to HRT 12 days, both recycling and HRT 6 days did not significantly affected the reactor performance. Moreover, the sedimentation process was not disturbed by higher upflow velocities, as TSS and COD_{susp} concentrations slightly decreased during 3rd and 4th phase.

Table 3.1 - Composition of the effluent during the different run phases

	Unit	1st phase	2nd phase	3rd phase	4th phase
pH	-	7,8	8,3	8,1	7,9
Temperature	°C	26,6	26,1	24,9	23,3
COD total	mg L^{-1}	1060	288	148	167
COD dissolved	mg L^{-1}	302	109	110	121
COD suspended	mg L^{-1}	758	179	38	46
TS	mg L^{-1}	2021	874	710	760
VS	mg L^{-1}	593	311	244	224
TSS	mg L^{-1}	275	91	59	58
VSS	mg L^{-1}	188	56	48	46
TKN	mgN L^{-1}	250	157	104	110
NH_4^+-N	mgN L^{-1}	219	135	96	109
N org	mgN L^{-1}	32	23	8	2
P tot	mgP L^{-1}	25	22	22	17

3.3. Solids removal

Solids removal was highly achieved with this kind of reactor (Figure 3.4; Figure 3.5; Figure 3.6). The percentage of removal of TS and VS was nearly constant during the experimental period (62% and 79%, respectively). The average removal efficiencies of TSS and VSS were 95% and 96% respectively, due to a complete sedimentation of suspended matter. This results in a low content of suspended solids in the effluent, with average concentrations of 77 mgTSS L^{-1} and 52 mgVSS L^{-1}. As a consequence, dissolved solids, which were about 30% of TS in the influent, raise up to 90% of TS in the effluent. While TSS and VSS percentage removal was nearly the same, VS removal was higher than TS. This implies that VS were removed both physically and biologically. Al-Jamal and Mahmoud (2008) obtained similar effluent quality for TSS and VSS at HRT of 2 and 4 days. Their efficiencies ranged between 74% and 78%, because the domestic sewage used as influent was characterized by low TSS and VSS concentrations (nearly 400 and 300 mg L^{-1}).

No significant difference on removals was noticed between 2nd, 3rd and 4th phase, even though influent TS and TSS varied between 1088-4120 mg L^{-1} and 490-3448 mg L^{-1}, respectively. Therefore, recycling and lower HRT did not affect the process efficiency.

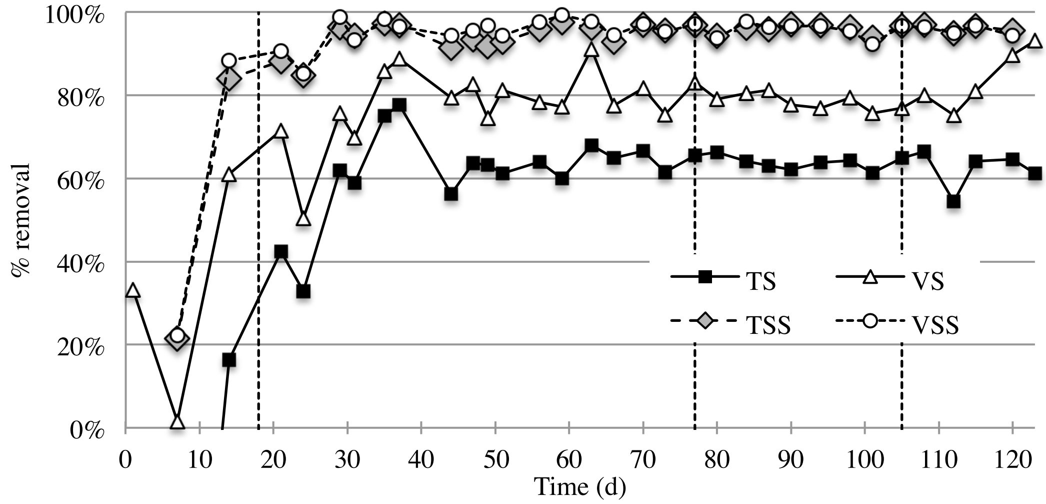


Figure 3.4 - Solids removal efficiencies (vertical dotted lines divide the different run phases).

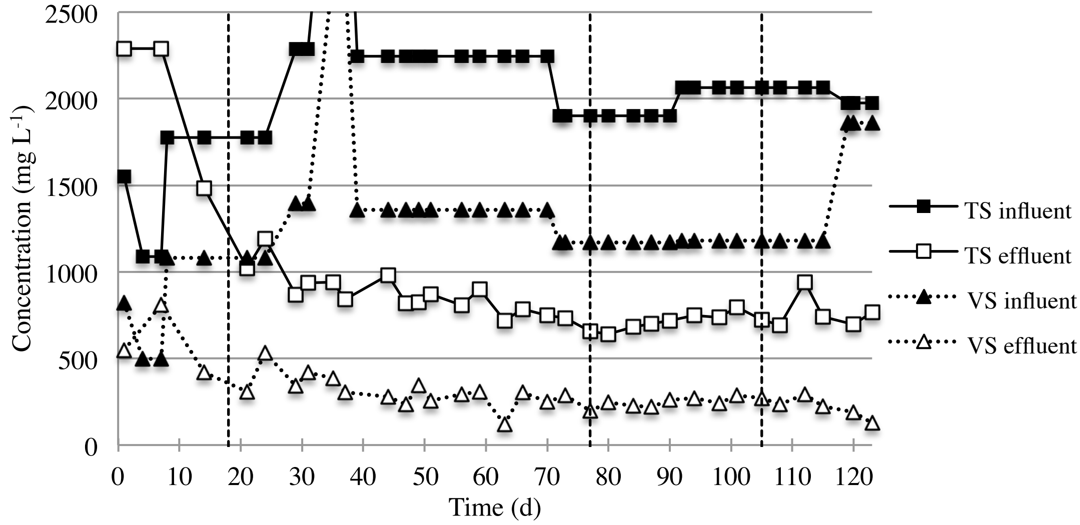


Figure 3.5 - TS and VS concentrations in influent and effluent (vertical dotted lines divide the different run phases).

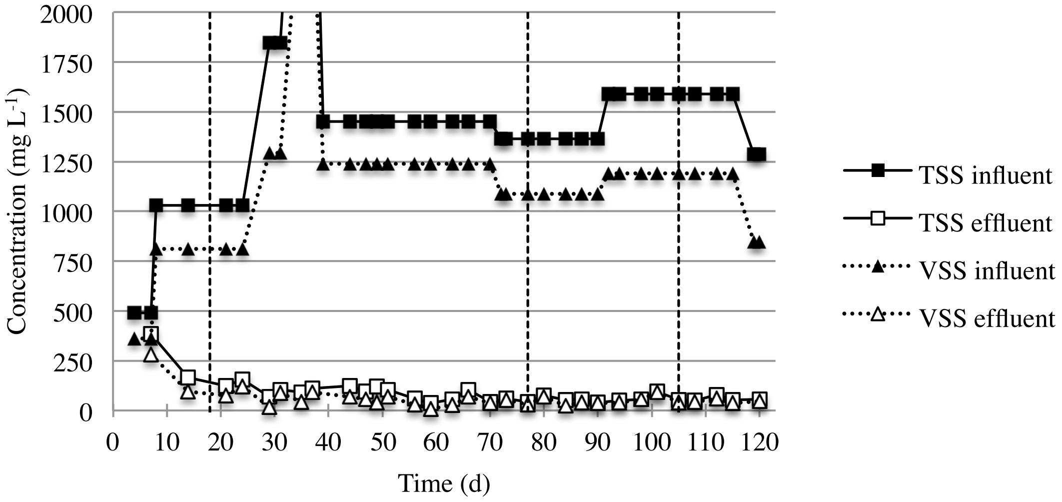


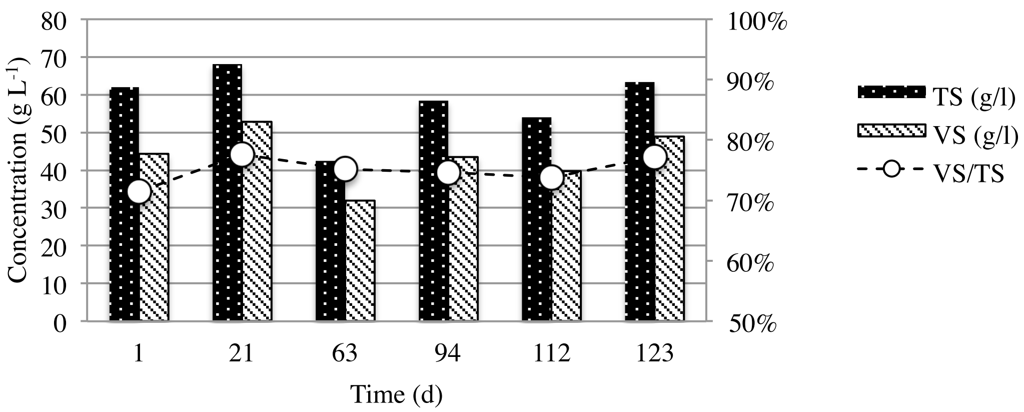
Figure 3.6 - TSS and VSS concentrations in influent and effluent (vertical dotted lines divide the different run phases).

3.4. Sludge bed development

The sludge bed development was monitored during the experimental period (Figure 3.7). The concentration of TS and VS remained nearly constant, while the volume of the sludge increased of about 50% (from 2,3 to 3,3 liters) because of particulate matter accumulation. This is a consequence of highly efficient sedimentation of non-degradable organics and inorganics, but also of slow hydrolysis of degradable organic matter.

The percentage of VS in the sludge slightly increased from the beginning of the experimental period. At the beginning, the ratio VS/TS was 71% and reached 77% after 123 days of operation. This indicates that sludge did not stabilize during the experimental period. The same behaviour was noticed by Kujawa and Zeeman (2005). In fact, inoculum sludge requires a long time to stabilize especially under anaerobic conditions at low temperatures, due to slow hydrolytic process (Metcalf and Eddy, 2004; Kujawa and Zeeman, 2005). According to Luostarinen *et al*. (2007), suspended solids can cause formation of scum layers and sudden washout of sludge, if they are only accumulated and not stabilised within the reactor. Long periods of operation would allow for better sludge bed development, thus improving the process performance of the reactor both in sedimentation and biological removal (Al-Shayah and Mahmoud, 2008; Al-Jamal and Mahmoud, 2009). Differently, Luostarinen and Rintala (2005), Halahsheh *et al.* (2005) and Moussavi *et al.* (2010) reported a VS/TS ratio between 47% and 61%, 66% and 57% respectively, indicating digestion and stabilization of the retained sludge. These positive results would be probably obtained in the long term period.

The sludge volume increased from 26% to 35% of the reactor volume during the experimental period. According to this result, it would take almost 1 year to fill 50% of reactor volume. For the anaerobic treatment of wastewaters with a large fraction of particulate matter, the hydrolysis of particulates is generally the rate-limiting step. Long sludge retention time (SRT) is therefore needed to provide a sufficient hydrolysis and methanogenesis (Kujawa and Zeeman, 2006). Solids retention time was evaluated for the overall experimental period and resulted to be around 700 days. As a consequence, the reactor desludging would be necessary only after several years of operation. Similarly, Luostarinen and Rintala (2005) reported that sludge volume increased, in a UASB-septic tank treating black water, from 20% to 65% after around 400 days of operation. According to literature (Zeeman *et al.*, 2000; Al-Shayah and Mahmoud, 2008; Al-Jamal and Mahmoud, 2009), the withdrawal of the sludge could be done once every 1 to 4 years or even more. This implies that the costs for sludge handling and treatment would be significantly reduced by using this kind of reactors.



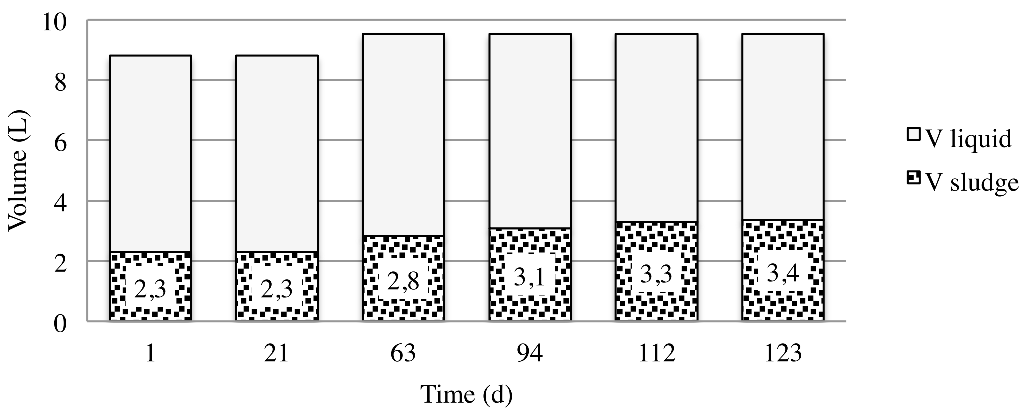


Figure 3.7 - Sludge bed development during the experimental period: concentration of TS and VS; sludge development.

3.5. BMP test results

The results of BMP tests on the influent wastewater are shown in Figure 3.8. Average values were obtained from the difference with blank tests. Specific cumulative biogas and methane productions were evaluated, in order to compare BMP test with the results of the experimental upflow-Imhoff tank. The substrate had a COD value of 4120 mg L^{-1}, while VS concentration was 2843 mg L^{-1}.

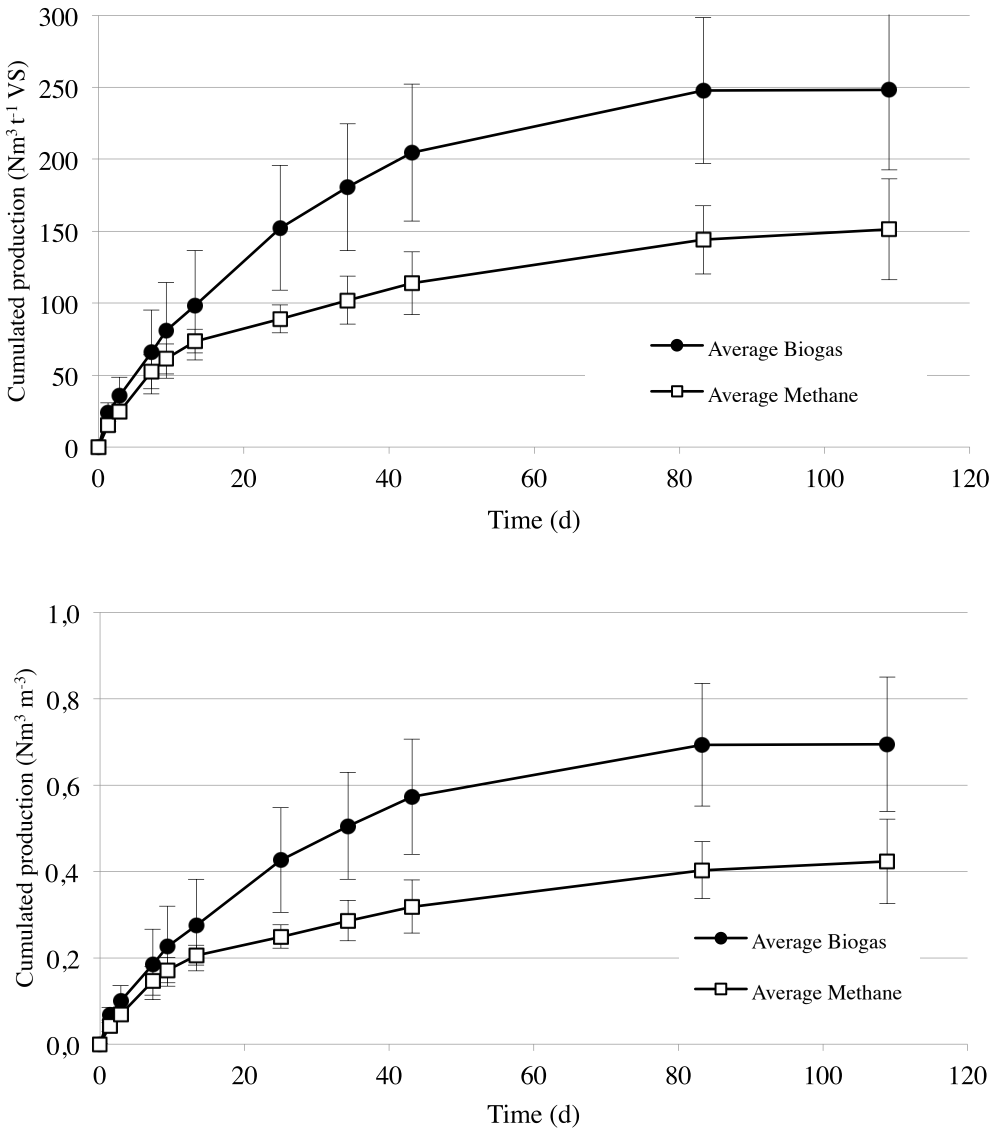


Figure 3.8 - Biogas and methane cumulated specific productions from BMP tests (VS and m^3 refers to the substrate used).

In order to estimate the maximum biogas and methane production, results from BMP tests were elaborated. The data on methane production were interpolated using an exponential function:

$$P(t) = P_\infty \bullet (1 - e^{-kt})$$

where P(t) is methane production at time t , P_∞ is the ultimate value of methane production and k is the rate of methane production. The OLS method (i.e. ordinary least squares) was used to determine P_∞ and k, using Libre Office Solver. Results obtained are shown in Figure 3.9. The resulting P_∞ values are 0,4 Nm^3 CH_4 m^{-3} and 143 Nm^3 CH_4 t^{-1} VS. At standard conditions, the volume occupied by one mole of any gas is 22,414 liters. Thus, it results that 400 NmL CH_4 produced per liter of substrate correspond to 1142 mg of COD removed per liter. Therefore, COD transformed into methane is about 28% of substrate COD (i.e. COD as CH_4 / COD substrate = 1142 mg L^{-1} / 4120 mg L^{-1}). This result indicates that the biggest fraction of influent COD is not easily biodegradable or inert, confirmed by the low BOD_5/COD ratio (0,09-0,14). Kujawa and Zeeman (2005) obtained 54% biodegradability of concentrated black water (COD_{tot} 10 g L^{-1}, VS 6 g L^{-1}) in batch test at 35°C.

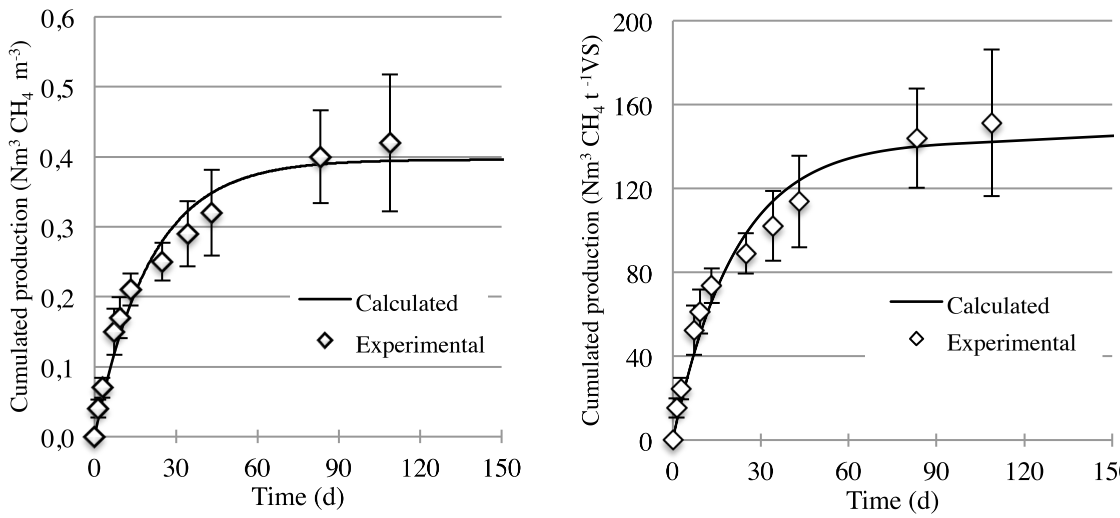


Figure 3.9 - Function evaluation of specific methane cumulated production from BMP tests (VS and m^3 refers to the substrate used).

It was possible to estimate the COD fractioning at the end of the test. The results are reported in Figure 3.10. Dissolved COD measured at the end of the test is supposed to be inert, as long time of retention allowed complete conversion of organics into biogas.

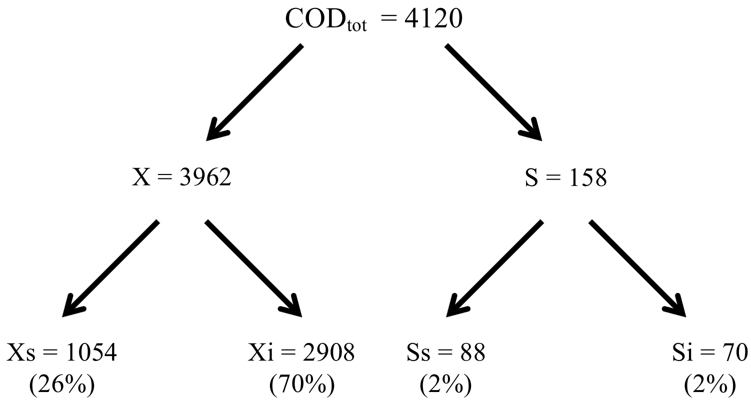


Figure 3.10 - COD fractioning from BMP test (mg L^{-1}; % on COD_{tot}).

In addition, analysis on the liquid from the BMP test reactors were performed in order to assess the percentage of biodegradability. At the beginning of the test organic nitrogen was 45 mgN L^{-1}, decreased to 5 mgN L^{-1} at the end. This result shows that the black water biodegradability is about 89%. The same result should be obtained from organic carbon mass balance. At the beginning of the test, organic carbon of filtered substrate (with TOC analyser, which cannot work with particulate matter in suspension) was 100 mgC_{org} L^{-1} (i.e. 25 mg C_{org}). The maximum specific production, as seen in Figure 3.9, was 0,4 Nm^3 CH_4 m^{-3}, corresponding to 54 mg C_{org} transformed into methane. This results shows that not only dissolved organic matter, but also particulate organic matter is converted into biogas.

3.6. Organics removal and biogas production

It clearly results that the main contributor to COD_{tot} in the effluent, with the exception of the first phase, is COD_{diss} varying between 38% and 74% (Figure 3.11). From day 60, the effluent COD_{diss} was higher than COD_{susp} because of effective sedimentation process. After 20 days of operation, as illustrated in Figure 3.12, the VFA monitored inside the reactor decreased and maintained stable values (below 60 mg CH_3COOH L^{-1}). Neither VFA accumulation nor increased biogas production was detected. According to Lew *et al*. (2004), domestic wastewater requires an initial hydrolysis step, which is significantly affected by temperature and is usually the rate-limiting step.

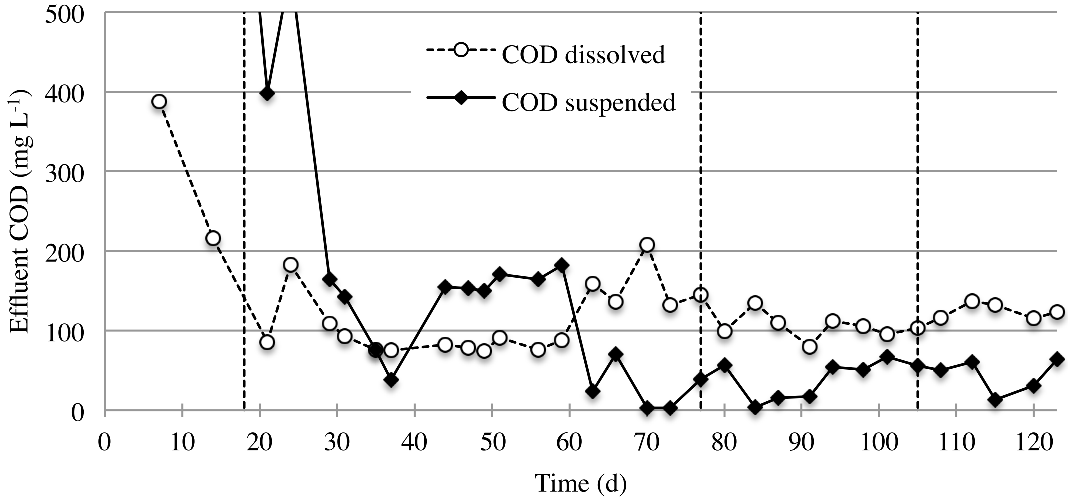


Figure 3.11 - COD fractions in the effluent (vertical dotted lines divide the different run phases).

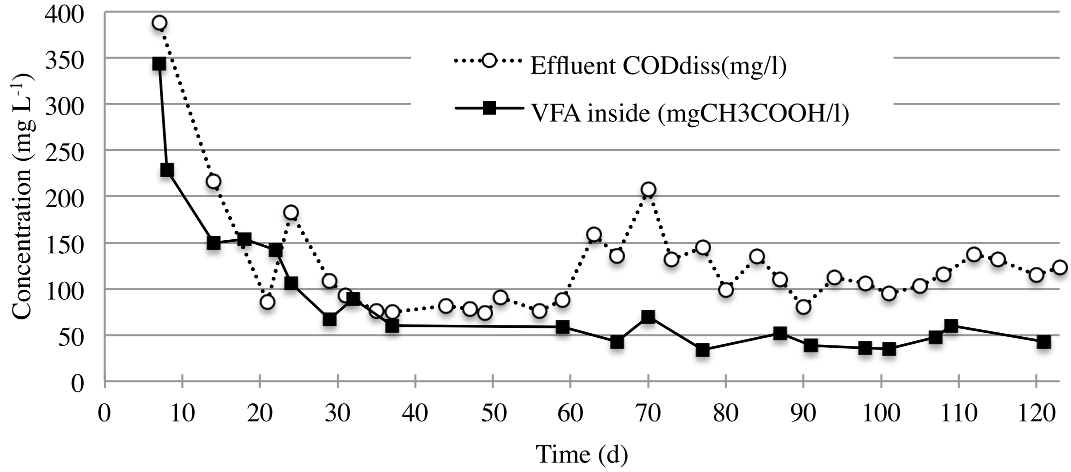


Figure 3.12 - Variation of dissolved COD in the effluent and VFA inside the reactor.

Table 3.2 - Comparison of COD removal efficiencies in present and earlier studies

Reference	Reactor	Wastewater	T (°C)	COD_{tot}	COD_{susp}	COD_{diss}
This study (2nd phase)	Upflow-Imhoff tank	Concentrated black water	21-32	89-96%	92-99%	23-44%
Al-Jamal and Mahmoud, 2009	UASB-septic tank	Domestic sewage	17	51-54%	83-87%	24-28%
Al-Shayah and Mahmoud, 2008	UASB-septic tank	Domestic sewage	24	56-58%	87-90%	20-22%
Ali *et al.*, 2007	UASB-septic tank	Domestic sewage	24	79%		43%
Elmitwalli *et al.*, 2003	Two phased upflow-hybrid septic tank	Concentrated sewage	13	94%	98%	78%
Luostarinen and Rintala, 2005	Two-phased UASB-septic tank	Synthetic black water	10	94%	98%	71%
Luostarinen *et al.*, 2007	Two-phased UASB-septic tank	Black water	14-18	71%	75%	44%
Kujawa and Zeeman, 2005	UASB-septic tank	Concentrated black water	15-25	61-78%	88-94%	

The removal efficiencies in terms of COD fractions are shown in Figure 3.13, 3.14 and 3.15. The results obtained were consistent with previous research and in some cases even higher (Table 3.2).

Dissolved COD in the influent accounted for less than 10% of total COD, thus suspended COD was comparable with total COD. It appears evident that, from day 28, the removal efficiency of COD_{tot} maintained very high values (above 90%). In particular, during 3rd and 4th phases COD_{susp} reached 99% removal. This result shows the effectiveness of the upflow-Imhoff tank in removing COD_{susp}, both with HRT 12 days and 6 days. While COD_{susp} was continuously removed, COD_{diss} was produced at the beginning of the experimental period and at the end of 2nd phase. This was probably due to the hydrolysis of particulate organic matter, which is a fundamental step before methanogenesis, or biomass release. However, as the COD_{diss} in the effluent was lower than in the influent during almost the experimental period, it results that the hydrolytic phase was the rate limiting process: the percentage of hydrolysis resulted to be only 6%. Previous research demonstrated that the performance of UASB-septic tanks at low temperatures (5–20 °C), treating concentrated sewage, is severely limited by the slow hydrolysis of solids that accumulate in the sludge bed (Zeeman and Lettinga, 1999). Elmitwalli *et al.* (2003) and Luostarinen and Rintala (2007) found that, despite high CODtot removal from concentrated sewage in upflow-hybrid septic tank, COD_{susp} was partly accumulated without further degradation. As a consequence of limited hydrolysis, methane production is compromised and the process may be deteriorated. However, other authors (Al-Jamal and Mahmoud, 2008; Al-Shayah and Mahmoud, 2009) obtained higher percentages of hydrolysis (between 16% and 26%) because of the lower content of COD_{susp}.

The pH monitored inside the reactor (Figure 3.16) was at optimal conditions, between 6,5 and 7,5 (Kujawa and Zeeman, 2005), during the second phase. Then, it slightly increased and fluctuated, but it was never of concern. No accumulation of VFA inside the reactor is a positive indicator of good methanogenesis, but it also suggests that hydrolysis was the rate limiting step as biogas production was very low.

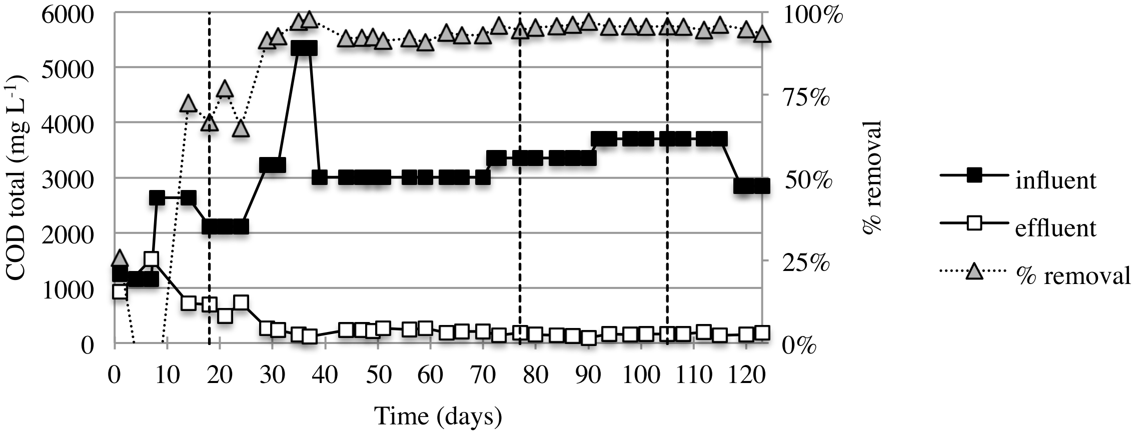


Figure 3.13 - Influent and effluent concentrations of total COD and removal efficiency. Vertical dotted lines divide the different run phases.

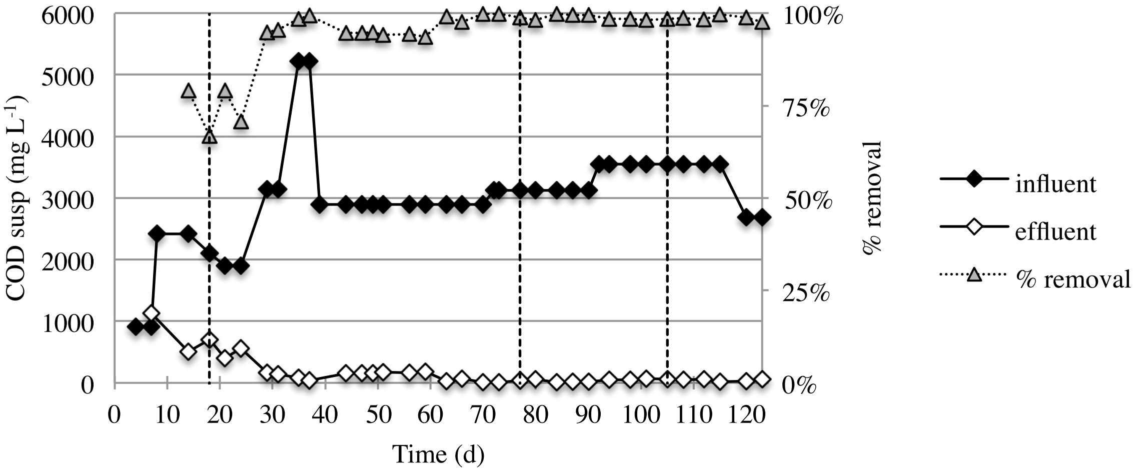


Figure 3.14 - Influent and effluent concentrations of suspended COD and removal efficiency. Vertical dotted lines divide the different run phases.

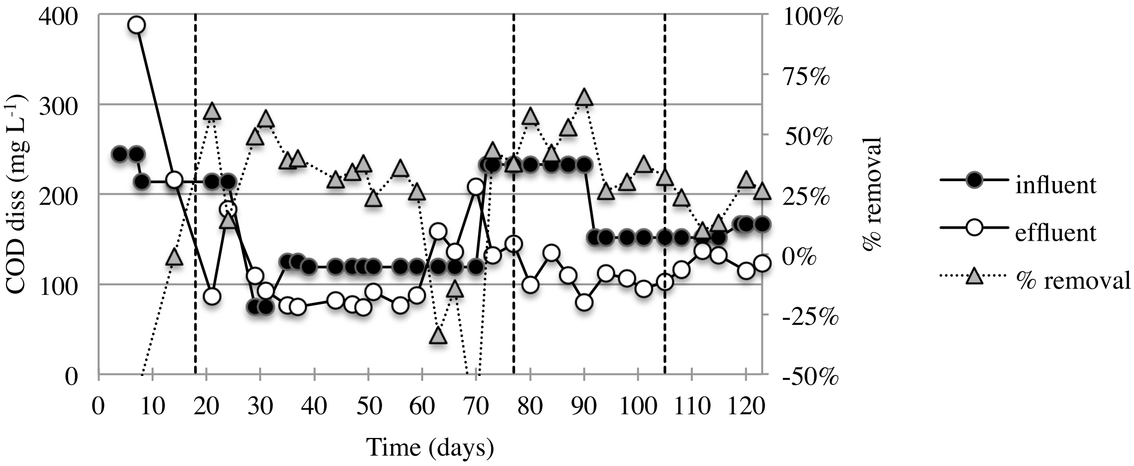


Figure 3.15 - Influent and effluent concentrations of dissolved COD and removal efficiency. Vertical dotted lines divide the different run phases.

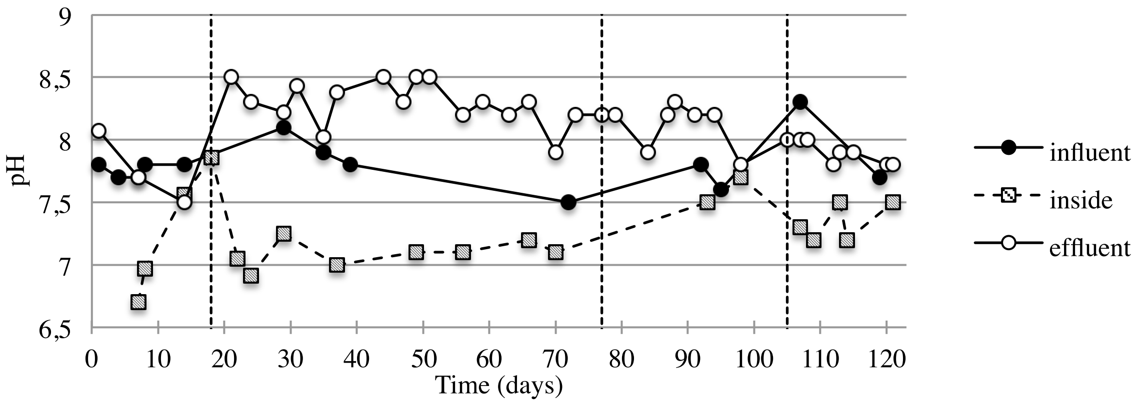


Figure 3.16 - pH behaviour in influent, effluent and inside the reactor during the experimental period. Vertical dotted lines divide the different run phases.

Biogas was firstly measured on day 22, at the beginning of 2nd phase. The cumulative biogas production is shown in Figure 3.17, while the specific production on VS is illustrated in Figure 3.18. During 2nd phase, biogas production followed a linear trend with 86 mL biogas d^{-1}, with 86% of methane content. The methanisation rate calculated as COD to $CH_4/COD_{influent}$ was 10%. The specific methane production was the highest obtained over the experimental period with 72 Nm^3 CH_4 t^{-1} VS. Biogas measurement faced some problems during 3rd phase when recycle was applied. Even though biogas production decreased sharply (with some days of no production at all), effluent quality remained on stable levels. For this reason, it seemed that biogas was still produced, but not detected by the gas counter. The average daily gas production was 33 mL biogas d^{-1}, with 87% of methane. The methanisation rate was only 2%. The specific methane production was 33 Nm^3 CH_4 t^{-1} VS. It was necessary to understand if there was a problem with the gas counter functioning, with the biomass or if the system was under fed. First, it was decided to decrease HRT to 6 days (4th phase), thus increasing the amount of COD_{diss} fed. As no difference in biogas measurement was detected, a 2-liters solution containing 3 g of acetic acid was injected, in order to evaluate if the biomass inside the reactor was still active. Suddenly, biogas started to be measured and about 2 liters of gas expected from the acetic acid solution were produced. As a consequence, it was probable that the recycle, being the only difference between 2nd and 3rd phase, caused some interferences with the gas measurement. During the last phase, recycle was stopped and seldom applied to mix the sludge and to avoid its compaction. Even if recycle lead to a better distribution of the influent in the sludge, no improvement in reactor performance was achieved. In Figure 3.19 it is possible to notice that, even though COD_{diss} increased in the influent, removal of COD as methane did not follow the same trend as expected.

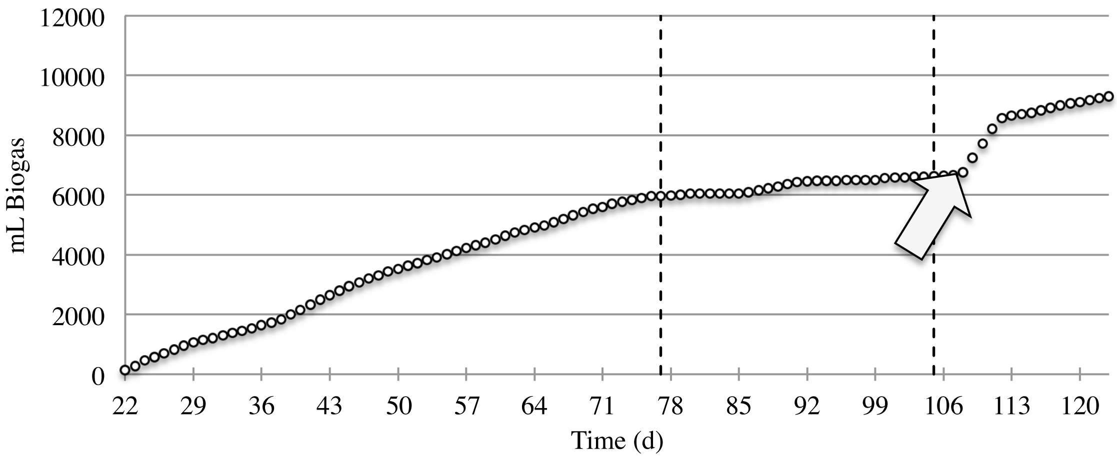


Figure 3.17 - Cumulative biogas production. Vertical dotted lines divide into 2nd, 3rd and 4th run phase. Grey arrow indicates injection of 2-liters solution with 3 g L^{-1} of acetic acid.

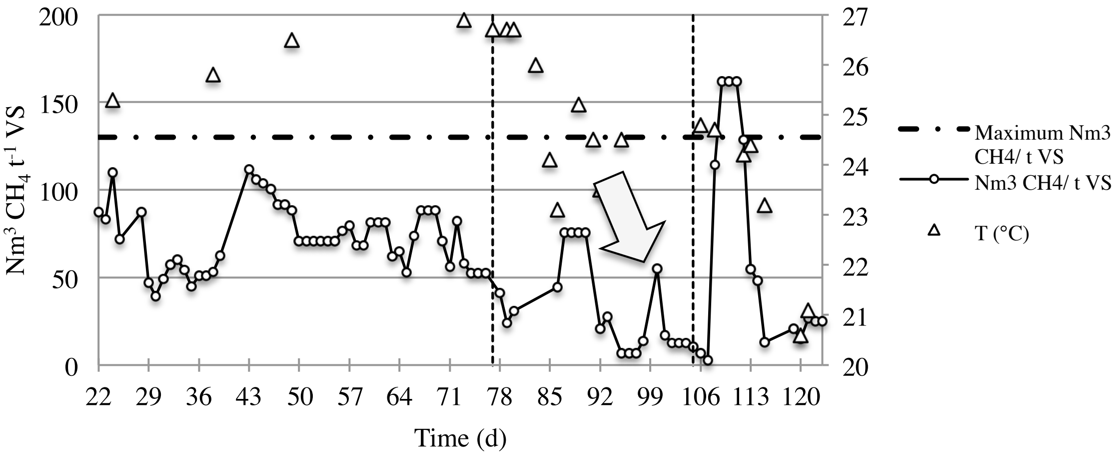


Figure 3.18 - Specific methane production compared to maximum value obtained from BMP tests. Vertical dotted lines divide into 2nd, 3rd and 4th run phase. Grey arrow indicates injection of 2-liters solution with 3 g L^{-1} of acetic acid.

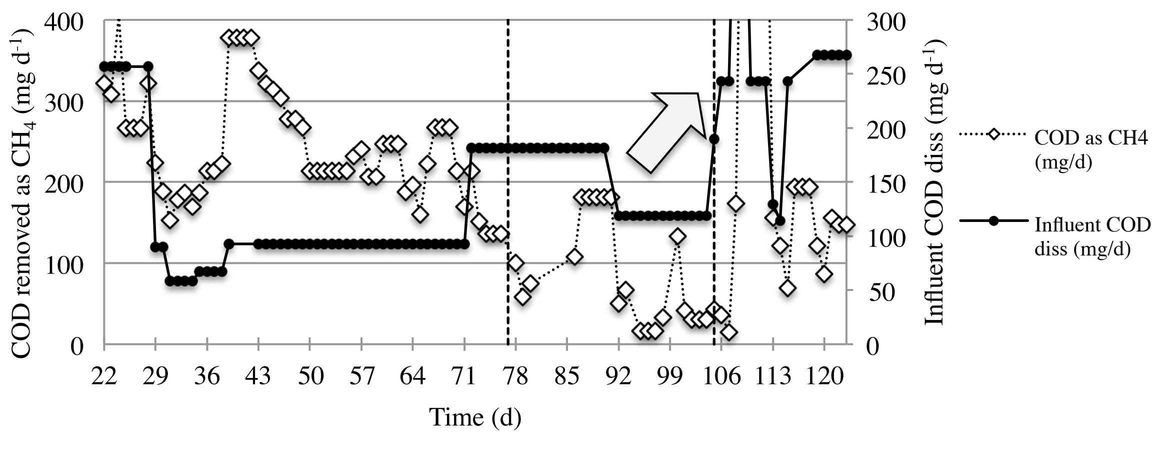


Figure 3.19 - Daily COD removed as methane and influent COD_{diss}. Vertical dotted lines divide into 2nd, 3rd and 4th run phase. Grey arrow indicates injection of 2-liters solution with 3 g L^{-1} of acetic acid.

3.7. COD mass balance

The result of the assessed mass balance is shown in Figure 3.20. According to this estimation, only 7,2% of the measured influent COD_{tot} was converted to gaseous methane, while 2,2% left the reactor through the effluent as methane in the liquid phase. Only 6,5% of influent COD was found in the effluent, 7% was subtracted to the system when collecting sludge to be analysed and the most important fraction (50,7%) was accumulated inside the reactor. This result shows again that the sedimentation of suspended particles was extremely effective.

The gap in mass balance (26,4%) is not surprising: Kujawa and Zeeman (2005) obtained similar results (29,7% and 40,7%) for two UASB-septic tanks treating concentrated black water at 15° and 25°C. According to them, these large gaps could be due to under measurement of biogas production: malfunctioning of the gas counter, escape from the GLS separator, entrapment of the biogas in the upper part of the reactor (Figure 3.3a) may explain this high missing fraction. To less extent, another reason could be the upper estimation of influent COD. Another possible explanation is related to sulphate content. In influent wastewater a maximum sulphate concentration of 250 $mgSO_4^{2-}\ L^{-1}$ was found. Oxidized sulphur compounds can serve as electron acceptors for sulphate reducing bacteria, which consume organic compounds in the anaerobic reactor and produce hydrogen sulphide. Metcalf and Eddy (2004) suggest that the amount of COD used for sulphate reduction is 0,89 $gCOD\ g^{-1}SO_4^{2-}$. Even though H_2S was not measured in the biogas, the maximum amount of COD used for sulphate reduction was estimated and resulted to be 222 $mgCOD\ L^{-1}$. In the experimental period 85 litres of influent were fed, resulting in about 19 gCOD consumed (i.e. 6,7% of input COD). Moreover, methane that left the system through the effluent was calculated according to Henry's law, which is valid at equilibrium conditions. In this way, methane in the liquid was underestimated. Singh and Viraraghavan (1998) obtained that 60% of methane produced was left through the effluent.

If the missing fraction was considered only as methane, its specific production would be 328 $Nm^3\ CH_4\ t^{-1}VS$. This value is not realistic, being roughly three times higher than BMP result on maximum specific production (143 $Nm^3\ CH_4\ t^{-1}VS$).

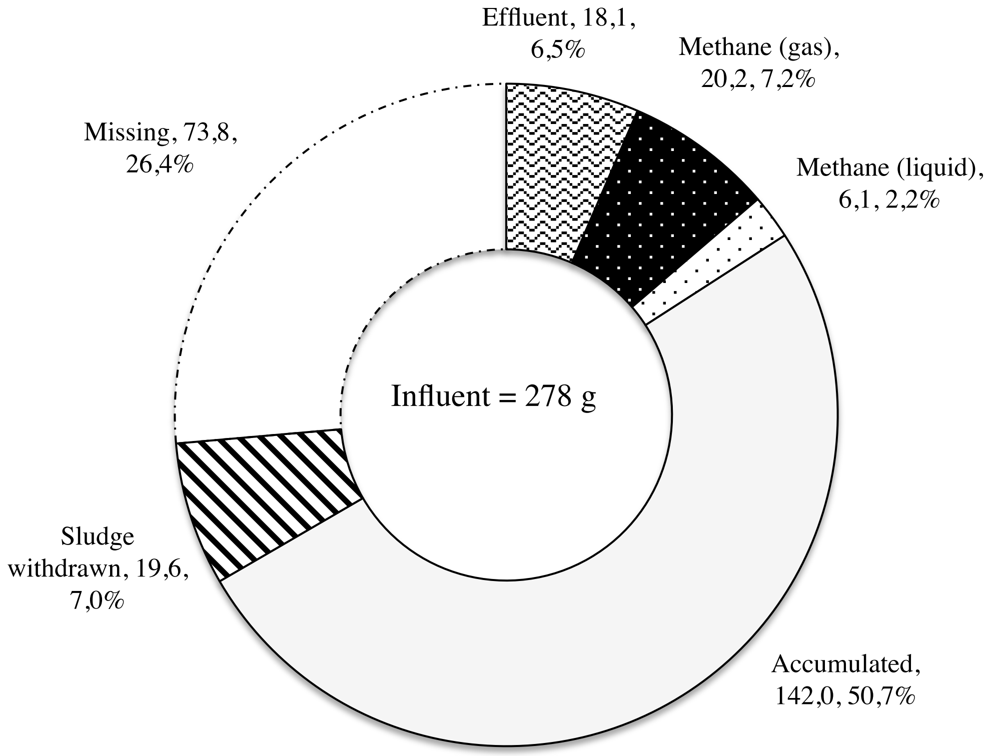


Figure 3.20 - COD mass balance on total influent COD over the experimental period of 123 (g; %).

3.8. Nutrients removal

The average influent TKN and NH_4^+-N was 237 mgN L^{-1} and 177 mgN L^{-1} respectively. This results in 60 mgN L^{-1} as organic N. The average effluent TKN was 152 mgN L^{-1}, with 87% of NH_4^+-N (Figure 3.21). Kujawa and Zeeman (2005*)* found that 90% of effluent TKN was ammonium, indicating high hydrolysis of proteins. Organic N in the effluent was 16 mgN L^{-1}, with an average removal efficiency of 73%. The average removal efficiencies of TKN and NH_4^+-N were 37% and 20% during 2nd phase. An increasing trend in nitrogen removal was monitored during 3rd phase (TKN 48% and NH_4^+-N 42%), probably due to lower influent concentrations. The effluent pH, being always higher than 7,5, may have influenced ammonia equilibrium, facilitating the release of gaseous ammonia in the atmosphere and a decrease of NH_4^+ in the effluent. Nevertheless, the difference in TKN and ammonium concentrations between the influent and the effluent was not significant.

The average effluent phosphorous was 21 mgP L^{-1}, with a removal efficiency of 40% (Figure 3.22). Phosphates (PO_4^{3-}-P) accounts for 90% of P_{tot} in the effluent.

Luostarinen and Rintala (2005) reported that little or no nutrient removal may be expected in UASB-septic tanks treating black water. The reason of the low nutrient removal is that they exist mainly in soluble forms (Kujawa and Zeeman, 2005). The very low nutrient removal in UASB-septic tanks treating strong sewage in Palestine was also reported by Al-Shayah and Mahmoud (2008) and Al-Jamal and Mahmoud (2009). The fact that the nutrients are present mainly in a soluble form (ammonium and phosphates), being readily available for the plants, make reuse of the effluent in agricultural field an attractive option (Kujawa and Zeeman, 2005).

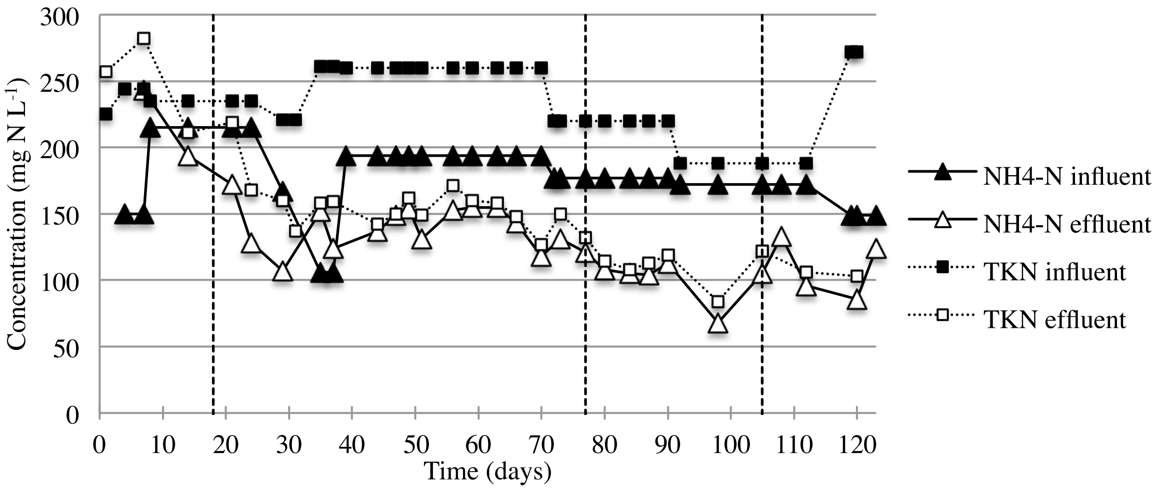


Figure 3.21 - Nitrogen course (TKN and ammonium) during the experimental behaviour (vertical dotted lines vertical dotted lines divide the different run phases).

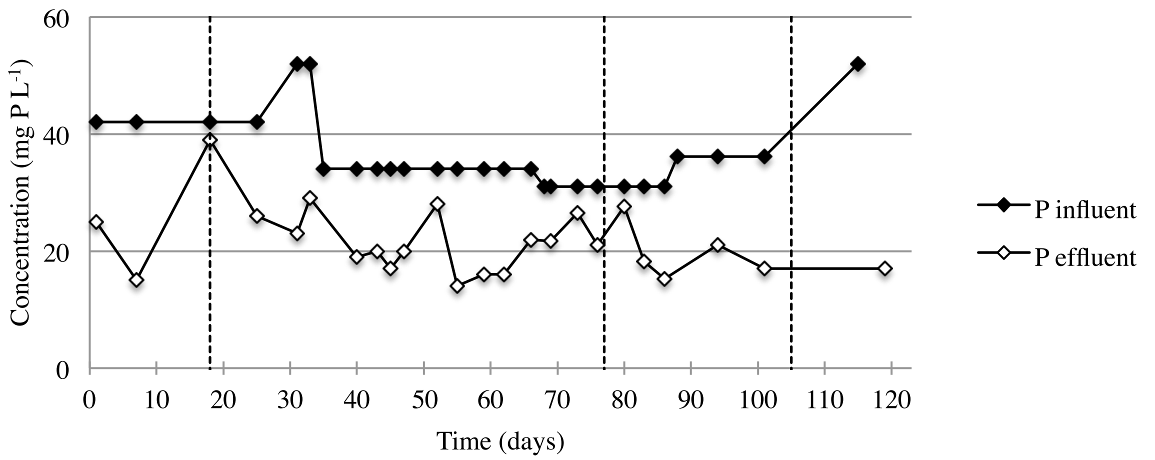


Figure 3.22 - Total phosphorous course during the experimental behaviour (vertical dotted lines vertical dotted lines divide the different run phases).

4. Conclusions

The aim of this experimental study was to assess the feasibility of upgrading existing Imhoff tanks into upflow-Imhoff tanks, in order to improve the quality of the effluent wastewater. The driving principle of this research was the decentralized sanitation and reuse concept, fitted with source segregation of different wastewater fluxes generated at household level. It was proved that this upgrading leads to better process performance. Thanks to the upward flow, both physical removal of particles and biological degradation are enhanced. This kind of reactor results to be a suitable option for decentralized wastewater treatment, in terms of compactness and easily operation (Elmitwalli *et al*., 2003). The results obtained in this research show high efficiencies in removing both solids and COD (TSS 95%, VSS 96%, COD_{tot} 89-96%, COD_{susp} 92-99%, COD_{diss} 23-44%). However, influent concentrated black water is rich in suspended matter, which accumulates inside the reactor. It is important to estimate the particulate inert fraction (i.e. X_i), because it determines the frequency of emptying the reactor. The filling period of the reactor resulted to be around 700 days, as sludge production is very low and sludge bed mainly increases because of particulate matter settling. Stabilization of sludge could be achieved in the long term operation.

According to BMP test results, COD of black water used was for 70% inert particulate, while 26% was biodegradable particulate. The very low fraction of dissolved COD determines low biogas production, because hydrolysis takes time to convert suspended organic matter. The maximum specific methane production obtained from BMP test was 143 $Nm^3CH_4\ t^{-1}VS$, while in the experimental reactor it ranged between 33 and 72 $Nm^3CH_4\ t^{-1}VS$. This gap is due to under measurement of biogas produced, but also to lower level of hydrolysis in the upflow-Imhoff tank (HRT 6-12 days) if compared to BMP tests (around 110 days). In order to obtain higher biogas production and reduce solids accumulation, hydrolysis should be further increased.

The mass balance on total input COD was carried out, taking into consideration the amount of COD accumulated inside the reactor (50,7%), methane production (9,4%), effluent COD (6,5%) and sludge withdrawn (7%). A missing fraction of 26,4% was attributed to under measurement of biogas produced and sulphate reduction.

As expected, low nutrient removal was achieved. The average effluent concentration were 152, 132 and 21 of TKN ($mgN\ L^{-1}$), ammonium ($mgN\ L^{-1}$) and phosphorous ($mgP\ L^{-1}$), respectively.

It was observed that, with the exception of the first phase, the process performance was almost constant. Changing HRT from 12 to 6 days and adding the recycle did not affect removal efficiencies. However, problems with biogas measurement occurred when recycle was applied. It was not understood why there was an interference with the gas counter, having proved that biomass was still active and COD removal stayed the same.

Finally, it must be underlined that effluent characteristics are not consistent with national regulations on free surface discharge (COD 125 mg L^{-1}; TSS 35 mg L^{-1}; TKN 15 mg L^{-1}; P 2 mg L^{-1}) (Decreto Legislativo 3 aprile 2006, n. 152; Allegato 5). In addition, heavy metals, pathogens and micropollutants make the effluent reuse a sensitive issue. As a consequence, further treatment is needed to remove residual COD, pathogens and to remove or recover nutrients depending on local requirements and reuse potentials. Several post treatments methods are proposed in literature: stabilization ponds, trickling filters, activated sludge and soil adsorption (Kujawa and Zeeman, 2006). Within the concept of on-site decentralized wastewater treatment system, phytotreatment appears to be the most suitable and sustainable post-treatment to meet the standards for reuse and discharge.

5. Bibliography

Ali, M., Al-Sa'ed, R., Mahmoud, N., 2007. *Start-up phase assessment of a UASB-septic tank system treating domestic septage.* The Arabian Journal for Science and Engineering, Volume 32, Number 1 C.

Al-Jamal, W., Mahmoud, N., 2009. *Community onsite treatment of cold strong sewage in a UASB-septic tank.* Bioresource Technology 100, 1061–1068.

Al-Shayah, M., Mahmoud, N., 2008. *Start-up of an UASB-septic tank for community on-site treatment of strong domestic sewage.* Bioresource Technol. 99, 7758– 7766.

Da Silva, F. J. A., Lima, M. G. S., Mendoça, L. A. R., Gomes, M. J. T. L., 2013. *Septic tank combined with anaerobic filter and conventional UASB – Results from full scale plants.* Brazilian Journal of Chemical Engineering 30 (1), 133-140.

Decreto Legislativo 3 aprile 2006, n. 152. Norme in materia ambientale. Gazzetta Ufficiale n. 88 del 14 aprile 2006 - Supplemento Ordinario n. 96.

De Graaff, M. S., Temmink, H., Zeeman, G., Buisman, C. J. N., 2010. *Anaerobic Treatment of Concentrated Black Water in a UASB Reactor at a Short HRT.* Water 2010, 2, 101-119; doi: 10.3390/w2010101.

Elmitwalli, T.A., Sayed, S., Groendijk, L., van Lier, J., Zeeman, G., Lettinga, G., 2003. *Decentralised treatment of concentrated sewage at low temperature in a two-step anaerobic system: two upflow-hybrid septic tanks.* Water Sci. Technol. 48 (6), 219–226.

Giménez, J. B., Martì, N., Ferrer, J., Seco, A., 2012. *Methane recovery efficiency in a submerged anaerobic membrane bioreactor (SAnMBR) treating sulphate-rich urban wastewater: Evaluation of methane losses with the effluent*. Bioresource Technology 118, 67-72.

Halalsheh, M., Dalahmeh, S., Sayed, M., Suleiman, W., Shareef, M., Mansour, M., Safi, M., 2008. *Grey water characteristics and treatment options for rural areas in Jordan.* Bioresource Technology 99, 6635–6641.

Henze M., Van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D., 2008. *Biological Wastewater Treatment: Principles Modelling and Design.* ISBN: 9781843391883. Published by IWA Publishing, London, UK.

Henze, M., Ledin, A., 2001. *Types, characteristics and quantities of classic, combined wastewaters.* In: Lens, P., Zeeman, G., Lettinga, G. (Eds.), Decentralised Sanitation and Reuse IWA Publishing, UK, pp. 57–72.

ISTAT, 2014. *Censimento delle acque per uso civile, Anno 2012*.

Kujawa, K. and Zeeman, G., 2005. *Anaerobic digestion of concentrated wastewater in DESAR concept*. STOWA report 2005-14.

Kujawa, K. and Zeeman, G., 2006. *Anaerobic treatment in decentralized and source-separation-based sanitation concepts.* Environmental Science and Bio/Technology (2006) 5:115–139.

Lew, B., Tarre, S., Belavski, M., Green, M., 2004. *UASB reactor for domestic wastewater treatment at low temperatures: a comparison between a classical UASB and hybrid UASB-filter reactor.* Water Science and Technology 49 (11-12), 295-301.

Lohani, S. P., Chhetri, A., Adhikari, J., Bakke, R., 2013. *Sustainable biogas production potential from urban wastewater in Nepal.* International Journal of Environmental Science and Development 4 (5), 595- 599.

Luostarinen, S.A., Rintala, J.A., 2005. *Anaerobic on-site treatment of black water and dairy parlour wastewater in UASB-septic tanks at low temperatures.* Water Res. 39 (2–3), 436–448.

Luostarinen, S.A. and Rintala, J.A., 2007. *Anaerobic on-site treatment of kitchen waste in combination with black water in UASB-septic tanks at low temperatures.* Bioresource Technol., 98: 1734–1740.

Luostarinen, S., Sanders, W., Kujawa-Roeleveld, K., Zeeman, G., 2007. *Effect of temperature on anaerobic treatment of black water in UASB- septic tank systems.* Biores. Technol. 98 (5), 980–986.

Metcalf and Eddy Inc., 2004. *Wastewater Engineering: Treatment and Reuse*. 4th ed. McGraw Hill, New York.

Moussavi, G., Kazembeigi, F., Farzadkia, M., 2010. *Performance of a pilot scale up-flow septic tank for on-site decentralized treatment of residential wastewater.* Process Safety and Environmental Protection 88 (2010) 47–52.

Singh, K. S., Viraraghavan, T., 1998. *Start-up and operation of UASB reactors at 20°C for municipal wastewater treatment.* Journal of Fermentation and Bioengineering 85 (6), 609-614.

Tilley, E., Ulrich, L., Luethi, C., Reymond, P., Zurbruegg, C., 2014. *Compendium of Sanitation Systems and Technologies*. 2nd Revised Edition. Duebendorf, Switzerland: Swiss Federal Institute of Aquatic Science and Technology (Eawag).

Zamalloa, C., Arends, J. B. A., Boon, N., Verstraete, W., 2013. *Performance of a lab-scale bio-electrochemical assisted septic tank for the anaerobic treatment of black water*. New biotechnology 30 (5), 573-580.

Zeeman, G., Lettinga, G., 1999. *The role of anaerobic digestion in closing the water and nutrient cycle at community level.* Water Sci. Technol. 39 (5), 187–194.

Zeeman, G., van Lier, J.B., Lettinga, G., 2000. *The role of anaerobic treatment at community and house on-site treatment of domestic waste (water). Effect of collection and transport systems*. In: Proceedings of the Technical Expert Consultation on Appropriate and Innovative Wastewater Management for Small Communities in EMR Countries. WHO/CEHA, Amman, Jordan, November 6–9, 2000.

Zeeman, G., Kujawa, K., de Mes, T., Hernandez, L., de Graaff, M., Abu-Ghunmi, L., Mels, A., Meulman, B., Temmink, H., Buisman, C., van Lier, J., Lettinga, G., 2008. *Anaerobic treatment as a core technology for energy, nutrients and water recovery from source-separated domestic waste(water).* Water Science & Technology, 57 (8), 1207-1212.

http://www.ritter.de/fileadmin/download/public/EN/Manuals/RITTER-EN-Manual-P02-MGC.pdf (last access 26/11/2014)

APPENDICES

APPENDIX A - Experimental data on temperature, pH, alkalinity and VFA.

Sampling date	**Day**	**Temperature** (°C)		**pH**			**Alkalinity** (mgCaCO3/l)	**Volatile Fatty Acids** (mgCH3COOH/l)
		IN	OUT	IN	INSIDE	OUT	INSIDE	INSIDE
01/07/14	1	27	27	7,8		8,1		
04/07/14	4	28,1		7,7				
07/07/14	7	26,7	26,7	7,7	6,7	7,7	1177	344
08/07/14	8	29,4	26,3	7,8	7,0		781	229
14/07/14	14	32,4	26,3	7,8	7,6	7,5	867	150
18/07/14	18				7,9		838	154
21/07/14	21					8,5		
22/07/14	22				7,1		708	142
24/07/14	24	27	25,3		6,9	8,3	717	106
29/07/14	29			8,1	7,3	8,2	739	67
31/07/14	31	31,5				8,4	788	89
04/08/14	35			7,9		8,0		
06/08/14	37				7	8,4	798	60
07/08/14	38	30,8	25,8					
08/08/14	39			7,8				
13/08/14	44					8,5		
16/08/14	47					8,3		
18/08/14	49	31,2	26,5		7,1	8,5		
20/08/14	51					8,5		
25/08/14	56				7,1	8,2		
28/08/14	59					8,3	739	59
04/09/14	66				7,2	8,3	716	43
08/09/14	70	30,9			7,1	7,9	711	70
10/09/14	72			7,5				
11/09/14	73		26,9			8,2		
15/09/14	77		26,7			8,2	626	34
17/09/14	79	33,6	26,7			8,2		
18/09/14	80		26,7					
22/09/14	84		26			7,9		
25/09/14	87		24,1			8,2	545	52
26/09/14	88		23,1			8,3		
28/09/14	90	29,2	25,2			8,2		
29/09/14	91						561	39
30/09/14	92			7,8				
01/10/14	93	31,8	24,5		7,5			
02/10/14	94	29,1	23,5			8,2		
03/10/14	95	28,6		7,6				
06/10/14	98	28,5	24,5		7,7	7,8	709	36
07/10/14	99		23					
09/10/14	101						702	35
14/10/14	106	27,7	24,8			8,0		
15/10/14	107	29,3		8,3	7,3	8,0	632	48
16/10/14	108	27,2	24,7			8,0		
17/10/14	109	27,0			7,2		543	60
20/10/14	112	27,0	24,2			7,8		
21/10/14	113	27,5	24,4		7,5	7,9		
22/10/14	114	25,9			7,2			
23/10/14	115		23,2			7,9		
27/10/14	119			7,7				
28/10/14	120	22,6	20,6			7,8		
29/10/14	121	26,6	21,1		7,5	7,8	769	43
31/10/14	123					7,8		

APPENDIX B - Experimental data on solids.

Sampling date	Day	SOLIDS							
		TS (mg/l)		VS (mg/l)		TSS (mg/l)		VSS (mg/l)	
		IN	OUT	IN	OUT	IN	OUT	IN	OUT
01/07/14	1	1550	2290	823	550				
04/07/14	4	1088		498		490		360	
07/07/14	7		2288	498	810	490	385	360	280
08/07/14	8	1775		1080		1030		810	
14/07/14	14		1485	1080	420	1030	165	810	95
21/07/14	21		1023	1080	308	1030	122	810	76
24/07/14	24	1775	1193	1080	535	1030	156	810	120
29/07/14	29	2285	868	1395	340	1846	68	1295	16
31/07/14	31	2285	938	1395	420	1846	104	1295	88
04/08/14	35	3764	940	2716	385	3459	92	2625	44
05/08/14	36								
06/08/14	37	3764	840	2716	306	3459	112	2625	92
07/08/14	38								
08/08/14	39	2245		1358		1452		1240	
13/08/14	44	2245	980	1358	280	1452	125	1240	70
16/08/14	47	2245	817	1358	235	1452	95	1240	55
18/08/14	49	2245	827	1358	347	1452	120	1240	40
20/08/14	51	2245	870	1358	255	1452	105	1240	70
25/08/14	56	2245	807	1358	295	1452	60	1240	30
28/08/14	59	2245	900	1358	308	1452	36	1240	8
01/09/14	63	2245	718	1358	120	1452	56	1240	28
04/09/14	66	2245	787	1358	305	1452	104	1240	68
08/09/14	70	2245	748	1358	248	1452	44	1240	36
10/09/14	72	1903		1170		1364		1088	
11/09/14	73	1903	732	1170	288	1364	60	1088	52
15/09/14	77	1903	655	1170	198	1364	44	1088	32
17/09/14	79								
18/09/14	80	1903	640	1170	245	1364	76	1088	68
22/09/14	84	1903	682	1170	228	1364	52	1088	24
25/09/14	87	1903	702	1170	220	1364	56	1088	40
26/09/14	88								
28/09/14	90	1903	720	1170	260	1364	40	1088	36
30/09/14	92	2065		1180		1588		1192	
01/10/14	93								
02/10/14	94	2065	748	1180	273	1588	50	1192	40
03/10/14	95								
06/10/14	98	2065	738	1180	243	1588	57	1192	55
07/10/14	99								
09/10/14	101	2065	798	1180	286	1588	96	1192	92
13/10/14	105	2065	724	1180	272	1588	54	1192	40
14/10/14	106								
15/10/14	107								
16/10/14	108	2065	692	1180	236	1588	49	1192	43
17/10/14	109								
20/10/14	112	2065	940	1180	293	1588	80	1192	60
21/10/14	113								
22/10/14	114								
23/10/14	115	2065	740	1180	224	1588	52	1192	38
27/10/14	119	1975		1860		1288		848	
28/10/14	120	1975	698	1860	190	1288	56	848	48
29/10/14	121								
31/10/14	123	1975	765	1860	128	1288	51	848	37

APPENDIX C - Experimental data on COD.

Sampling date	Day	COD					
		COD tot (mg/l)		COD dis (mg/l)		COD susp (mg/l)	
		IN	OUT	IN	OUT	IN	OUT
01/07/14	1	1258	934				
04/07/14	4	1157		244		913	
07/07/14	7	1157	1522	244	388	913	1134
08/07/14	8	2635		214		2421	
14/07/14	14	2635	723	214	216	2421	507
18/07/14	18	2110	701			2110	701
21/07/14	21	2110	484	214	86	1896	398
22/07/14	22						
24/07/14	24	2110	737	214	183	1896	554
29/07/14	29	3224	273	75	109	3149	164
31/07/14	31	3224	236	75	93	3149	143
01/08/14	32						
04/08/14	35	5350	152	125	76	5225	76
05/08/14	36						
06/08/14	37	5350	113	125	75	5225	38
07/08/14	38						
08/08/14	39	3010		119		2891	
13/08/14	44	3010	237	119	82	2891	155
16/08/14	47	3010	231	119	78	2891	153
18/08/14	49	3010	224	119	74	2891	150
20/08/14	51	3010	262	119	91	2891	171
25/08/14	56	3010	240	119	76	2891	164
28/08/14	59	3010	270	119	88	2891	182
01/09/14	63	3010	183	119	159	2891	24
04/09/14	66	3010	206	119	136	2891	70
08/09/14	70	3010	211	119	208	2891	3
10/09/14	72	3358		233		3125	
11/09/14	73	3358	135	233	132	3125	3
15/09/14	77	3358	184	233	145	3125	39
18/09/14	80	3358	156	233	99	3125	57
22/09/14	84	3358	139	233	135	3125	4
25/09/14	87	3358	126	233	110	3125	16
26/09/14	88						
28/09/14	90	3358	97	233	80	3125	17
29/09/14	91						
30/09/14	92	3703		152		3551	
01/10/14	93						
02/10/14	94	3703	166	152	112	3551	54
03/10/14	95						
06/10/14	98	3703	157	152	106	3551	51
07/10/14	99						
09/10/14	101	3703	162	152	95	3551	67
13/10/14	105	3703	159	152	103	3551	56
15/10/14	107						
16/10/14	108	3703	166	152	116	3551	50
17/10/14	109						
20/10/14	112	3703	198	152	137	3551	61
22/10/14	114						
23/10/14	115	3703	145	152	132	3551	13
27/10/14	119	2850		167			
28/10/14	120	2850	146	167	115	2683	31
31/10/14	123	2850	187	167	123	2683	64

APPENDIX D - Experimental data on nutrients.

Sampling date	Day	Nitrogen				Phosphorous (mg P/l)	
		TKN (mgN/l)		NH4+ (mgN/l)			
		IN	OUT	IN	OUT	IN	OUT
01/07/14	1	225	257				
04/07/14	4	244		150			
07/07/14	7	244	282	150	243		
08/07/14	8	235		215			
14/07/14	14	235	211	215	194	42	25
18/07/14	18						
21/07/14	21	235	219	215	172	42	15
22/07/14	22						
24/07/14	24	235	168	215	128	42	39
29/07/14	29	221	160	167	107	42	26
31/07/14	31	221	137				
01/08/14	32						
04/08/14	35	261	158	106	152	52	23
05/08/14	36						
06/08/14	37	261	159	106	124	52	29
07/08/14	38						
08/08/14	39	260		194		34	
13/08/14	44	260	142	194	137	34	19
16/08/14	47	260	150	194	149	34	20
18/08/14	49	260	162	194	154	34	17
20/08/14	51	260	149	194	131	34	20
25/08/14	56	260	171	194	153	34	28
28/08/14	59	260	160	194	155	34	14
01/09/14	63	260	158	194	155	34	16
04/09/14	66	260	148	194	143	34	16
08/09/14	70	260	127	194	118	34	22
10/09/14	72	220		177		31	
11/09/14	73	220	150	177	131	31	22
15/09/14	77	220	132	177	121	31	27
17/09/14	79						
18/09/14	80	220	114	177	108	31	21
22/09/14	84	220	108	177	105	31	28
25/09/14	87	220	113	177	104	31	18
26/09/14	88						
28/09/14	90	220	119	177	113	31	15
29/09/14	91						
30/09/14	92	188		172		36	
03/10/14	95						
06/10/14	98	188	84	172	68	36	21
07/10/14	99						
09/10/14	101						
13/10/14	105	188	122	172	105	36	17
14/10/14	106						
15/10/14	107						
16/10/14	108			172	133		
17/10/14	109						
20/10/14	112	188	106	172	96		
21/10/14	113						
27/10/14	119	272		149		52	
28/10/14	120	272	103	149	86		
29/10/14	121						
31/10/14	123			149	124		17