Doctoral School in Environmental Engineering

On the use of Constructed Wetlands in mountain regions: innovative tools and configurations

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"Que teu coração voe
cada dia mais alto
cada hora mais vivo
cada instante mais solto
e acabe virando
um pássaro."

(Wishes for me from my professor and friend Jorge Jacó)

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Acknowledgments are the only part of the thesis that you can write with your emotions and this should be easy. However, when I arrived to this part, things were not so easy. Writing the acknowledgements has been a deep trip into my past. I know it would be easier to be formal and just say thanks to a list of people who have stayed with me during these almost four years. But I am slightly more complex than that and there are things that I want to describe, maybe because this has been a period when most of the things that I knew in my life changed.

I am Brazilian. I lived in Brazil most of my life but, as many Brazilians do, I have decided to explore life far from my birth place. Italy is somehow similar to southern Brazil, so I was not afraid to come here even if such a decision was not obvious. My parents have never travelled to a foreign country. However, this did not stop me: when you have a dream, you just follow it. At the moment of the decision, I just did not know that all the certainties I had were about to be left behind when I took the plane that took me to the Big Boot. And then the train, which took me right to this small city surrounded by mountains, the highest I had ever seen in my life.

I have learned a lot of things in these years about the life as a foreigner, as a professional and, of course, as a PhD student. When you are not in your natural environment, your survival skills start to play an important role, and you start to adapt, develop and grow, grow as a new person. There are travels that, when you start them, you do not know how much it will hurt you, you do not know how much you are going to learn, you just know that, it does not matter how strong the life will hit on you, you are going to hit it back. Because you are Brazilian and Brazilians never give up! It is clear that I did not do it alone and writing these acknowledgments made me think about all the choices and people in my life that directly or indirectly put me in the conditions to arrive here. Now I will try to include them all in here.

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And now the travel continues. I do not know where I am going to go, but thank you all for being part of my life!

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Summary

The use of Constructed Wetlands (CWs) has been increasing over the last twenty years for decentralized wastewater treatment projects (e.g. rural communities, isolated houses, etc.) because of the low maintenance requirements and operational costs, efficiency in terms of organic matter, nitrogen and suspended solid removal. Nevertheless, the application of these systems in mountain areas is faced with some issues related to the specific characteristics of these areas, namely: the complex morphology with steep slopes and limited extensions of flat land, low temperatures and, in tourist contexts, population variations throughout the year. Limited availability of suitable land is a key issue for the application of a technology requiring considerable surfaces to produce effluents of good quality. Land area requirements constitute a well-known problem of CWs that is related to a lack of knowledge on the biological reactions occurring inside the bed. In fact, usually CWs are designed by considering simple first order decay models and specific surface area requirements, while the real requirements are not taken into account, leading most of the times to an overestimation of the area required. The limited knowledge on the processes and relative efficiencies of CW leads to overdesign of CW, mainly in low temperatures contexts and where there is a fluctuation on the resident population. Despite the efficiency that could be achieved through overestimation, those systems would be underutilized for a large part of the year. Ultimately, overestimated CWs consume more land than needed, eventually leading to the decision of switching to other systems.

This research aims to identify approaches and configurations that may improve the applicability of CWs for wastewater treatment of mountain communities. These approaches try to overcome the cross-cutting issue of land area requirement, as well as those related to the variation of temperature and population through the year. This was done by exploring the use of respirometric techniques for the estimation of kinetic and stoichiometric reactions inside the bed and by testing, in a pilot plant, the influence of the tourist presence and low temperatures on the efficiency of innovative CW configurations.

The research was developed at both the lab and the field scale. At the lab scale, two different tests were used in order to estimate the oxygen consumption in CW filter material: liquid respirometry and the off-gas technique. Liquid respirometry proved to be a reliable method when used to measure kinetic and stoichiometric parameters of the CW's biomass. The off-gas technique was applied at the lab scale showing promising results, though further research is needed to improve the applicability of the method to CWs. Along with that, at the lab scale, a modified AUR method was applied on the CW material to quantify the nitrification rate of real systems at different temperatures and therefore to predict the removal efficiency throughout the year.

At the field scale, several tests were performed in a pilot plant composed by two hybrid CWs (VSSF+HSSF). Among these: operation under continuous and discontinuous winter conditions,

operation with overload during the summer (to simulate the presence of tourists) and the application of innovative configurations (Recirculated and Aerated VSSF). All these tests were designed with the purpose of dealing with the trade-off between the reduction of a CW's land area requirement and the enhancement of its efficiency. Two innovative configurations were tested in the pilot plant: Recirculated VSSF CW and Aerated VSSF CW. Both configurations can provide saturated and unsaturated conditions, which allow the nitrification/denitrification inside the bed. During the period when experimental configurations were tested, the traditional VSSF CW was operated with an average specific surface area to 3.5 m^2/PE, the Recirculated VSSF of 1.5 m^2/PE and the Aerated VSSF of 1.9 m^2/PE on average. The results showed that the CW's surface can be considerably reduced without a significant reduction in the removal efficiency. The extra investment needed to equip VSSF CWs with aeration/recirculation would be compensated by a lower area requirement.

This study explored some of the problems associated with the application of traditional CWs under the physical and social conditions that characterize mountain contexts, providing important information for future research and application. First of all, a reliable tool, the respirometric technique, was explored for the estimation of kinetic and stoichiometric parameters that will allow a more precise estimation of the land area required for these systems. Moreover, two innovative configurations (the use of recirculation and aeration in CWs) were proposed to be used where traditional configurations, though well designed, are still too large to be applied. Such configurations can also be used as a temporary solution to increase the treatment capacity during tourist peak seasons, while a traditional configuration is kept over the rest of the year. While this research focused on mountain environments, the configurations and results contained therein could be applied to a wide variety of settings where shortage of land or difficult climate conditions would exclude CWs from the list of wastewater treatment options available.

Chapter 1

Scope and outline of the thesis

1.1 Introduction

Reducing the impact caused by human settlements on water bodies is undeniable for the preservation of the environment and the protection of human health (inter alia UNEP et al., 2004; Corcoran et al., 2010). Among various measures that can be adopted to achieve this goal, wastewater treatment plays a major role. Despite such importance, however, adequate wastewater treatment is still inadequate in many parts of the world due to the economic cost of facilities and the technological challenges associated with the context of application. In western countries, where wastewater treatment is generally efficient and widespread, mountain areas still present several challenges to the implementation of high quality wastewater treatment facilities. Reliable solutions for the wastewater treatment in these areas have been proposed, among others, by the Austrian Water and Waste Association in 2000 (OEWAV, 2000) and by the EcoSan Club in 2011 (Müllegger et al., 2011). The first one describes general approaches for wastewater treatment in mountain areas, while the second one deals with the specific case of refuges or mountain huts. Several technical solutions have been proposed for wastewater related problems, but hardly any other field of wastewater treatment is dominated by the specific boundary conditions that are present in mountainous regions, e.g. difficult access to certain sites, shortage of sites and challenging load variations caused by varying seasonal and weather conditions (OEWAV, 2000).

The wastewater produced in the mountain can be transferred to treatment plants located in the valley, depending on the distance between the sources and plants (OEWAV, 2000). However, domestic wastewater produced by mountain communities cannot always be collected to a centralized wastewater treatment plant (WWTP) as a consequence of the difficult geomorphologic conditions and the need for long collecting pipes. This situation calls for alternative solutions that ensure high quality standards, low energy consumption and adaptability to an environment characterized by steep slopes, limited space and eventually considerable natural value.

In some mountain communities, after collection, wastewater is simply treated by sieving and settling in a septic tank/Imhoff tank, even though this kind of treatment provides effluents that may be characterized by a significant presence of suspended solids, thus calling for an improved/secondary treatment that may preserve water resources. There is not a standard solution, and each context has its own best option according to ecological, social and economic criteria. Among technologies that are widely accepted as a post treatment for septic tanks, Constructed Wetlands (CWs) may represent a good option in this context.

CWs are commonly used in small and decentralized communities due to their low maintenance requirements, reduced operation costs when compared to conventional systems and their efficiency in the reduction of organic matter, nitrogen and suspended solids. CWs neither involve complicated and expensive technology, nor require specifically trained technicians for operation. CWs are also one of the most sustainable wastewater treatment technologies, requiring very little maintenance to achieve a good treatment quality. Another advantage is their reliability: when properly designed, they can cope with large fluctuations in wastewater influent, both in terms of hydraulic and organic loading (Paing and Voisin, 2005; Molle et al., 2005). On sufficiently sloping sites there can be no power requirements (Paing and Voisin, 2005), while construction, capital and operational costs are lower than those of other systems, such as activated sludge.

However, when applying CWs to small mountain communities, two issues can arise, that are related to the peculiar characteristics of these lands, namely: cold temperatures and flow variations. Mountain areas are exposed, at least during part of the year and particularly in some geographic regions, to significantly low temperatures. This may considerably reduce the efficiency of CWs, which must be designed to guarantee high effluent standards even in the winter season. Boosting the knowledge on the temperature influence on the biological activity in CWs is essential for ensuring their efficiency during the year. Further, mountain villages that are popular tourist destinations experience flow variations due to the fluctuation of population throughout the year. In many mountain regions around the world, including the Alps, the population of tourist villages increases dramatically during the high season. As it may be very expensive to design CWs based on the high organic load discharged during such season (i.e. very large area requirement), the capability of a CW to deal with higher loads for short periods of time, as well as its possibility to quickly recover after long idle periods (that usually occur during the winter) should be tested.

A cross-cutting issue to the above-mentioned ones is that of the large surface required by CWs to guarantee high effluent standards. While this problem is relevant to any context for the resulting conflict with other land uses (e.g. agriculture, urban, etc.), it becomes particularly binding in mountain areas, where the extension of flat land is limited.

The need to overdesign CWs is often related to a lack of knowledge on the biological reactions occurring inside the bed and partly due to the conservative approaches normally used by designers. The design is usually based on the use of simple first order decay models or specific surface area requirements (e.g. 4 m^2/PE). Even though several models have been developed to evaluate the organic matter, nitrogen and phosphorus removal in CWs (inter alia Rousseau et al., 2004; Langergraber et al., 2009), often the kinetics and stoichiometric parameters of bacterial biomass appearing in the models are theoretically assumed and not based on real measurements. Therefore, a better measurement of kinetic and stoichiometric parameters is needed to allow designers to better estimate the area that ensures high removal performances. Respirometric tests are an option to directly measure the kinetic parameters (e.g. maximum oxidation rate of biodegradable COD, heterotrophic yield coefficient, etc.) used in mathematical models and this may help to design CWs with a reduced land area requirement. Although respirometric tests are widely used in activated sludge processes to evaluate kinetic and stoichiometric parameters (Ubay Çokgör et al., 1998; Majone et al., 1999), the application of respirometric tests in CWs is still limited to a few experiences, and an in-depth study is needed to optimize this technique (Giraldo and Zarate, 2001; Andreottola et al., 2007, Morvannou et al., 2011).

In order to reduce the surface required by these systems, different approaches have been proposed. Some of them are based on the improvement of removal rates, by means of artificial aeration (Ouellet-Plamondon et al., 2006; Nivala et al., 2007), the use of alternative feeding periods, the modification of the filter material's thickness, or the recirculation of a fraction of the HSSF outlet wastewater to the VSSF inlet (Tunçsiper, 2009; Ayaz et al., 2012).

1.2 Objectives

This research aims to improve the applicability of CWs to treatment of mountain communities' wastewater by identifying approaches and configurations that can tackle some of the limitations of these systems, like the large land area requirement and poor performance under cold climates.

This was done through a comprehensive investigation of biological processes occurring in CWs, an analysis of how CWs are affected by the specific conditions characterizing mountain regions and a study of whether and how innovative configurations can reduce the area requirement of CWs.

The research has three specific objectives, which are listed below along with related research questions.

Objective 1: Providing reliable tools for the estimation of kinetic and stoichiometric parameters in CWs that might be used in the design phase.

Several models have been developed to estimate the removal of pollutants in CWs but the kinetic parameters of bacterial biomass appearing in the models are often assumed theoretically, rather than based on real measurements. In this research, respirometric techniques were applied to the CW filter material for the estimation of these parameters.

Research questions:

- Are respirometric tests a reliable tool for the measurement of stoichiometric and kinetic parameters associated to heterotrophic and autotrophic bacteria in CWs?
- Can liquid respirometry capture the changes in the biomass during the acclimatization period of CW lab cores?
- Is it possible to estimate kinetic and stoichiometric parameters in CW using the off-gas technique (normally used in activated sludge)?

Objective 2: Assessing the performance of Vertical Sub Surface Flow (VSSF) CWs under conditions commonly found in mountain communities.

Mountain communities may be exposed to significantly low temperatures and peculiar fluctuations in flow due to the presence of tourists. CWs that are applied to mountain communities should be able to deal with these conditions, without reducing the quality of the effluent. A better knowledge of how CWs work under these conditions may help to design systems that can maintain high quality standards without requiring unreasonable surfaces.

Research questions:

- Can a hybrid CW plant designed on the basis of just the resident population treat the additional pollutant load produced during the tourist period?
- How does the removal efficiency of a VSSF CW vary under cold climates and flow variations?
- Is an enhanced AUR (Ammonia Uptake Rate) test an effective tool for the estimation of the maximum specific nitrification rate (v_N) of the CW biomass under different temperatures?

Objective 3: Proposing alternative configurations that can reduce the area of a CW without reducing its efficiency.

The land area requirement is still one of the main constraints to the application of CWs. This becomes even more binding in contexts, like mountainous ones, where the extent of flat land is limited. In order to deal with this issue, two hybrid CWs (each one composed by a VSSF and a HSSF) with different filter material in the VSSF CW were compared.

Research questions:

- Does the filter material play a major role on the efficiency and cost of the system?
- Is the recirculation of the VSSF's effluent effective for the treatment of higher organic loads?
- Does aeration of VSSF-CWs increase the efficiency in the treatment of higher organic loads?

1.3 Outline of the thesis

The thesis was divided into 12 chapters. Figure 1shows the structure of the thesis. The literature review on CWs, including an overview on applications and common configurations, is found in Chapter 2, while Chapter 3 provides a detailed description of the respirometric tests and the pilot plant used in this research.

As a new approach in CWs, respirometric tests were used in this research to estimate kinetic and stoichiometric parameters with greater precision. Once included in mathematical models, these parameters should allow design to be performed with greater accuracy. Chapter 4 and Chapter 5 regard the application of liquid respirometric tests (saturated conditions) in VSSF-CW lab cores. The application of liquid respirometry was carried out during the acclimatization of the lab cores (Chapter 4). Respirometric tests were also performed with acclimatized CW lab cores (Chapter 5), where kinetic and stoichiometric parameters of heterotrophic biomass and wastewater biodegradability were evaluated, providing information that may support the optimisation of design procedures or the estimation of the maximum oxygen requirements in CWs.

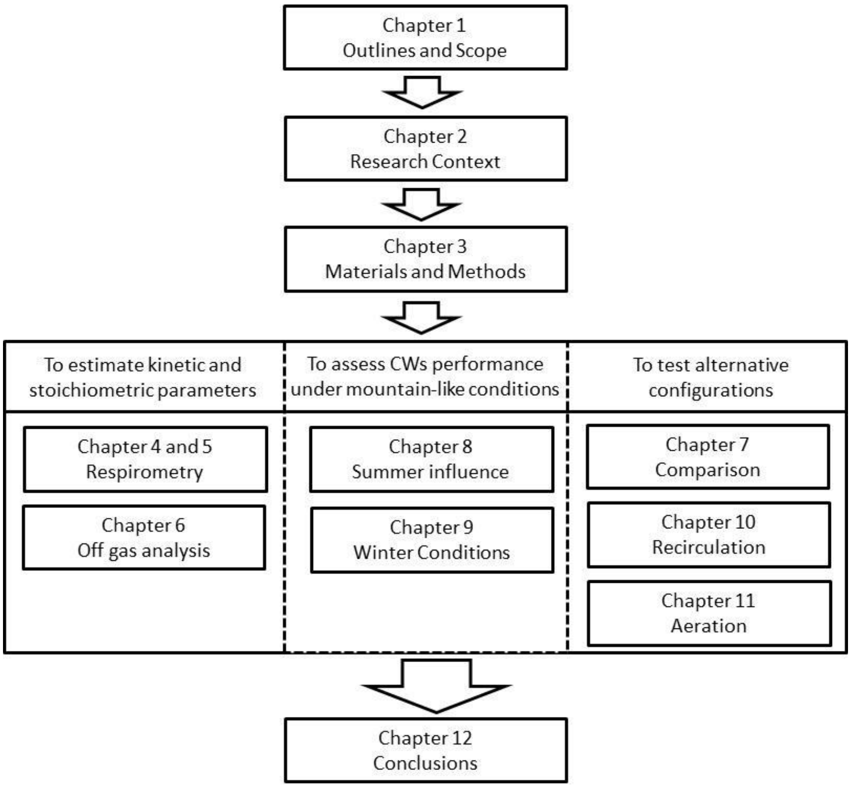


Figure 1 Outline of the thesis

Although the application of liquid respirometry for the measurement of oxygen consumption provided reliable results, the use of a saturated test on unsaturated material could sound controversial. Hence, other tests were conducted in order to estimate the oxygen consumption in the gas phase. Among these, the off-gas technique, which is widely used for evaluating the oxygen transfer efficiency in activated sludge, was applied in CWs (Chapter 6).

Moving from the design phase to the goal of testing the behaviour of CWs under specific physical conditions, part of this research relied on the use of a pilot plant. Experiments done in the pilot plant are described in Chapters 7 to 11. Chapter 7 is a technical and economic comparison between two kinds of VSSF-CWs used in the pilot plant.

The presence of a variable population, which is a common issue in tourist areas, and its influence on the performance of a CW were evaluated during summer and winter periods. During summer, a hybrid CW system designed for the resident population only, was operated for a period of 2 months with higher loads, simulating the presence of tourists (Chapter 8). In order to simulate winter conditions, the pilot plant was tested under regular operation and discontinuous operation during the winters of 2010 and 2011 (Chapter 9). Moreover, in this period, AUR tests were also performed to estimate the temperature influence on the nitrification capacity of CW cores.

Pursuing the objective of making CWs more suitable for mountain communities, and with the knowledge that the land area requirement is an important constraint on it, innovative CW configurations were proposed. These were specifically aimed at increasing the oxygen transfer and subsequently reducing the land area requirements of VSSF CWs. The first configuration tested was the Recirculated VSSF CW (Chapter 10). This configuration is based on the recirculation of the wastewater inside the VSSF CW: the wastewater fed in the system is maintained inside the system by closing an electronic valve, and it is recirculated from the bottom to the top of the system every hour during the cycle (6 hours/cycle). The second configuration tested was an Aerated VSSF CW (Chapter 11): the wastewater is also kept inside the VSSF, which receives an aeration pulse of 5 minutes every half an hour, during a 6 hours cycle. In both systems the valve is opened at the end of the cycle and wastewater discharged to the HSSF for 4h.

Chapter 12 discusses the main findings of the research as well as the pros and cons of tested configurations. Recommendations for future research are also presented.

Chapter 2

Research context

2.1 Domestic wastewater treatment in the European Union

Domestic wastewater is generated in residential settlements and services and originates predominantly from the human metabolism and household activities. The pollutant content in wastewater can be divided in three main groups: dissolved substances, colloids and suspended solids (Wiesmann et al, 2006). Dissolved substances can be divided in organic (e.g. Chemical Oxygen Demand and Biological Oxygen Demand - COD and BOD) and inorganic (e.g. nutrients, metals and heavy metals) substances. The colloids are suspension of small particles like droplets of oil or other insoluble liquids, like water-in-oil emulsions and solid in water colloids (turbid water). Colloids are not separated from suspended solids. Suspended solids are measured using a graduated Imhoff cone and the mineral and organic fractions are defined after filtration, by drying, weighing, incinerating at 500°C and re-weighing the ash. Standardized generation rates per-person (population equivalent) when the black water and grey water are not separated is showed in the Table 1.

Table 1 Standardized production rates per-person (population equivalent) from Wallace et al (2006).

Parameter	Production per person (raw average)
BOD_5	60 grams/day
Nitrogen	12 grams/day
Suspended solids	70 grams/day
Phosphorus	2 grams/day
Average flow	220 litres/day (United States)
	110 litres/day (others developed countries)
	60 – 80 litres/day (developing countries)

All these components could be naturally degraded if they were released in limited quantities into water bodies. However, in the case of urban settlements, water bodies do not have the capability to degrade the enormous amount of pollutants generated, thus leading to environmental problems, and threat to human safety. In this context, wastewater treatment is a fundamental tool to prevent environmental degradation, water pollution and health problems in the communities.

Each country has its own institutional framework that guarantees environmental protection. The European Commission's Department for the Environment has produced a Water Policy that includes various recommendations aimed at protecting water quantity and quality in the European Union. In the field of Water Pollution, there is the Urban Wastewater Directive (91/271/EEC), which ensures that the environment will be protected from adverse effects of the discharge of wastewater. Member states are required to establish systems of prior regulation or authorization for all discharges of urban and industrial wastewater into urban sewage collecting systems. Following the member states' regulation, agglomerations with more than 2000 PE must be provided with wastewater collecting systems. Agglomerations with a population equivalent of less than 2000 must be equipped with a collecting system and appropriate treatment must be provided. Any process or disposal system, which after discharge allows the receiving waters to meet the relevant quality objectives, is intended to be an appropriate treatment of urban waste water (industrial and domestic wastewater, and run-off rain water).Small communities (less than 2000 PE) often cannot afford full time services as well as operational and maintenance staff. Plants for such communities, though having as little mechanical equipment as possible and being constructed with local materials, should produce an effluent quality according to the standards. In communities that are near to urban areas the wastewater treatment is generally efficient and widespread, but this condition is not always present in mountain areas, where several challenges to the implementation of high quality wastewater treatment facilities are still present.

The use of Constructed Wetland (CW) has soared in decentralized wastewater treatment projects, single home projects and rural communities (Wallace et al., 2006). This is also related to the very advantages of these systems, namely low maintenance requirements and operational costs, lower costs compared to conventional systems and the considerable efficiency in terms of BOD, nitrogen suspended solid removal (Langegraber, 2008).

In Italy, the use of CW as a solution for the wastewater treatment of small communities was officially introduced with the Decree-Law 152 (1999, May 11th). This establishes that constructed wetlands and stabilization ponds are indicated for urban areas with populations between 50 and 2000 people equivalent (P.E.). In 2002, the Autonomous Province of Trento introduced a guideline for the design, construction, management and use of constructed wetlands (Delibera 992 della Giunta Provinciale). Among the main obstacles towards the construction of these systems in Italy is the lack of trust by administrators due to some unsatisfactory early applications. Besides that, the Italian name for constructed wetland ("fitodepurazione"), which means "purification by means of plants", does not give biomass and filtration their real importance, further decreasing the trust in these systems.

2.2 State of art in Constructed Wetlands

Wetlands are transition zones between water and land where vegetation has developed in response to saturated conditions, occurring for at least part of the year. They are an unique ecosystem with unique hydrology, soils, and vegetation. Wetlands can be divided into two major types: Natural and

Constructed Wetlands. Natural Wetlands are portions of a landscape that exist due to natural processes rather than a direct or indirect anthropogenic influence (Fonder and Headley, 2010) and Constructed Wetlands (CWs) are engineered systems that optimize and control the processes that occur in natural wetlands.

The common or traditional classification of CWs divides them directly in surface flow and subsurface flow CW (Cooper et al, 1996). More recently, Fonder and Headley (2010) introduced a wider classification system encompassing the Restored Wetlands (i.e. areas which were formerly natural wetlands that were lost or heavily degraded in the past and now support a near-natural wetland ecosystem), the Created Wetlands (i.e. non-wetland areas which have been converted to a wetland ecosystem by civil engineering works) and Treatment Wetlands (i.e. artificially created wetland systems designed to provide a specific water treatment function). The treatment wetlands were previously known as CWs.

Constructed Wetlands (in this thesis, CW are intended as treatment wetlands in the classification of Fonder and Headley, 2010) provide an enhancement / optimization of the physical, chemical and biological processes in order to remove pollutants from the water. According to Fonder and Headley (2010), three characteristics are common to all CWs: the presence of macrophytic vegetation, the existence of water-logged or saturated substrate conditions for at least part of the time and the inflow of contaminated waters with constituents that are to be removed. In the research context, sometimes treatment wetlands may be “unplanted” in order to exclude the effect of plants in the system. However, apart from the research context, treatment units without wetland vegetation, such as gravel or sand filters, should not be classified as a CW (Fonder and Headley, 2010).

According to hydraulic conditions of the system (water position), constructed wetlands can be divided in two main categories: surface flow and subsurface flow. The main difference between these systems is that subsurface flow systems are media-based systems. Surface Flow Constructed Wetlands (SFCW) are defined as aquatic systems in which the majority of flow occurs through a water column overlying a benthic substrate. In Subsurface Flow Constructed Wetlands (SSFCW) the water flows through the porous medium. Figure 2shows the classification tree proposed by Fonder and Headley (2010) for Constructed Wetlands. In this research, just the SSF CWs are going to be explored.

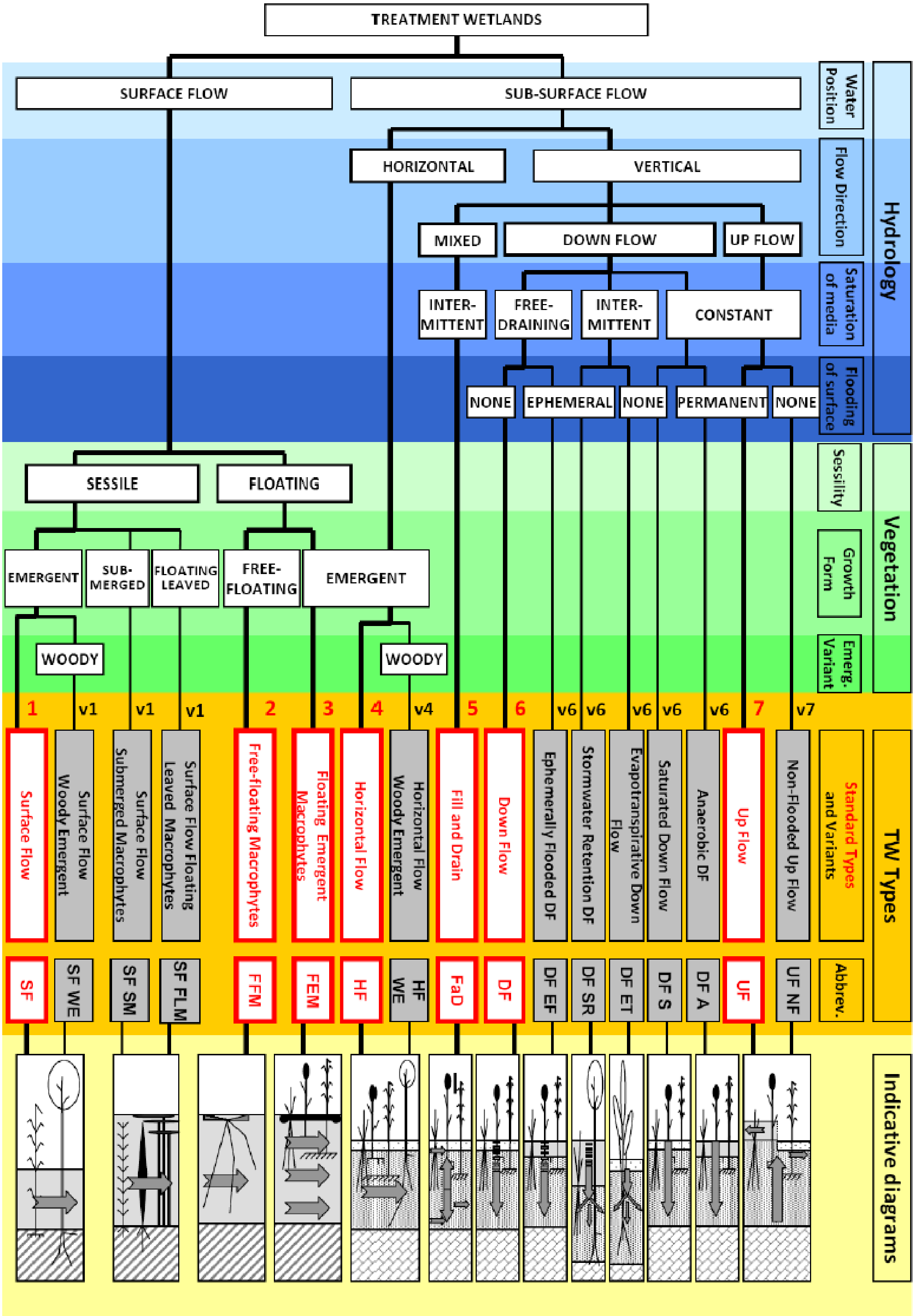


Figure 2 CW's classification tree proposed by Fonder and Headley (2010).

2.2.1 Subsurface flow CW

In the Subsurface Flow Constructed Wetland (SSFCW) the majority of the flow passes through the porous media, where most of the biogeochemical processes of wastewater treatment takes place. Subsurface flow systems are sub-classified based on flow direction into those with a horizontal flow and those with a vertical flow.

a. Horizontal Subsurface Flow Constructed Wetland (HSSF CW -Figure 3): In 1952, Kathe Seidel started the investigation of the potential capability of bulrush in treating wastewater in artificial environments. A new wetland treatment was developed in 1960 and it was called root-zone method. The use of a common reed was based in the theory that it would increase the hydraulic conductivity of the soil matrix. HSSF is a large gravel and sand-filled channel through which wastewater flows horizontally, the filter material filters out particles and microorganisms degrade organic matters (Healy et al; 2006). HSSFCW presents anoxic/anaerobic conditions (saturated filter bed),which provide good conditions for denitrification (Vymazal, 2007). The bed is usually planted with herbaceous emergent macrophytes. The macrophyte used in Europa is the Common Reed (*Phragmites australis*) and elsewhere, the genera *Schoenoplectus*, *Cyperus*, *Typha*, *Baumea* and *Juncus*.

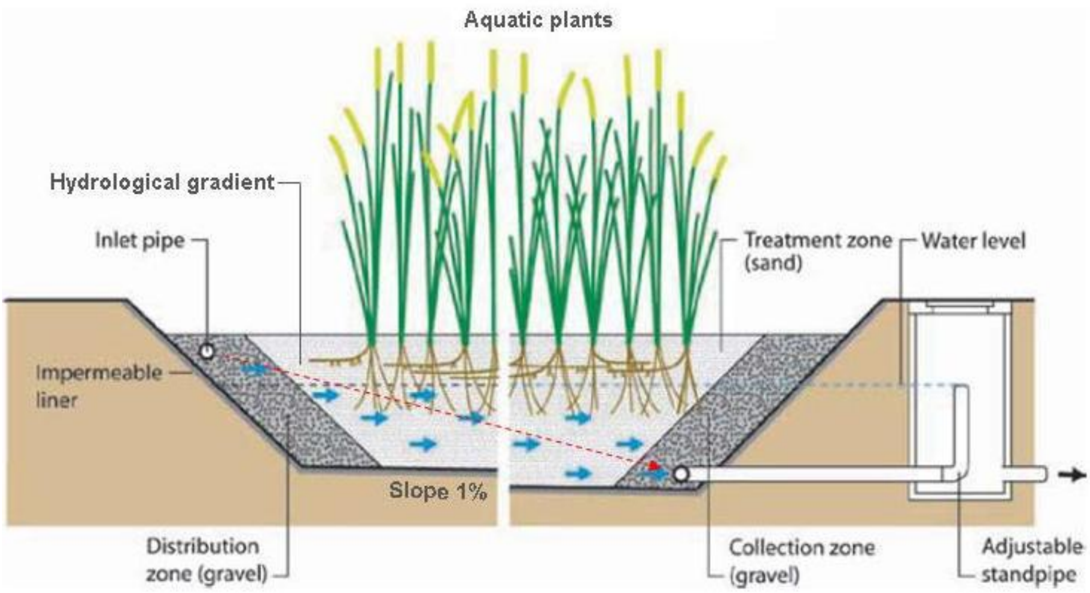


Figure 3 Schematic representation of HSSF CW (Morel and Diener, 2006).

b. Vertical Subsurface Flow Constructed Wetland (VSSF CW –Figure 4): it is a new type of constructed wetland that became widespread in the mid-1990s even though research on it had started also with Kathe Seidel in 1952. At that time, this system was called Max Planck Institute Process. In this process, a gravel media served as the rooting media for the plants that were considered to be the main mechanism in this process (this idea is now criticized). In VSSF the wastewater is dosed onto the wetland surface using a mechanical dosing system. The water flows vertically down through the filter matrix (Healy et al.; 2006). During the feeding, the air pressure increases inside the bed, because the air is trapped between the two layers of water (on the top and on the bottom), it increases the dissolution of oxygen in the water. After the feeding, the water drainage between the filter material creates under pressure conditions that sucks air inside the bed. Due to the higher availability of oxygen, VSSFCW has good rates in ammonia-N removal, but very limited denitrification takes places in the bed (Vymazal, 2007).

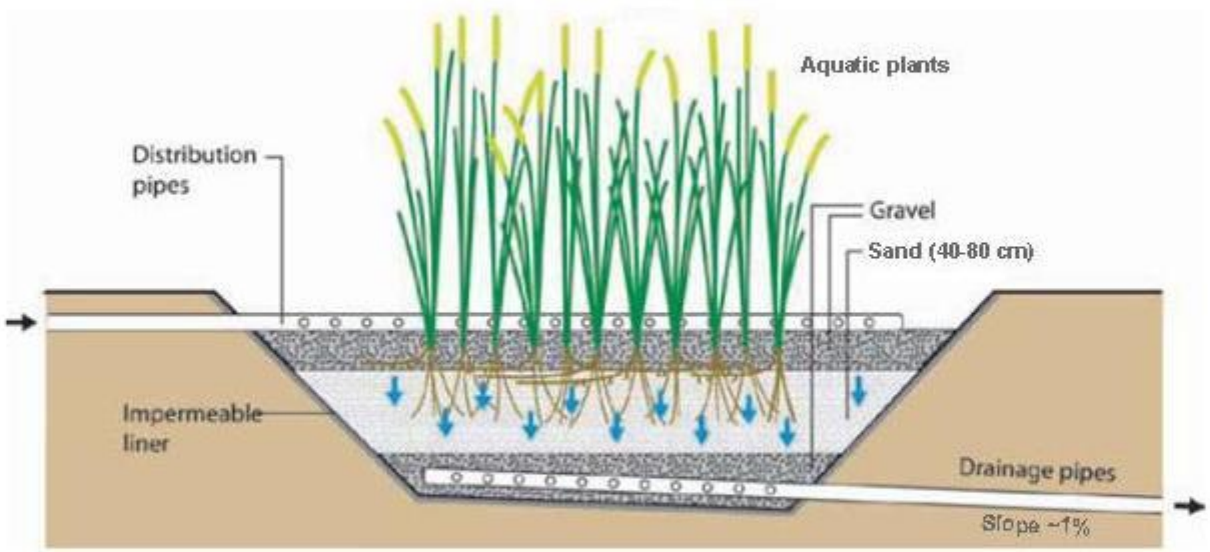


Figure 4 Schematic representation of VSSF CW (Morel and Diener, 2006)

Fonder and Headley (2010) further divided the VSSF in three standard types of CW: Down Flow (DF) CW (free-draining and without surface flooding), Up Flow (UP) CW (flooded surface) and Fill and Drain (FaD) CW (mixed flow direction, normally alternating between up and down flow), as following:

The Down Flow (DF) CW is the normal VSSF CW with free-drainage (open outlet) that remains unsaturated (also called in this thesis VSSF). Pipes distributed the flow on the top surface of the bed and the surface flooding is avoided. On the bottom, a network of perforated drainage pipes is located in a layer of coarse media (Cooper et al., 1996). Sometimes the drainage pipes on the bottom are connected to the atmosphere in order to promote passive aeration of the substrate. Influent distribution pipes may be located above the substrate, or, in cold climates, buried within the granular media bed or under a layer of insulating mulch.

The Up Flow (UF) CW (sometimes referred to as Anaerobic Bed) is a constantly saturated media permanently flooded over the surface. The wastewater is introduced at the bottom of the media bed via a series of distribution pipes and moves slowly upwards to the substrate surface. For practical reasons of conveying the effluent to the outlet, these systems have a flooded surface.

In the Fill and Drain (FaD) CW (also Tidal Flow and Fill and Draw wetlands) the flow alternates between upward and downward flow. The media in these systems has an intermittent saturation level (saturated and unsaturated conditions resulting of the filling and draining sequences). Normally the upper surface of the media is not flooded. Their application at full scale is increasing due to the relatively high rates of oxygen transfer and nitrogen removal: in one reactor, nitrification occurs under aerobic conditions while the bed is drained, and denitrification with anaerobic condition and carbon source provided by the second filling sequence (Austin, 2006).

Different kinds of CW can be combined in order to enhance the performance of the system. A combination of CW is normally called a Hybrid system, and it usually involves different sequences of VSSF and HSSF CWs, and the most commons are VSSF+HSSF and HSSF+VSSF. In a Hybrid plant composed by a VSSF followed by a HSSF, the first stage of the plant is responsible by providing suitable conditions (aerobic) for nitrification while the second stage provides a suitable condition (anoxic/anaerobic) for denitrification. Conversely, when applying a Hybrid system composed by a HSSF followed by a VSSF, the organic load removal will take place in the first stage and the ammonia removal in the second stage. In this second Hybrid system, the removal of the total nitrogen would be very low due to the nearly zero denitrification in the last stage. The ammonia nitrogen is oxidized to nitrate in the vertical-flow stage but without recycling is then discharged (Vymazal, 2007).

2.2.2 Design parameters in VSSF and HSSF CWs

Subsurface flow CWs are normally a part of a treatment process. Normally it comes after a primary treatment (e.g.: septic tank, settling tanks) that removes the settleable and floating solids prior to entering the CW bed. In some cases, a CW can be used also after secondary treatment with a polishing function (e.g. after stabilization ponds). The design of CWs could be divided roughly in two categories: sizing calculations (which require characterization of the incoming water, regional climate and goals of the CW) and physical specifications (basic data recorded over the years as depth, plants, media size, etc.) (Wallace et al., 2006). The 4 following approaches on the design of CWs are an evolution of the experience in CW construction:

a) Sizing methods/ Scaling factors.

Scaling factors are the simplest approach to CW design. They are the most used method in Europe, where national guidelines encourage the use of these factors that specify the superficial area of the system with appropriate filter material and wastewater concentrations. Indeed the use of scaling factor is highly dependent on experience in the use of the CW for the same wastewater flows and characteristics and under the same climatic conditions. Guidelines that support the design of constructed wetlands can be found in Table 2 and Table 3. Normally, factors like the contribution of a person equivalent and the superficial area requirement are taken into account. Different countries developed their own legislation that fixed a minimum value for design parameters, as shown in Table 2 and Table 3 for the HSSF and the VSSF (intended as down flow CW), respectively.

b) Loading specifications

Loading charts can be used as a design tool to size CW: an influent loading rate is selected to produce a target effluent concentration and the CW area is calculated from the influent mass load (Wallace et al., 2006). A prescription of a specified area-loading rate for biochemical oxygen demand (BOD) is a common method for design, but there are also charts available for TSS and TKN (for both HSSF and VSSF), and Organic N, Ammonia N, Total N, total P and faecal coliforms (for HSSF).

The use of charts that specify the Average Load Rates (ALR) applied per unit area in relation to the final effluent concentration as design criteria is possible, if CWs have similar wastewater characteristics and climatic conditions. For designing a VSSF or HSSF, graphs are expressed as ALR in g $m^{-2}d^{-1}$, and are drawn by multiplying the volume of wastewater applied (m^3/d) and the wastewater concentration (for example mg COD/L), and dividing this by the superficial area (m^2). The estimation of the outlet data can be just considered as a central tendency for the final concentration of the effluent and not as a real concentration at all times (Wallace et al., 2006). One of the limitations of the loading chart method is the need of a functional relationship between the inlet loading and the effluent concentration (which does not occur for example with TSS where only the first part of the bed is involved in its removal). Another limitation regards the scatter in loading chart data, because CW design, climatic and loading parameters are not the same for all the system that composed the dataset and a large component of the CW rely on the designer’s judgment (as depth, filter material and others).

c) First-order models

In performance-based design, the effects of degradation coefficients rate (k-rates), temperature (θ) and the combined effects of internal hydraulics and pollutant weathering are considered. US EPA (1983) was the first one to introduce simple first-order models of pollutant removal in the wetland literature. In 2004, Rousseau et al. (2004) confirmed that, according to the state of art, k-C* models seem to be the best available design tool for HSSF if all the assumptions are fulfilled. In their book, Wallace et al. (2006) used the first-order model as the primary tool in the design process. First-order kinetics (*k-C**) model use equation 1 for the estimation of the area of a HSSF and VSSF CW:

$$A = \left(-\frac{Q}{k_T} \right) \cdot \ln \left(\frac{C_e - C^*}{C_i - C^*} \right) \tag{1}$$

Where A is the area in m^2; Q is the inflow (m^3 yr^{-1}), C_i is the wastewater concentration at the inlet (mg/L), C_e is the wastewater concentration at the effluent (mg/L), C* is the background concentration and k_T is the removal rate for a specific temperature and area (m^3 m^{-2} yr^{-1}). Among these parameters, the estimation of k_T is rather important during the design phase.

The biological activity in the subsurface CW is extremely correlated with temperature: a decrease of temperature results in a lower bacterial growth and metabolic rates are reduced as well. A modified van't Hoff-Arrhenius equation is used to estimate temperature effects on the biological reaction rates:

$$k_T = k_{20}\theta^{T-20} \tag{2}$$

where k and k_{20} are the reaction rate (d^{-1}) at temperature T (˚C) and at 20˚C and θ is the temperature coefficient. In the case of HSSF, it is also possible to calculate the volume of the system (V represents the void volume of the system – the porosity volume)

$$V = -\frac{Q.\ln\left(\frac{C_e - C^*}{C_i - C^*}\right)}{k_T} \tag{3}$$

One of the most important issues in using a first-order kinetic model is that one must be sure that the kinetics parameters available in literature are representative of the system that is going to be designed and the context where the system will be located, weather conditions and filling material (porosity, diameter, etc.).

d) Advanced mathematical modelling

Computer models have been developed to support the desisgn of CW. Langergraber and Simunek (2005) developed the CW2D and the updated version CWM1 (Langergraber et al., 2009). Both models are implemented in the platform Hydrus-2D flow model (Simunek et al., 1999), that can simulate saturated and unsaturated flow and describe the biochemical transformation and degradation processes in subsurface-flow constructed wetlands. CW2D, the first version, describe the processes related to the organic matter, nitrogen and phosphorus degradation based on 12 components and 9 processes. The CWM1 is a general model to describe biochemical transformation and degradation processes for organic matter, nitrogen and sulphur in HSSF and VSSF CWs. It is based on the mathematical formulation introduced by the IWA Activated Sludge Models (ASMs). CWM1 describes aerobic, anoxic and anaerobic processes within 17 processes and 16 components (8 soluble and 8 particulate). Various calibration studies have been conducted and the model seems to have a good potential as a design tool. However, also in the case of this design approach, it is important to make sure that the kinetic and stoichiometric parameters used are reliable.

Table 2 Sizing parameters in the design of HSSF CW.

Reference		Surface Area Requirements	Depth	Filter material	Permeability (main layer)	Hydraulic load	Organic load
Germany	ATV, 1998	5 m^2/PE	0,5 m	U= d_{60}/d_{10}<5	$K_s = 10^{-3} - 10^{-4}$ m/s	4 cm/d	-
Germany	DWA, 2006			U= d60/d10<5 and 0.2mm ≤d_{10}≤ 0.4mm	$K_s = 10^{-3} - 10^{-4}$ m/s		
Austria	Onorm, 1998	6 m^2/PE	-	Distribution: gravel 16/32 (4/8); main layer 4/8 (2/4)	-	5 cm/d	112 kg/ha/d
Czech Republic	Vymazal, 1998	2nd treatment: 5 m^2/PE 3rd treatment: 1 m^2/PE	0,6 - 0,8 m	Washed gravel: 3-16 mm	$K_s = 10^{-3}$ - 3.10^{-3} m/s	-	< 80 kg/ha/d
United Kingdon	Cooper, 1996	2nd treatment: 5 m^2/PE 3rd treatment: 0,5 - 1 m^2/PE	0,6 m	Washed gravel: 3-6 mm, or 5-10 mm, or 6-12 mm	$K_s = 10^{-3}$ m/s	2nd treatment <5 cm/d 3rd treatment <20 cm/d	-
United Kingdon	GBG 42 (Griggs and Grant, 2001)	-	-	Washed gravel: 3-16 mm	-	-	-
France	CEMAGREF-EC, 2001	5 m^2/PE (BOD in 150-300) 10 m^2/PE (BOD in 300-600)	0,6 m	Washed gravel: 3-6 mm, or 5-10 mm, or 6-12 mm	$K_s = 10^{-3}$ -3.10^{-3} m/s	-	-
France	CEMAGREF-EC, 2004						
Denmark	Brix, 2004	5 m^2/PE	0,6 m	U= d_{60}/d_{10}<4 0,3<d_{10}<2mm 0,5<d_{60}<8mm	K=10^{-4} m/s -	-	-
Italy	PAT, 2002	6 m^2/PE	0,6 m	Main layer: 60 cm 4/8 o 2/4	< $K_s = 2x10^{-3}$ m/s	-	-

Table 3 Sizing parameters in the design of VSSF CWs.

Reference		Surface Area Requirements	Depth	Filter material	Permeability (main layer)	Hydraulic load	Organic load
Germany	ATV, 1998	2,5 m^2/PE	0,8 m	U= d_{60}/d_{10}<5 and $d_{10} \geq 0.2$mm	$K_s = 10^{-3} - 10^{-4}$ m/s	60 mm/d	20 -25 g BOD^5/m^2
Austria	Onorm, 2008	4 m^2/PE	0.7-0.8 m	From the top to the bottom: 5/10 cm washed gravel 4/8 or 8/16mm; 50 cm sand 0/4mm (0.2mm $\leq d_{10} \leq$ 0.4mm); 5-10 cm gravel 4/8mm; 20 cm gravel 8/16 or 16/32 mm	-		
Czech Republic	Vymazal, 1998	2^{nd} treatment: 1° stage: 0,8- 2m^2/PE 2° stage: 2 - 5 m^2/PE 3^{rd} treatment: 2 - 5m^2/PE	0,6 m	Sand and Gravel (0 - 12 mm) U= d^{60}/d^{10}<5	$K_s = 10^{-3} - 10^{-4}$ m/s	20 - 80 mm/d	-
United Kingdon	Cooper, 1996	1 -2 m^2/PE<100 PE: 1° stage: 3,5 x $PE^{0,35}$ +0,6 x PE 2° stage: 50% of 1° stage	1 m	From the top to the bottom: 8 cm sand; 15 cm gravel 6 mm; 10 cm gravel 12 mm; 15 cm gravel 3/6 mm	$K_s = 10^{-3} - 10^{-4}$ m/s	70 - 80 mm/d	23 -25 g BOD^5/m^2
United Kingdon	GBG 42 (Griggs and Grant, 2001)	-	-	0.2/0.5mm con $d_{<0.1\%}$ = 0.1mm	-	-	-
France	CEMAGREF-EC, 2001	1° stage: 1,2 - 1,5 m^2/PE 2° stage:0,8 m^2/PE	0,6 - 0,8 m	From the top to the bottom: 1° stage 40 cm gravel 2/8 mm; 30 cm gravel 10/20mm; 20 cm gravel 20/40mm; 2° stage: the first 40 cm are sand	-	30 mm/d	24 -25 g BOD^5/m^2
Denmark	Brix, 2004	2 m^2/PE	1,2 m	From the top to the bottom: 15 cm insulation; 90 cm sand; 15 cm gravel	-	100 mm/d	30 g BOD^5/m^2
Denmark	Brix and Arias, 2005	3 m^2/PE	1 m	From the top to the bottom: 0.2 m insulation; 1 m filtersand (0.25mm<d10< 1.2mm; 1mm<d60<4mm; U=d60/d10<3.5); 0.2 m drainage (Ø 8-16mm)	-	45-50 mm/d	20 g BOD^5/m^2 /d
Italy	PAT, 2002	4 m^2/PE	-	From the top to the bottom: 15-20 cm gravel 8/16mm; 60 cm sand 0/4mm; 20 cm gravel 8/16mm o 16/32mm	K_s = 2x10^{-3} - 10^{-4} m/s	30-40 mm/d	-

2.2.3 Advantages and drawbacks of CW application

Constructed wetlands offer several advantages for use in small and decentralised wastewater treatment plants. One of these advantages is their efficiency in the reduction of BOD, nitrogen and suspended solids. Constructed wetlands normally do not require complicated or expensive technology, nor specifically trained technicians. They are also one of the most sustainable technology, requiring very little maintenance or management to achieve a good treatment quality. Another advantage of constructed wetlands is their reliability: when properly designed, they can cope with large fluctuations in wastewater influent, both in hydraulic and organic loading (Paing and Voisin, 2005; Molle et al., 2005). On sufficiently sloping sites there can be no power requirements (Paing and Voisin, 2005) and maintenance may be limited to cleaning the influent pipe (Molle et al., 2005). Construction, capital and operational costs are lower than those of other systems, such as activated sludge. The lifetime of a constructed wetland used for wastewater treatment will be determined by wastewater loadings, the capacity of the wetland to remove and store contaminants, and the buildup of litter: there are systems operating for more than 20 years with little, if any, loss of effectiveness.

Besides the above mentioned advantages, CWs do have limitations as well. Some limitations are hardly connected to their biological activity. The removal of pollutants in CW undergoes various processes, like: filtration, sedimentation, adsorption and biological degradation. The biological activity is extremely correlated with temperature, as the decrease of temperature reduces the bacterial growth and its metabolic rates. Although, several studies have shown that seasonal temperature variation does not always affect COD and BOD removal (Kadlec and Reddy, 2001) the activity of nitrifying bacteria is considered strongly limited below 10˚C and denitrification activity is detected only above 5˚C (Brodrick et al., 1988; Herskowitz et al., 1987; Werker et al., 2002).

Another drawback is the clogging of the filter material. In CW, pre treatment of the influent, mostly made with a septic tank, is required to avoid clogging problems in the bed (Langegraber, 2008). However, clogging is a complex process and its mechanisms are not completely clear. The total accumulated matter within CW systems which occupies pore volume is considered to be one of the major factors of clogging, but both biofilm growth and suspended solid accumulation could occupy the pore space. It occurs in nearly half of the wetlands after running for 5 years. Appropriate operation that allow feeding and rest periods can be adopted for ensuring enough oxygen supply inside the bed for the mineralizationof the accumulated organic matters by microorganisms and the reoxygenation of the substrate (Zhao et al, 2009).

Along with other limitations, large land area requirements might be considered as the single major drawback of CWs. The design procedures used (Table 1 and 2) are usually based on specific surface area requirements usually overestimate the area in order to protect the surface water. However this specific area requirement sometimes limited the use of CW in mountain regions where it is not always available due to the geomorphologic conditions, natural slopes and also agricultural uses. The main problem is that the CW are still being seen as black box systems, which are designed by considering simple first order decay models. Several models have been developed to evaluate the organic matter, nitrogen and phosphorus removal in CWs (inter alia Rousseau et al., 2004, Langergraber et al., 2009), but the kinetics parameters (e.g. growth rate and decay rate of heterotrophic biomass or nitrifiers) required in the models are often assumed theoretically and not based on real measurements.

The monitoring of microbial biomass, substrate consumption and/or product formation provides the information needed to estimate certain kinetics parameters such as the cell growth, production and uptake rates, and as well corresponding yields. The respirometric approach seems to be promising to measure directly some kinetic parameters used in the mathematical models, such as the maximum oxidation rate of readily biodegradable COD, the maximum nitrification rate or the endogenous respiration (Ortigara et al., 2010). On other hand, innovative CWs have been proposed to deal with the compromise of reducing the area of CWs systems without decreasing their efficiency. This would allow a wider application of CWs, even in sensitive areas. Innovative configurations are normally based on alternate feeding periods which may increase the loads applied, on the conditions of saturation in CW systems being varied, on the recirculation of the treated wastewater and eventually the forced aeration. Among the most promising techniques utilized in order to improve the nitrogen removal and reduce area requirement simultaneously are the recirculation and the forced aeration.

2.3 Innovative configurations for reducing the area of CWs

The main problem of CWs is associated with their considerable land area requirement. In fact, guidelines suggest that surfaces up to 4 m^2/PE have to be considered when designing a CW. This is mostly due to the fact that, given the current knowledge on CWs, only large surfaces are supposed to guarantee the desired removal rates. Unfortunately, such land area requirements may constitute a significant problem where the cost of land is particularly high or the availability of land is limited. The latter is typically the case of mountain regions, where the geomorphologic context may not offer suitable conditions for a CW to be hosted in the vicinity of a community. Hence, new configurations are needed, that can reduce the size of a CW, while maintaining or even enhancing its nitrification and nitrogen removal performances. Among the most promising techniques, utilized in order to improve the nitrogen removal and reduce area requirement simultaneously, are the recirculation and forced aeration.

2.3.1 Recirculation of wastewater

Recirculation in CWs in usually intended as taking part of the CW outlet effluent and transfering it to the intlet CW, or also to the septic tank. The effluent recirculation may improve the treatment efficiency of the CW in terms of BOD_5 and TSS, due to the enhancement of microbial activity, through intense interactions between pollutants and microorganisms. Adapting full-scale CW facilities to the recirculation modification may increase operation costs, because of additional energy consumption for pumping (Stefanakis et al., 2009).

A possible configuration of recirculation in hybrid systems composed by HSSF + VSSF is the recirculation of the nitrified VSSF effluent in the HSSF where denitrification occurs (Tunçsiper, 2009; Ayaz et al., 2012). This kind of recirculation has the advantage of performing the organic matter removal in the HSSF and achieving nitrification in the VSSF, due to the low level of organic matter in the inlet wastewater. The denitrification of the VSSF effluent will happen because of the recycle in the HSSF.

Stefanakis et al. (2009) tested the recirculation of 50% of a HSSF effluent to a second stage HSSF and found out that effluent recirculation at a rate of 50% seems to have negatively affected the performance of the HSSF: pollutant removal rates were reduced after recirculation application, especially for nitrogen and phosphorus.

In VSSF CWs, recirculation can be applied to VSSF treating pre-settled wastewater. In particular, a fraction of the nitrified VSSF effluent wastewater is recirculated in the primary settler (or septic tank) where nitrate is denitrified due to anoxic conditions and the availability of biodegradable organic matter (Brix and Arias, 2005; ÖNORM B 2505, 2008). Another VSSF recirculated configuration was developed to treat domestic wastewater produced from single houses, in order to reutilize in irrigation. Groos et al. (2007) tested a combination of a VSSF CW (with recirculation) and a trickling filter: the effluent of VSSF CW was collected in a storage tank and then recirculated at the top of the VSSF to extend the contact time in the CW (Sklarz et al., 2009). Efficient removal was observed for TSS and BOD. Nitrogen was converted to nitrate and could partially fulfill plant nutrient requirements, reducing the need for fertilizer and hence providing environmental and economic benefits.

2.3.2 Artificial aeration in CW

Biological degradation of organic matter and transformation of ammonium–nitrogen are processes limited by the oxygen availabity (Vymazal J., 2007). The insufficient oxygen supplying through the CW surface or plants transfer results in poor nitrogen removal in various types of CWS. These conditions are mainly observed in HSSF systems, where saturated conditions limit the oxygen diffusion from air to water. Moreover, oxygen availability in HSSF is even more limiting in winter when the plants are dormant.Hybrid systems have been used in order to increase the oxygen concentration in CWs. In the hybrid system, the presence of a VSSF CWs before the HSSF CWs, improve the aerobic concentrations in the HSSF, enhancing the nitrogen removal in the final effluent (Norvee et al., 2007).

Artificial aeration is an alternative and has been proposed as a solution in the enhancement of removal performances in HSSF CW. Compressed air can be insufflate in the bed by pipes located at the bottom of the bed(Nivala et al., 2007) or in the initial section of the bed (Ouellet-Plamondon et al., 2006). The application in the initial section is related to the organic load, that is usually high in the first meters of a HSSF. Aeration can be applied continuously or discontinuously, depending on the dissolved oxygen concentration in the bed (Zhang et al., 2010) and also on the purpose of the treatment. The possibility of designing a CW with aerobic and anaerobic regions in the wetland bed would improve nutrients removal from the system.

The additional energy demand associated with artificial aeration is compensated by an improvement in the removal of pollutants. In particular, artificial aeration may improve nitrogen and organic matter removal, especially in winter, when it stimulates heterotrophic bacterial activity without reducing denitrification (Ouellet-Plamondon et al., 2006). TSS removal may also be improved, because aeration maintains empty spaces in the lower part of the gravel bed (Ouellet-Plamondon et al., 2006). Zhang et al. (2010) stated that a limited artificial aeration in CWs may be a cost-effective method for treating domestic wastewater, because it requires smaller land areas as compared to a traditional HSSF CW.

2.4 Respirometric Technique

The dynamic of the Oxygen Uptake Rate (OUR) is widely used in activated sludge processes to evaluate kinetic and stoichiometric parameters and to characterise wastewater biodegradability (Ubay Çokgör et al., 1998; Majone et al., 1999). The relationship between the specific growth rate or the specific substrate removal rate and the substrate concentration has been considered to determine the maximum specific growth rate for heterotrophs (μh). To this purpose, a linearized form of the Monod equation was used, and overall VSS and COD measurements enabled biomass and substrate to be defined (Williamson and McCarty, 1975, Ubay Cokgor et al, 1998). However, due to difficulties in the interpretation of COD and VSS measurements, experimental procedures based on respirometric measurements for the assessment of kinetic constants were developed. Ekama et al.(1986), in one of the pioneering studies in this field, proposed to make aerobic or anoxic reactors run at suitably low initial food/microorganism ratios for the assessment of readily biodegradable substrate (Ubay Cokgor et al, 1998). More recently, Andreottola et al. (2007) suggested the application of the respirometric techniques to measure kinetics of organic matter oxidation and nitrification using columns that simulate cores of VFCW.

Respirometric tests are based on the oxygen consumption in the substrate degradation in biological systems. In a respirometric test, during biological degradation, the oxygen concentration is measured through time in order to obtain the Oxygen Uptake Rate (OUR). The OUR dynamics allow the calculation of kinetic parameters, such as: the maximum oxidation rate of readily biodegradable COD, the maximum nitrification rate and the endogenous respiration.

The first applications of equipments, that perform oxygen measurements in environmental matrices (respirometers), started with Jenkins and Montgomery in the 60s, but the first application in the wastewater treatment plants was in the 80s, mainly for the characterization of wastewater biodegradability. According to Andreottola et al (2002), respirometers can be classified in manometric (the oxygen consumption creates a pressure difference inside the reactor), electrolytic (after the oxygen consumption, electrolytic compounds add new oxygen in the reactor to compensate the pressure created) and electrochemical (the oxygen concentration is measured by a probe whose sensors are submerged in an electrolytic solution).

A normal respirometer has two phases: liquid (L) and gaseous (G) where the oxygen can be measured (as dissolved oxygen in water or saturation in the air). These phases can have exchanges with the atmosphere (open respirometers – flow of gaseous and/or liquid phase from inside to outside of the reactor) or not (in the case of closed respirometer –static gaseous and/or liquid phase). Spanjers et al. (1998) and Andreotolla et al (2002) classified the respirometers according to the phase where the oxygen is measured (in the liquid or in the gaseous phase) and based on the gaseous and liquid flows inside the reactor. This resulted in a 3 letter classification, where the first letter identifies where the oxygen is measured (L or G), the second letter refers to flow in the gaseous phase (S – static or F-with Flow) and the third letter refers to flow in the liquid phase (S or F). For example, an

LFS reactor is a respirometer in which the measurement is made in the liquid phase (L), the gaseous phase is open to the atmosphere or the reactor is aerated (F) and the liquid phase is static in the reactor (S) (Figure 5).

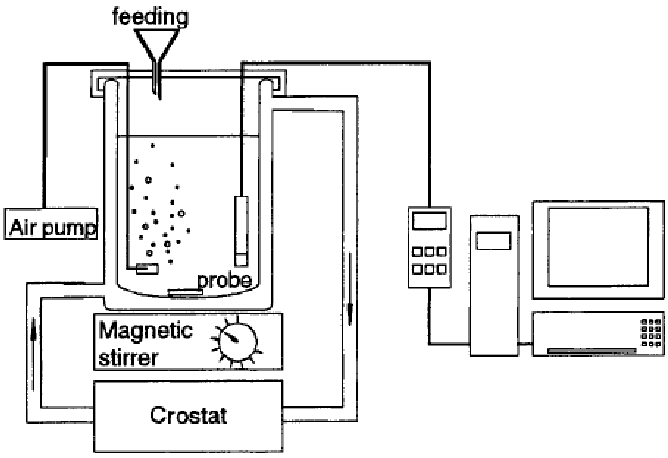


Figure 5 LFS respirometer (Ziglio et al., 2001)

Figure 5 shows the respirometer that is most commonly used to obtain kinetic parameters in activated sludge. It consists of a batch reactor, with temperature control, constant mixing and aeration. The data collected by the probes (oxygen values trough time) is acquired by a computer system. Oxygen can be introduced in the reactor in a continuous or intermittent way. The aeration is an important mechanism in the OUR test: dissolved oxygen should be maintainedabove 2mg/L, asbelow this value, the oxygen is a limiting factor in the system.

Figure 6 shows the OD measurements during a respirometric test with continuous aeration.A respirometric test starts with the aeration of the activated sludge, to allow the aerobic degradation of the organic material, which is still present in the sludge. When all the biodegradable materials have been consumed by the biomass, the OUR values represent the endogenous respiration.In Figure 6, endogenous respiration is the line before the addition of substrate, which is characterized by the OD saturation level. During the endogenous phase, biodegradable substrate can be added to the activated sludge and the OUR values obtained from that moment (substracting the endogenous OUR) represent the exogenous respiration of the biomass (maximum substrate consumption rate). During this phase the biomass activity continues at this maximum level until all external substrate is taken up for storage and growth. After the substrate depletion, OUR curves change the slope and the OUR values drop from the maximum level to a level around the endogenous values.

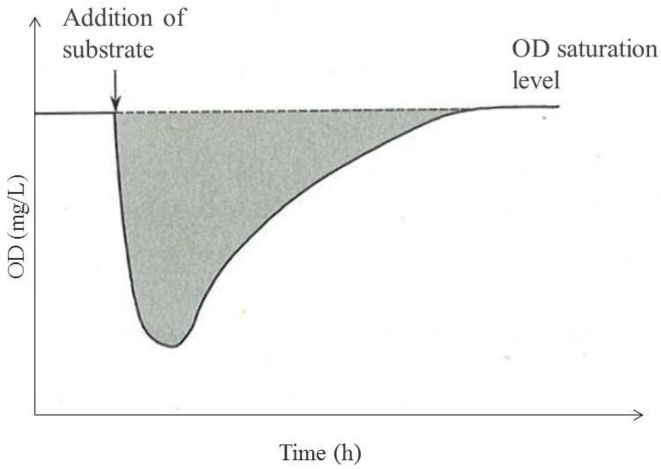


Figure 6 OD behavior after the addition of substrate during a respirometric test with continuous aeration (Andreottola et al., 2002a)

In the test with intermittent aeration two ranges of oxygen concentration are fixed, the maximum value (aeration stops) and the minimum value (aeration starts again). The activated sludge is aerated until the maximum values are reached and then the aeration stops. The dissolved oxygen concentration starts to decrease due to the biomass consumption until it reaches the minimum value, and then the aeration starts again (Figure 7). The OUR (Oxygen Uptake Rate) value is calculated as the average slope of the OD line (during the decreasing phase) associated with the average time of descending tract.

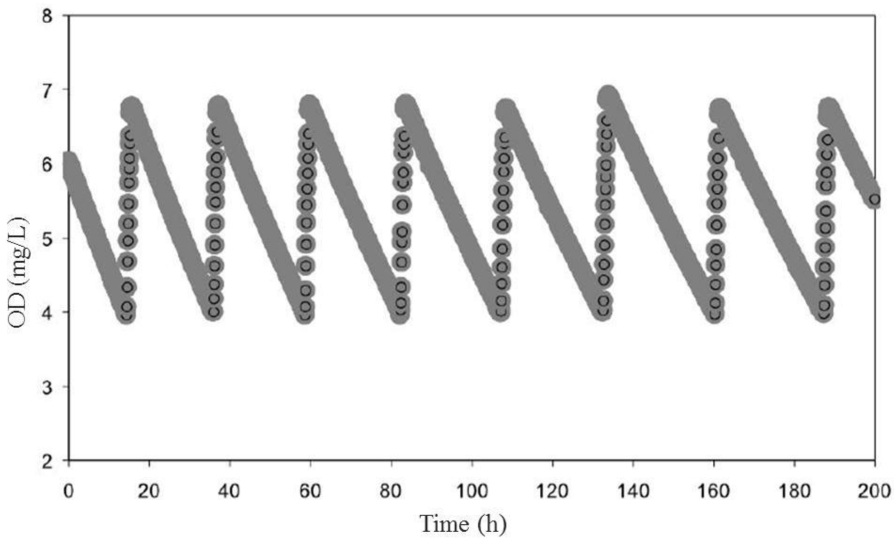


Figure 7 OD behavior during a respirometric test with continuous aeration. OUR is calculated for each decreasing line (Foladori et al., 2004)

An OUR value is associated to each decreasing line of the oxygen measurement. The respirogram (
Figure 8) is a sequence of OUR values, which represents the trend of the values of OUR through time.

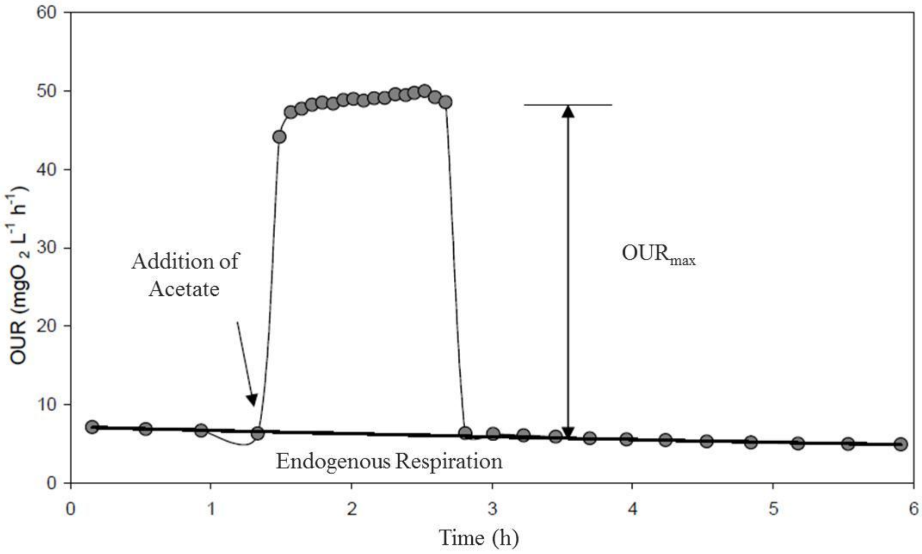


Figure 8 Typical respirogram for acetate consumption in activated sludge (Foladori et al., 2004)

During the substrate consumption under dynamic conditions, the formation of intracellular polymers (storage process) is the main mechanism for the removal of the readily biodegradable carbon sources. Dynamic conditions are characterized mainly by feast (presence of external substrate) and famine (absence of external substrate) conditions, where the microorganisms are exposed to the substrate for a relatively short period of time. During feast conditions, microorganisms store the substrate as intracellular products period and then consuming the stored substrate under famine conditions (*inter alia* von Loosdrecht et al, 1997; Ni and Yu, 2007). Figure 9 is a representation of the difference in the respirogram between substrate consumption with and without storage (Majone et al., 1999).

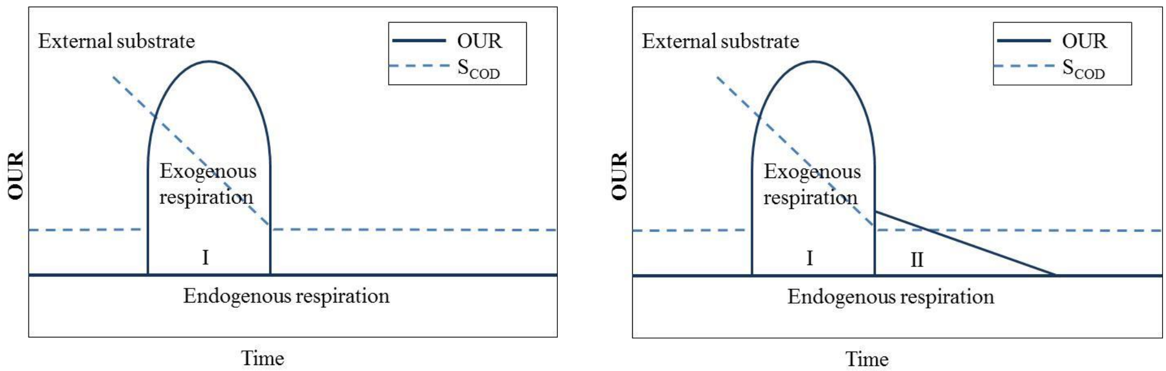


Figure 9 Substrate consumption: on the left hand the consumption is without storage (I) and with the storage effect (II) (adapted from Majone et al., 1999).

According to Sin et al. (2005), the accumulation rate of storage products is linearly correlated to the difference between the maximum substrate uptake rate and the substrate uptake rate required for growth: when the maximum substrate uptake rate of the biomass is higher than the amount needed for the growth rate, the difference between them is converted into storage products. The kind of storage polymers that is formed depends on the substrate type. For example, acetic acid is a simple substrate that is stored as polyhydroxyalkanoates (PHA) (Karahan et al., 2008 and Ni and Yu, 2007) and soluble starc, which is a hydrolysable organic compound, can be stored simultaneously as poly-glucose (glycogen like substances) and/or utilized for direct growth (Karahan et al., 2005, Karahan et al., 2006).

The description of this process has important consequences on activated sludge modelling. The Activated Sludge Model No. 1 (ASM1) and Activated Sludge Model No. 3 (ASM3) incorporate hydrolysis of complex substrates as rate limiting processes using surface reaction kinetics. These models describe the utilization of hydrolysis products with different mechanisms: in ASM1 the compounds resulting from hydrolysis are utilized only for heterotrophic growth (Henze et al., 1987 apud Karahan et al., 2005), while the ASM3 considers that the readily biodegrable substrate is firstly stored and then the stored material is utilized for growth in the famine phase (Karahan et al., 2005, Ni and Yu, 2008). However, experiments indicate that the storage processes and the level of internal hydrolysis depend much on system operation parameters and on feast and famine conditions. Modelling efforts attempt to account for simultaneous substrate storage and growth during the feast phase (Karahan-Gül et al., 2003; Ni and Yu, 2008; Ni and Yu, 2007; Karahan et al., 2008) in order to obtain a better interpretation of the experimental results.

The basic stoichiometric for the storage process is expressed in the equation 4.

$$\Delta O_{STO} = (1 - Y_{STO})\Delta S_S \tag{4}$$

After the consumption of the readily biodegradable COD (S_{S1}), equation 4 can be manipulated in order to obtain the storage yield (Y_{STO}), as shown in equation 5:

$$Y_{STO} = \left(1 - \frac{\Delta O_{STO}}{S_{S1}}\right) \tag{5}$$

Karahan-Gül et al. (2002) calculated Y_{STO} graphically using the OUR curve as show in Figure 10.

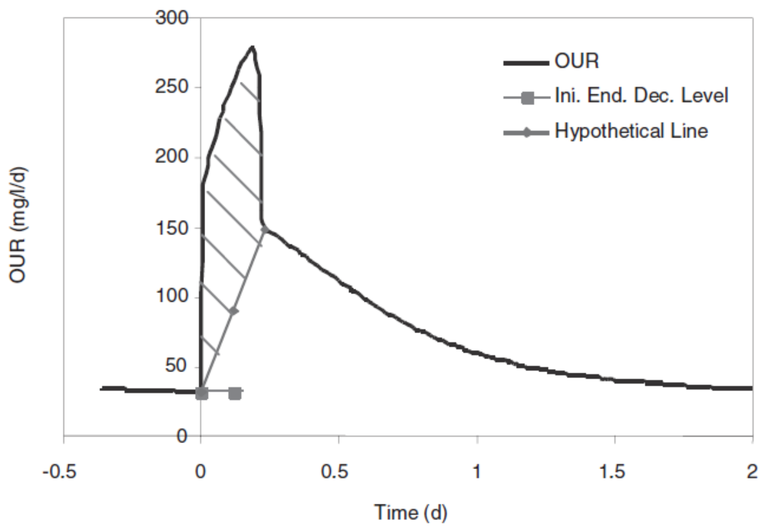


Figure 10 Graphical procedure for the determination of ΔO_{STO} (Karahan-Gul et al., 2002a).

The area under the whole OUR curve and above the endogenous OUR level represents the amount of oxygen utilized for the consumption of the specific amount of substrate fed to the system (ΔO_2). The Y_{STO} (storage yield) is determined using the area under the OUR curve and above the straight line drawn from the endogenous decay level to the inflection point on the OUR curve, which is the end of the feast phase according to ASM3 (Karahan-Gül et al., 2002a).

Karahan et al. (2005 and 2006) studied the utilization of starch by activated sludge for simultaneous microbial growth on external substrates and formation of storage products. The ASMGS (Activated Sludge Model for Growth and Storage) was proposed for the utilization of starch and reflects an appropriate combination and adaptation of ASM1 and ASM3, with simultaneous substrate storage and growth concept, together with the addition of adsorption (Karahan et al., 2005 and Karahan et al., 2006).

2.4.1 Respirometric experimental techniques in CWs

Current experimental techniques for the measurement of oxygen consumption in CW are the following: solid respirometry *in-situ* (Giraldo and Zarate, 2001), solid respirometry *off-site* (unsaturated conditions), applied on samples taken from the CW system (Giraldo and Zarate, 2001; Morvannou et al., 2010) and liquid respirometry *off-site* (saturated conditions), applied on samples taken from the CW system (Andreottola et al., 2007, Ortigara et al., 2010).

a) Solid respirometry in-situ:

In situ measurements of gas content (CO_2, CH_4 and H_2S) during an operation cycle are performed directly on CW bed assuming three different depths: 20 cm, 40 cm and 60 cm. The measurements are taken after feeding, when the oxygen content reached 21%. The observation of CO_2 profile showed that two reactions with different kinetic rates occurred: a fast one (sorption of particulate and dissolved organic matter straight after the feeding) and a slow one (biological oxidation that continues several hours after the discharge). The biological oxidation is divided in two steps: hydrolysis (solubilization of particulate organic matter) and the biological oxidation of dissolved organic matter. Regarding the CH_4 and H_2S, their concentration increases after the feeding and starts to decrease some time after it. The presence of methane in the bed indicates that anaerobic reaction is taking place, despite the oxygen concentration inside the bed (about 12%) (Giraldo and Zarate, 2001). This test was done in a vertical subsurface flow constructed wetland (VSSFCW), but could also be performed in a horizontal subsurface flow constructed wetland (HSSF CW) because the measurements are done directly on the bed and in the air phase.

b) Solid respirometry off-site (unsaturated conditions):

Two main studies describe this category of respirometric tests:

(1) Giraldo and Zarate (2001): unaltered sample of the sand was taken from the wetland bed straight after the feeding and put into a Erlenmeyer. A respirometric test was carried out to measure the metabolic activity, inside the sand once the sorption of BOD has occurred, in terms of oxygen consumption, CO_2 production, methane concentration and organic matter degradation. The oxygen concentration decayed with time and when the oxygen has reached a constant value, the Erlenmeyer was opened for reaeration. Afterthe reaeration of the Erlenmeyer, the slope increase drastically indicating a dependence of organic matter degradation kinetics and the concentration of oxygen in the atmosphere. The authors do not estimate the kinetic rates based on their tests.

(2) Morvannou et al. (2010): unsaturated samples of the porous media taken from VSSF-CW were used to estimate biological process parameters of models like HYDRUS/CW2D (Langergraber et al.,2009). The biological method was adapted from thatapplied to household waste characterization and is aimed to measure the Dynamic Respiration Index (DRI). DRI is the difference in oxygen concentration between the entrance and the exit of the reactor in % of O_2 and it allows to estimate the nitrification rate as well. The oxygen demand is measured versus time in the gas passing through a reactor, containing a mixture of an organic matrix mixed and a bulking agent (wood). Different sample/bulking agent ratios were considered to increase the porosity (for aeration efficiency) and maintain the humidity (for bacterial growth). As opposed to the former study (Giraldo and Zarate, 2001),

this research adopted a continuous air supply not to limit oxygen uptake rates, and the kinetic rates of nitrification were calculated.

c) Liquid respirometry off-site (saturated conditions):

This test could be applied on samples taken from the CW system and CWs cores. The filter material is extracted from the filter bed and put into the core. The core is filled with water from the bottom to avoid air entrapment. The liquid is recirculated by a peristaltic pump and passed in front of two oxygen probes (one at the top and another at the bottom). The respirogram is obtained bycomputing the difference between these two probes. The Oxygen Uptake Rate (OUR) was measured in the liquid phase in terms of Dissolved Oxygen (DO) in order to obtain the OUR dynamic, which is known as respirogram (Andreottola et al., 2007, Ortigara et al., 2010). From the respirogram, exogenous and endogenous respiration can be distinguished and kinetics of organic matter oxidation (due to the heterotrophic biomass) and nitrification can be calculated. This test was done with VSSF CW samples.

Chapter 3

Materials and Methods

3.1Liquid respirometry off-site (saturated conditions)

A new concept of respirometer suitable for the application with CW cores developed by Andreottola et al. (2007) was used in this research (Figure 11).The respirometer consists of a saturated CW core being connected to a pump which forces a continuous recirculation of the water flow from the bottom to the top of the core. Aeration is supplied continuously on the top of the core. During the recirculation, wastewater passes in front of two dissolved oxygen (DO) probes located on the top and on the bottom of the core (Hach Lange 5740 sc).

The probe on the top is immersed and the fluid is aerated by a compressor, the sensor measures the concentration of oxygen in the liquid above the material. The probe located on the bottom of the core is placed within a cell that does not allow the entry of outside air to the circuit and it measures the concentration of oxygen of the liquid that has already passed through the core. Figure 12 shows the behaviour of both probes after the addition of an organic readily biodegradable substrate (acetate). The difference in concentration between the two probes (probe located on the top minus probe located on the bottom) is assumed to represent the oxygen consumption by the system.

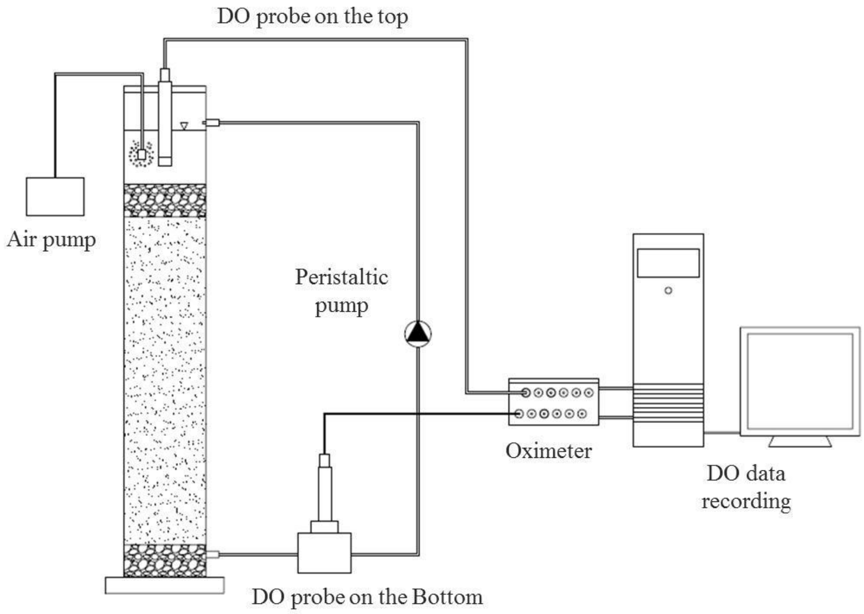


Figure 11 Scheme of the respirometers used to test CW cores (adapted from Andreottola et al., 2007).

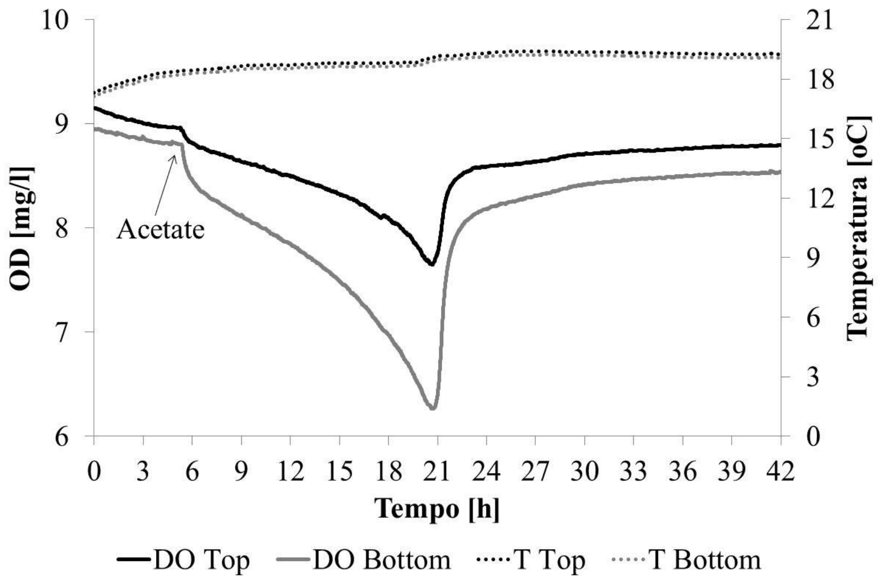


Figure 12 Behaviour of OD and temperature after the addition of acetate.

Oxygen Uptake Rate (OUR) expressed as $mgO_2L^{-1}h^{-1}$ (Figure 13), was calculated as the difference between the DO concentration on the top and the bottom of the core and taking into account the hydraulic retention time (equation 6).

$$OUR\left(mgO_2L^{-1}h^{-1}\right) = \frac{DO_{top} - DO_{bottom}}{HRT} \tag{6}$$

where:
DO_{top}= dissolved oxygen measured on the top of the column, expressed as $mgO_2\ L^{-1}$;
DO_{bottom}= dissolved oxygen measured on the bottom of the column, expressed as $mgO_2\ L^{-1}$;
HRT= hydraulic retention time (contact time in the core, from the top to the bottom of the core, calculated as the ratio V_L/Q and expressed as hours).

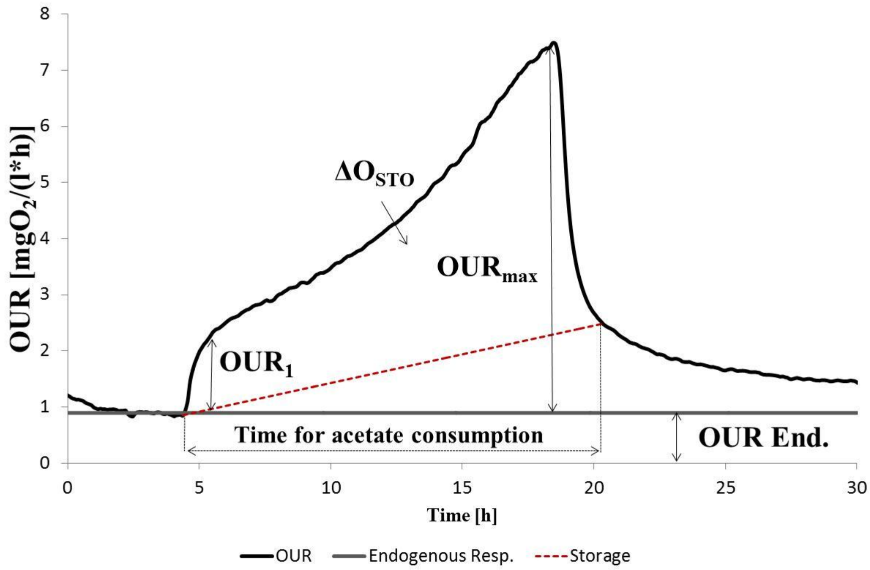


Figure 13 Respirogram from acetate consumption during the acclimatization of lab cores

Some kinetic parameters related to the bacterial activity (heterotrophic and autotrophic) can be obtained by respirometric tests:
(1) endogenous respiration (OUR_{end}): OUR measured in the absence of external substrates, such as biodegradable COD and ammonia, coming from raw wastewater;
(2) maximum oxidation rate of biodegradable COD($v_{COD,max}$): OUR related to the oxidation of biodegradable organic matter present in the wastewater (readily and slowly biodegradable);
(3) maximum nitrification rate ($v_{N,max}$): OUR related to the oxidation of ammonia;
(4) heterotrophic yield coefficient (Y_H): represents the fraction of substrate converted into heterotrophic biomass.

The maximum oxidation rate of biodegradable COD ($v_{COD,max}$) and the heterotrophic yieldcoefficient (Y_H) can be obtained by adding, after conditions of endogenous respiration have been reached, a rapidly biodegradable COD source (RBCOD), represented by an acetate solution (CH_3COONa). The addition of acetate results in an immediate reduction of the oxygen content as measured by the probe. Consequently, the value of OUR increases until it reaches a peak value (OUR_{max} - Figure 13). After the peak has been reached, values start decreasing and once the substrate has been consumed, values become those characterizing the endogenous phase.

OUR values during the endogenous phase are associated with an exponential curve extrapolated for the entire duration of the test; and the contribution in terms of oxygen consumed in this phase is calculated as the area under the exponential line that characterizes it. Instead, the total consumption of oxygen needed to degrade organic matter (ΔO_2) is obtained by calculating the area under the respirogram since the addition of substrate (t_o) until the return of the curve to endogenous conditions (t_f), which is subtracted from the endogenous decay (equation 7):

$$\Delta O_2 \left(mgO_2 / L\right) = \int_{to}^{tf} \left[OUR(t) - OUR_{end}(t)\right] dt \tag{7}$$

The maximum oxidation rate of biodegradable COD($v_{COD,max}$) and the heterotrophic yield coefficient (Y_H) can be calculated as follows:

$$v_{COD,\max} \left(mgO_2 L^{-1} h^{-1}\right) = \frac{OUR_{\max}}{1 - Y_h} \tag{8}$$

$$Y_H \left(mgCOD / mgCOD\right) = 1 - \frac{\Delta O_2}{S_s} \tag{9}$$

Where S_s is the amount of rapidly biodegradable COD added at the beginning of the test, and OUR_{max} is the OUR value obtained in the peak of COD consumption minus the OUR value during the endogenous phase.

The Y_{STO} (storage yield) is determined using the area under the OUR curve and above the straight line drawn from the endogenous decay level to the inflection point on the OUR curve (ΔO_{STO} - Figure 13), which is the end of the feast phase according to ASM3 (Karahan-Gül et al., 2002). The Y_{STO} can be estimated through equation 10.

$$Y_{STO}(gCOD / gCOD) = 1 - \frac{\Delta O_{STO}}{S_S} \tag{10}$$

In the same way, a known amount of ammonia (NH_4-N) can be added in the system in order to achieve the parameters related to the ammonia consumption. The resulting respirogram can be seen in Figure 14.

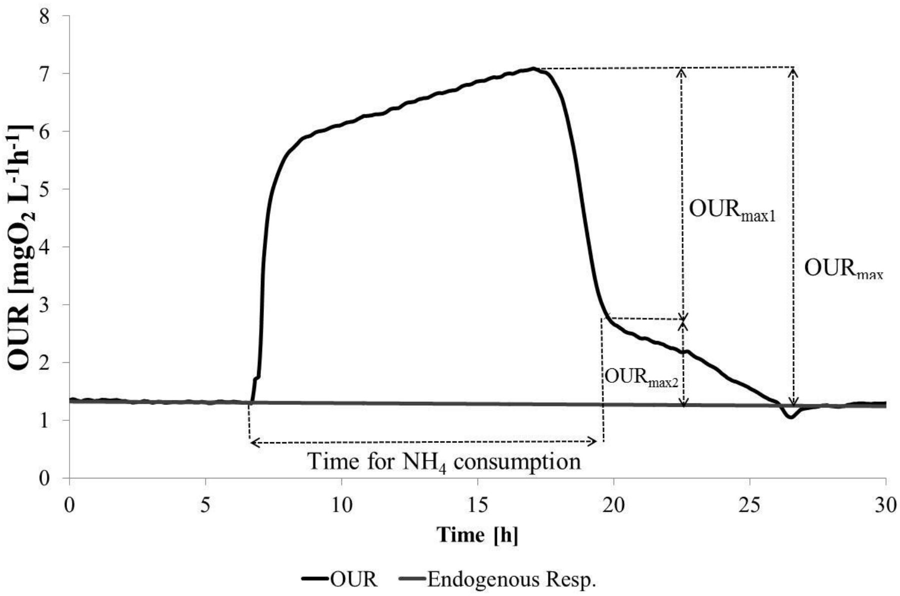


Figure 14 Respirogram obtained in a CW core after the NH_4 addition.

The respirogram is obtained from the resulting peak value of OUR from which you subtract the new value of endogenous respiration ($OUR_{end,N}$) to find the maximum value of OUR ($OUR_{max,N}$). The $OUR_{max,N}$ is used to calculate the maximum nitrification rate ($v_{N,\ max}$) (1 g of ammonia transformed into nitrate needed 4,57 gO_2):

$$v_{N,\max}\left(mgN-NH_4L^{-1}h^{-1}\right)=\frac{OUR_{\max,N}}{4{,}57} \tag{11}$$

The rates of consumption for AOB (Ammonia Oxidizing Bacteria) and NOB (Nitrate Oxidizing Bacteria) can be also obtained from a respirogram of ammonia consumption when nitrite storage can be observed (Foladori et al., 2012) as shown in Figure 14. Equation 12 and 13 introduce the AOB and NOB consumption rate calculation.

$$v_{AOB}=\frac{OUR_{\max,1}}{3.43} \tag{12}$$

$$v_{NOB}=\frac{OUR_{\max,2}}{1.14} \tag{13}$$

Figure 15 shows two lab cores under respirometric test.

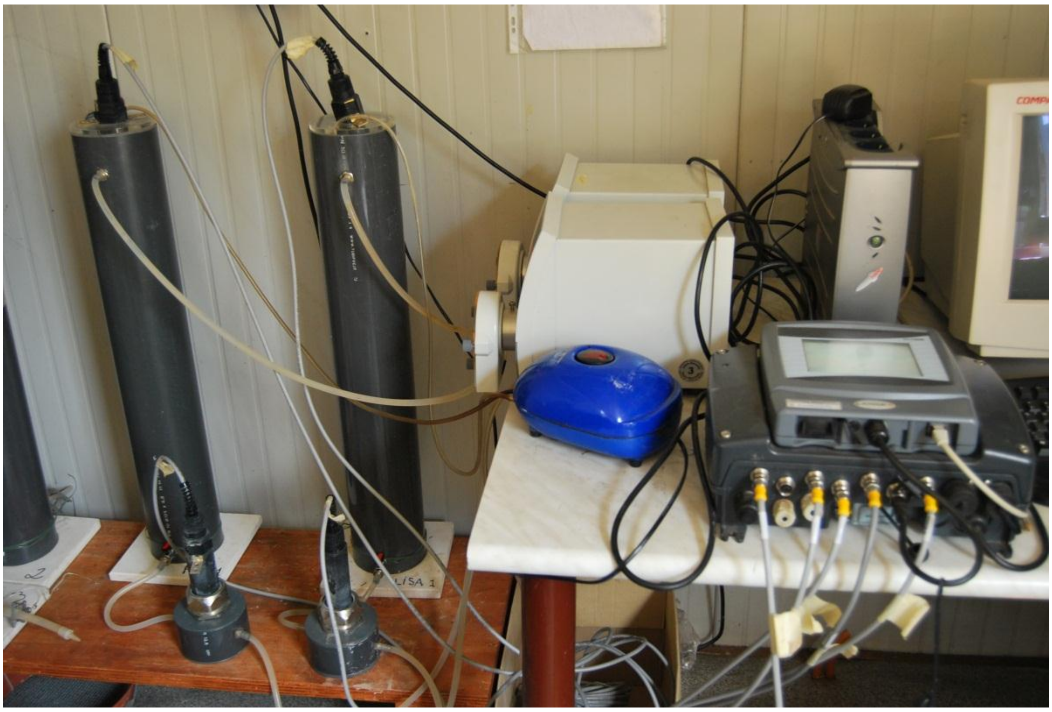


Figure 15 View of the cores under respirometric test.

3.1.1Addition of biodegradable substrate

Sodium acetate (CH_3COONa) was used as a readily biodegradable carbonaceous substrate. For the respirometric test an amount of acetate correspondent to 200 mgCOD/L was added in the core through a spiked addition (60 mL of acetate 10 g/L).The contribution of autotrophic microorganisms was evaluated by addition of 30 mL of NH_4-N (2g/L) in the CW cores. For tests realized with the material collected from the pilot plant (described later on in this chapter), the amount of NH_4-N added was 30 mL of Ammonia solution (10g/L).

Wastewater was added in the CW lab cores in order to evaluate its consumption. Wastewater with COD concentration of 324-465 mgCOD/L was used. For the respirometric test the core was aerated until endogenous conditions were reached and then 1.5 L of wastewater was gently added in the water column on top of the core, replacing 1.5 L of liquid which was extracted from the bottom of the core, while ensuring that no air was trapped in.

3.1.2CW cores

Four lab cores were made to use in the lab experimentation. All the cores have the filling material that represents a VSSF CW used in the pilot plant (that will be described later). Two cores were built similar to the VSSF in the E-lineand two, with the VSSF in theC-line. These two configurations were choosen in order to represent the conditions actually found in the pilot plant located in Ranzo (which is described latter). The cores have a height of 0.74 m high, a diameter of 0.125 m and a volume of 9.08 L (Figure 16). The liquid volume (V_L) in the respirometer was 4.2 L, including the porosity of the media, water in the pipes and in the cell where de DO is measured and a small water column on top of the core. The velocity of the recirculation used in these test was 300 mL/L.

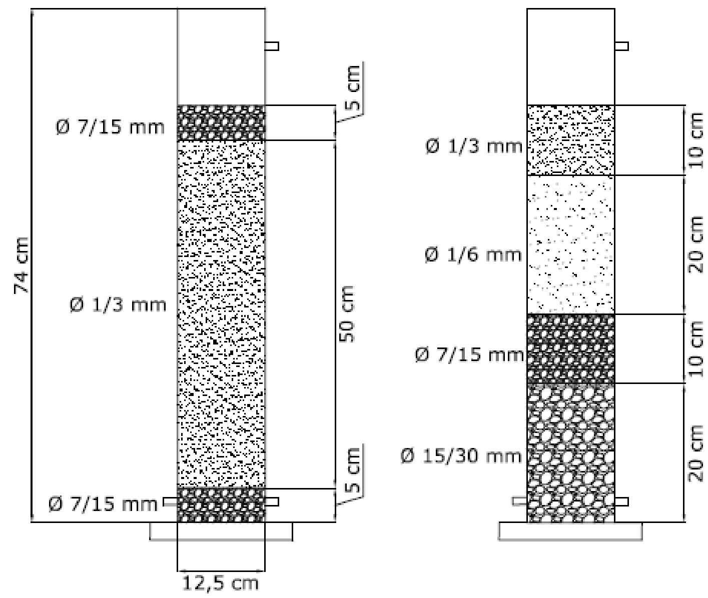


Figure 16 View of the lab cores configuration: the column in the left hand side is the C-line configuration and the column in the right hand side is the E-line configuration.

The E-line configuration (cores are called VSSF 1 and VSSF 2) included the following layers (from the bottom to the top): 0.2 m of gravel with grain size of 15 - 30 mm (porosity p= 31%); 0.1 m of gravel with grain size of 7 -15 mm (p= 30%); 0.2 m of sand with grain size of 1-6 mm (p= 28%); 0.1 m of sand with grain size of 1-3 mm (p= 31%). The liquid volume in the column was 2.2 L inside of the filter material.

The C-line configuration (also called VSSF 3 and VSSF 4) included the following layers instead (from the bottom to the top): 0.2 m of gravel with grain size of 15-30 mm (porosity p= 31%); 0.1 m gravel 7-15 mm (p= 30%); 0.2 m of sand with grain size of 1-6 mm (p= 28%); 0.1 m of sand with grain size of 1-3 mm (p= 31%). The liquid volume in the column was 2.14 L.

The columns had been acclimatised under unsaturated conditions for several months using pre-settled municipal wastewater and applying various COD loads (40 gCOD m^{-2} d^{-1}; 2.75

m^2/PE). During the acclimatisation period, oxygen transfer was provided only spontaneously during filling and discharge of the cores.

3.1.3 Biodegradable COD estimation

Traditional respirometers, as described previously in Ziglio *et al*. (2002), were also used in this research. The closed-respirometers used in this research consisted in batch reactors with temperature controlled where 1.2 L of activated sludge (taken from the oxidation tank of Trento Nord WWTP, Italy) was aerated intermittently between two DO set-points. Aeration and mixing were provided by compressed air and magnetic stirrer. DO was monitored by oximeters (OXI 340, WTW GmbH, Weilheim, Germany) connected to a data acquisition system. OUR was calculated as the slope of DO concentration during a phase without aeration and between the two set-points. In order to inhibit nitrification, allylthiourea was added at the beginning of the respirometric tests.

24-h respirometric tests were carried out according to the approach proposed by Vanrolleghem *et al.* (1999) for COD characterisation in raw wastewater. The measurement of biodegradable COD in influent and effluent wastewater taken from the CW units was performed by respirometry. The respirogram obtained for a conventional activated sludge used as reference was compared to the respirogram obtained after the addition of a known amount of wastewater to the activated sludge. The comparison allows calculation of the amount of biodegradable COD in the tested wastewater on the basis of the oxygen consumed for its oxidation.

3.2 Pilot plant description

The outdoor CW pilot plant is located in Ranzo, a small village in the eastern Alps (Province of Trento, Italy) at an elevation of 739 m above the sea level (coordinates 46° 03' N ande 10° 56' E). It is located 22 km away from the city of Trento and 120 km away from the city of Verona. Meteorological data for the whole period were collected from the nearest meteorological station located at 2 km from the pilot plant (S. Massenza, Province of Trento, Italy). The geographic location is shown Figure 17, while the village is shown in Figure 18.

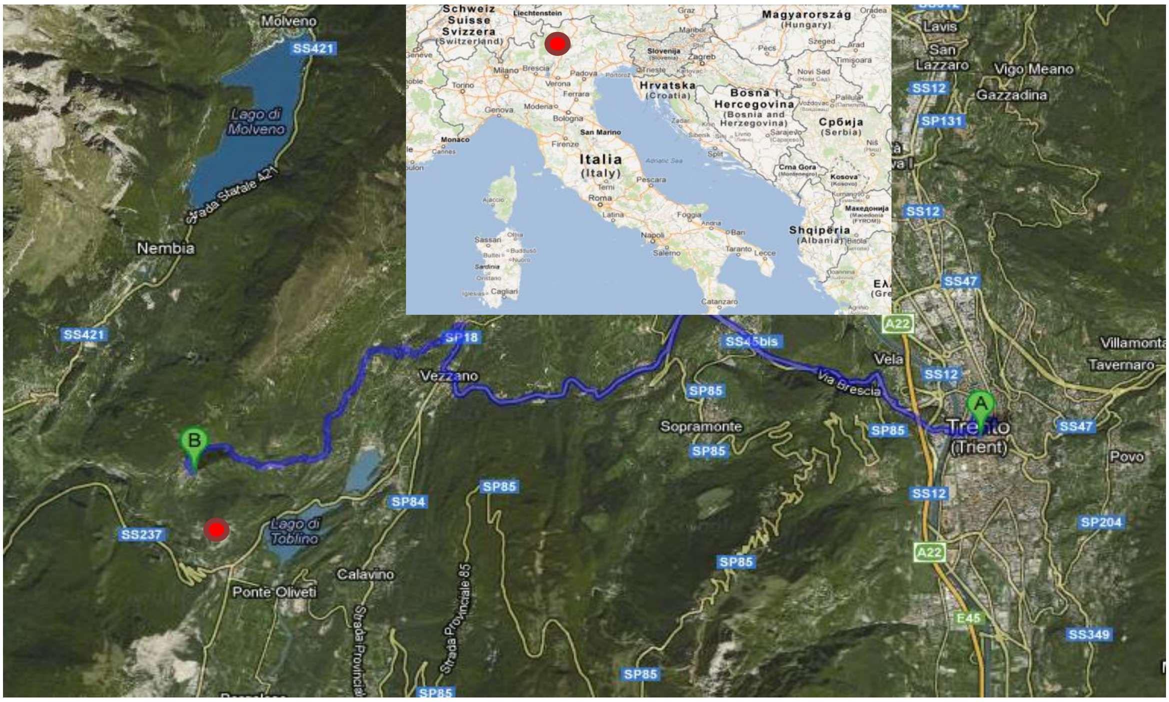


Figure 17 Geographic location of Ranzo (B – red dot) and Trento (A).


Figure 18 View of the village of Ranzo.

3.2.1 Control line and Experimental line

Ranzo is a small community of 435 inhabitants with no commercial or industrial activities in it. The wastewater produced by people living in the village is collected by pipes (rainfall water is collected separately) and transported by gravity until the pilot plant. Wastewater passes through a mechanical grid and a two chamber Imhoff tank in order to minimize the risk of clogging. After that part of the wastewater from the Imhoff tank is pumped to the pilot plant (Figure 19). Figure 20 and Figure 21 show a view of the pilot plant installation and the Imhoff tank, respectively.

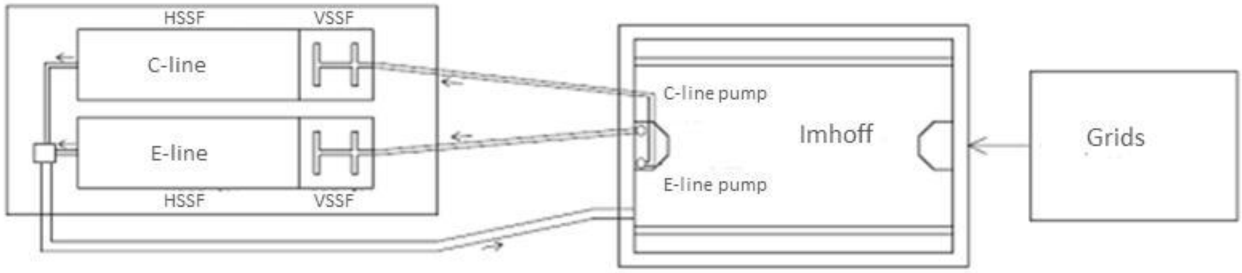


Figure 19 Scheme of the pilot plant.

Figure 20 View of the pilot plant installation

The pilot plant consists of two lines in parallel composed by a Hybrid CW: a VSSF followed by a HSSF (Figure 22). The pilot plant is divided into two main lines: one is the control line called C-line (whose design characteristics follow the indications provided by the Province Law n. 992/2002) and the other is the experimental line called E-line. In the E-line the innovative configurations were tested, while the C-line has the same operation conditions during all the period. Figure 24 describes the layers of the VSSF and HSSF and Figure 23 shows the distribution system placed above the VSSF.
Table 4 shows the description of the filter material layers used in VSSF and HSSF.


Figure 21 View of the Imhoff tank

Table 4 Description of the filter material layers used in VSSF and HSSF.

	Units	VSSF		HSSF
		E-line	C-line	E-line and C-line
Depth (filter material)	m	0.60	0.80	0.60
Superficial Area	m^2	2.25	2.25	9.00
Width	m	1.50	1.50	1.50
Length	m	1.50	1.50	6.00

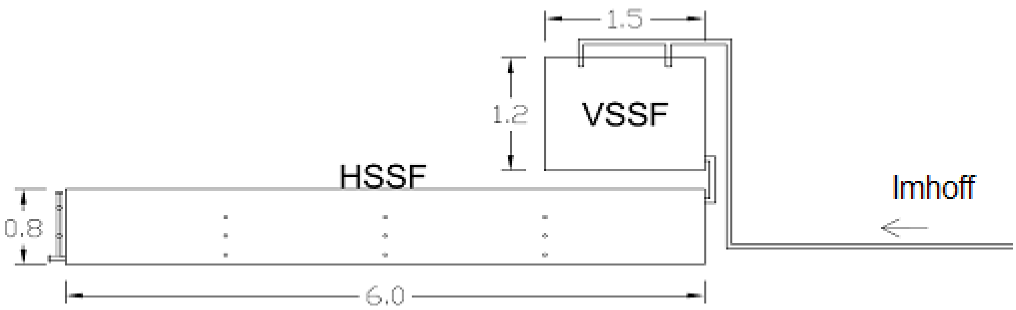


Figure 22 Scheme of the Hybrid CW used in the pilot plant.

The main physical difference between these two systems lies in the kind of filter material used in the VSSF (Figure 24). The E-line configuration presents the following layers from the bottom to the top: 0.2 m of gravel with grain size of 15 - 30 mm (porosity p= 31%); 0.1 m of gravel with grain size of 7 -15 mm (p= 30%); 0.2 m of sand with grain size of 1-6 mm (p= 28%); 0.1 m of sand with grain size of 1-3 mm (p= 31%). Initially two separate layers were on top of the VSSF with grain size of Ø1-3 mm and Ø1-6 mm, but these were turned into one single layer with size distribution Ø1-6 mm after few months of operation.

Figure 23 Distribution system above the VSSF on both lines: E-line and C-line.

The C-line configuration was made of the following layers (from the bottom to the top): 0.2 m of gravel with grain size 15-30 mm (porosity p= 31%); 0.1 m of gravel with grain size 7-15 mm (p= 30%); 0.5 m of sand with grain size 1-6 mm (p= 28%); 0.1 m of sand with grain size of 1-3 mm (p= 31%). Both VSSFs are unplanted.

The HSSF (Figure 25) had the same configuration in both systems: a 0.5 m wide gravel-made drainage layer with grain size of 15-30 mm (porosity p=30%) on both sides of the filter and in the middle a 5 m wide layer with gravel of grain size 3-7 mm (p=26%). The HSSF is equipped with a group of three taps at a distance of 1.5 m from each other that allow the effluent to be sampled along the side at different heights (0.2 m, 0.4 m and 0.6 from the bottom) (Figure 25). HSSF is planted with 4 plants/m^2 of *PhragmitesAustralis*.

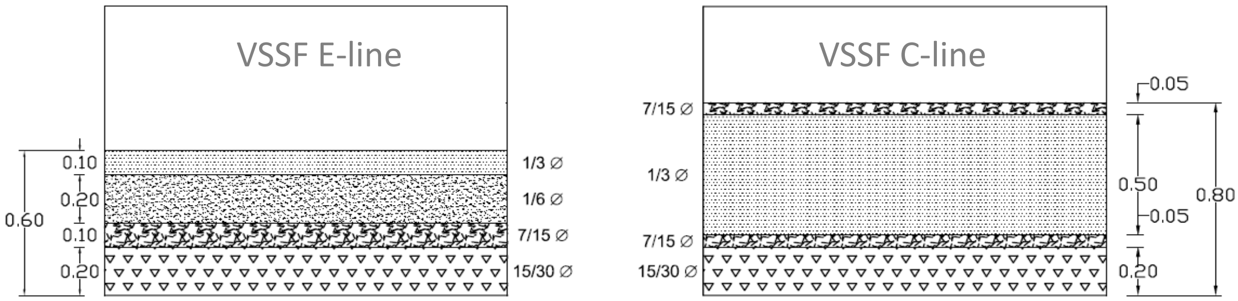


Figure 24 Layout of the VSSF for theE-line and C-line configurations

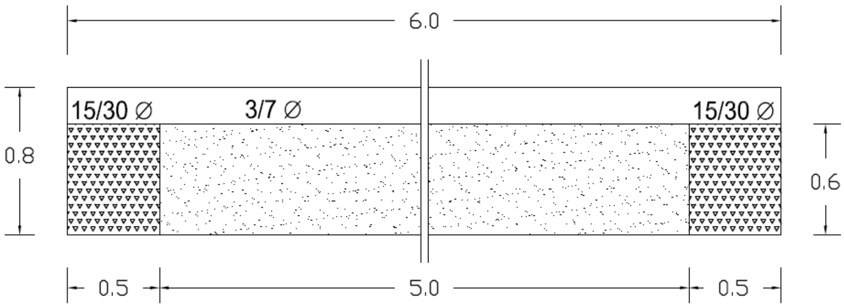


Figure 25 Layout of the HSSF

The pilot plant is equipped with a control panel (Figure 26) for the regulation of various parameters, such as: the feeding and resting period of the feeding pump; operation and resting period of the recirculation pump; the duration of the cycle (intended as intervals between feeding periods) and the control of the automatic valve.

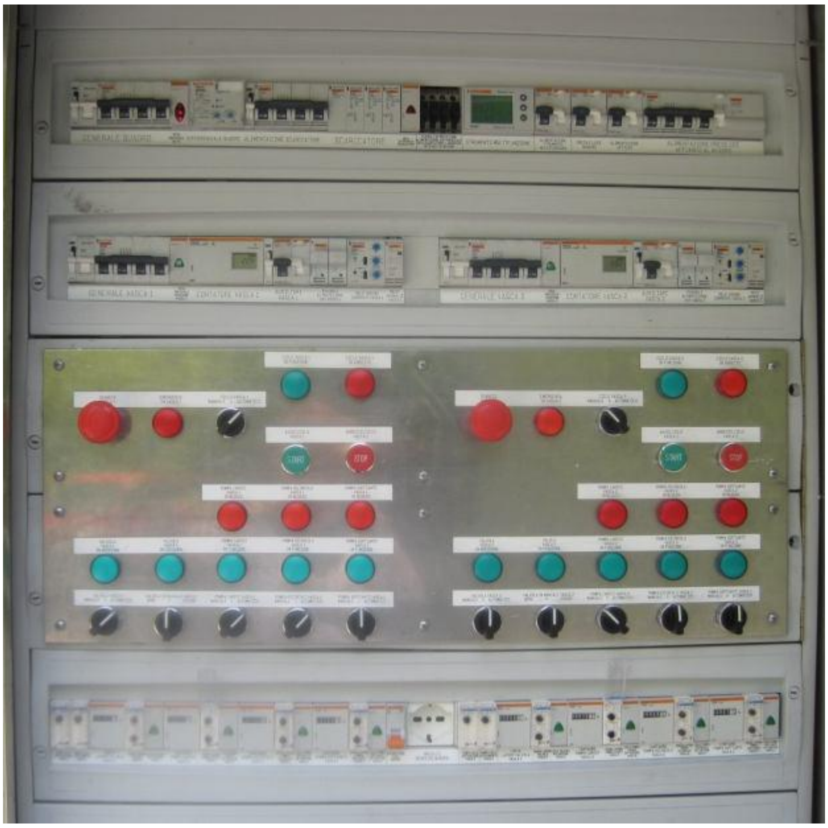


Figure 26 Control panel of the pilot plant: E-line controlsare located on the left hand side, whileC-line controlsare located on the right hand side.

Different working phases in the E-line are controlled by a timer as well as by level probes positioned in the pipe outside the bed (Figure 27) that work as a piezometer.

Figure 27 Piezometer pipe where the level probes are installed in the E-line

Figure 28 and Figure 29 show a view of the pilot plant on March 2010 and July 2011, respectively.

Figure 28 View of the pilot Plant in March 2010.

Figure 29 View of the pilot Plant in July 2011, before and after plants were cut.

3.2.2 Operational periods

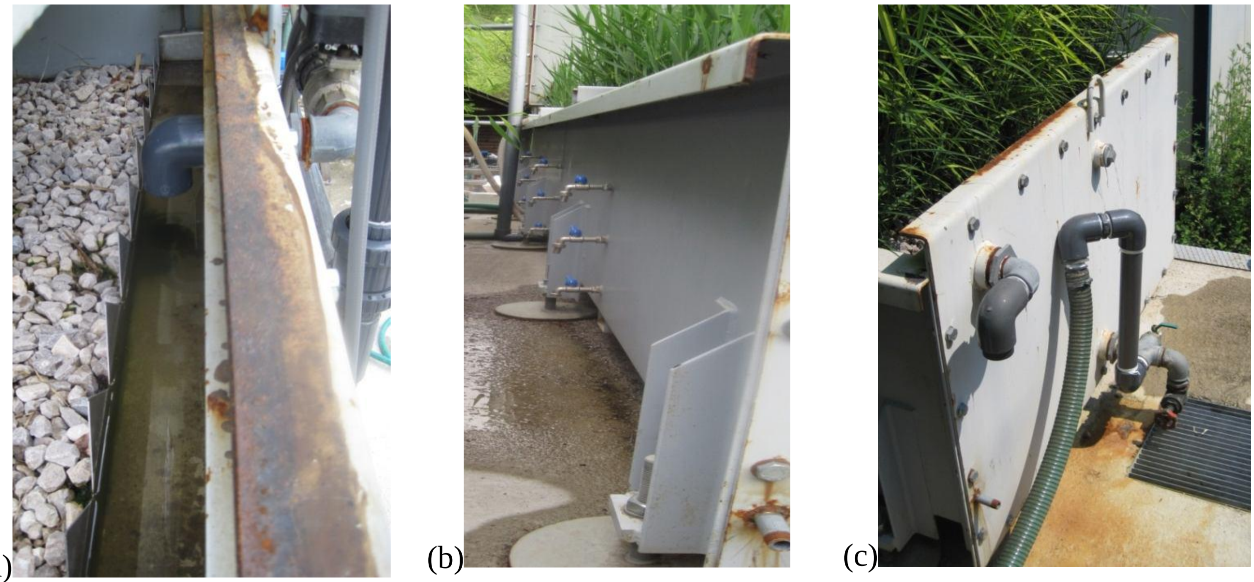
The pilot plant was operated for two years (2010 and 2011).In this period, different configurations were tested in the E-line. The C-line has been operated with the same configuration over the entire research period. The initial configuration maintained that both lines were operated with the same organic and hydraulic load. After 3 months, the hydraulic and organic loads in the E-line were increased in order to explore the limits of the configuration. Further, recirculation of wastewater and aeration were tested in the VSSF. Table 5 shows the main parameters of the different configurations.

Table 5 Main parameters for the different configurations adopted in this study

Configuration	VSSF			HSSF	
	Hydraulic Load ($L\ m^{-2}\ d^{-1}$)	Organic Load ($gCOD\ m^{-2}\ d^{-1}$)	Specific Surface (m^2/PE)	Hydraulic Load ($L\ m^{-2}\ d^{-1}$)	Specific Surface (m^2/PE)
Low Load VSSF+HSSF (C-line)	63	33	3.5	16	19.6
Low Load VSSF+HSSF (E-line)	55	37	3.2	14	12.8
High Load VSSF+HSSF(E-line)	123	87	1.3	31	5.2
High Load VSSF+HSSF / recirculated VSSF (Recirculated E-line)	169	82	1.5	42	5.8
High Load VSSF+HSSF / aerated VSSF (Aerated E-line)	135	58	1.9	34	8.7

The value of COD, TSS, NH_4-N, NO_3-N, NO_2-N, TKN, Total P, P-PO_4^{3-} was assessed in the influent and effluent of the CW pilot plant by chemical analysis. Samples of influent and effluent from VSSF and HSSF systems were collected once to twice a week over a 2 year period. Figure 30 shows the sampling points in the pilot plant. Particulate COD was calculated as difference between total COD and filtered COD. Analyses were performed according to Standard Methods (APHA, 2005).

Intensive monitoring campaigns (track-studies) were conducted during the VSSF normal operation cycle to obtain the concentration time-profiles of wastewater effluent from the VSSF over 8 different time frames (track-studies): 0-5 minutes, 5-10 minutes, 10-20 minutes, 20-30 minutes, 30 minutes-1 hour, 1-2 hours, 2-4 hours and 4-6 hours. Samples along the HSSF unit were taken from taps to obtain the longitudinal profile of concentrations in the bed (3 sample points).



(a) (b) (c)

Figure 30 Sampling point: (a) Distribution from the VSSF to the HSSF; (b) Taps on the side of the HSSF; (c) Collection point of the HSSF final effluent.

The operation periods tested in this research are described as follows:

a) Low Load VSSF+HSSF C-line and E-line:

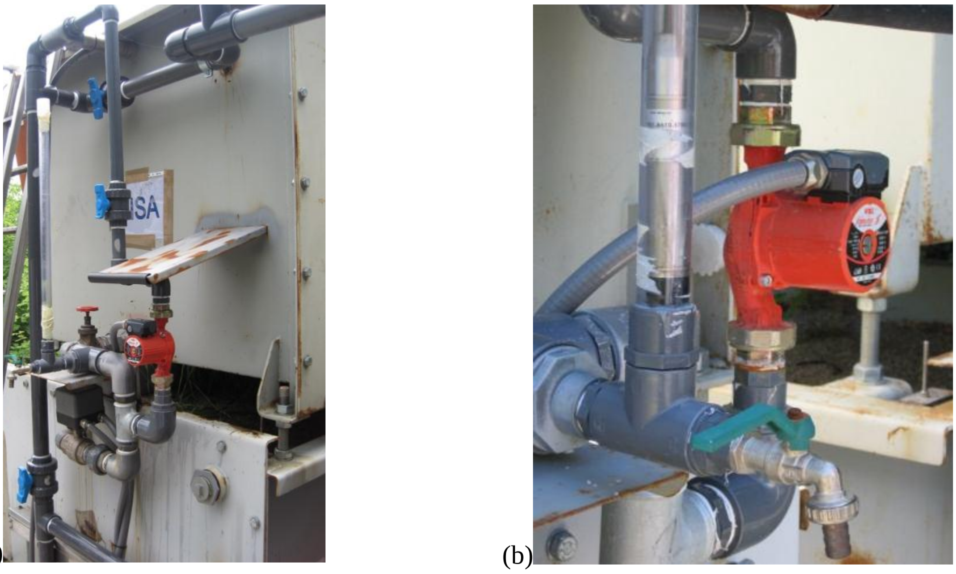
The VSSF system was operated with a conventional down-flow configuration with a single feeding per cycle applied on top of the VSSF bed (6.6 h/cycle, 3.6 cycles/day on average) and the VSSF effluent flows to the HSSF. The VSSF C-line: operated with about 3.7 m^2/PE in the period 2009-2011 and configurationE-line operated with 3.2 m^2/PE(results are shownin Chapter 7);

b) High load VSSF+HSSF (E-line):

The VSSF of theE-line was operated with a conventional down-flow configuration with a single feeding per cycle applied on top of the VSSF bed (6.6 h/cycle, 3.6 cycles/day on average). The specific surface area of the VSSF in the E-line and in the C-line was 1.3 m^2/PE and 3.2 m^2/PE, respectively (results are shownin Chapter 8);

c) High load recirculated VSSF + HSSF (Recirculated E-line)

The VSSF of the E-line was operated with a recirculated configuration with a single feeding per cycle applied on top of the bed (10.5 h/cycle, 2.3 cycles/day on average). The specific surface area of the E-line was 1.5 m^2/PE on average, while in the C- line it was 3.6 m^2/PE on average. Recirculation in the VSSF CW was performed closing the automatic valve (Figure 31) at the bottom of the bed to maintain saturated conditions in the bottom layers for approximately 6 h/cycle (6-h-phase). Periodic short recirculations (5 minutes per hours, 6 times per cycle) were performed from the bottom to the top of the bed in an attempt to improve the spontaneous aeration and favour nitrification. At the end of the cycle the valve was opened and the discharge of wastewater occurred from the bottom of the bed to the HSSF for 4 hours (results are shown in Chapter 10);



(a) (b)

Figure 31 Pump for the recirculation of wastewater: (a) General view and (b) Close-up view.

d) High load aerated VSSF + HSSF (Aerated E-line)

The aerated VSSF in the E-line was based on the saturation of the bottom of the VSSF bed, where the aeration is applied. The aeration allows the alternation of anoxic and aerobic condition on the saturated bottom of the bed. VSSF system was operated with an aerated configuration with a single feeding per cycle applied on top of the bed (10.8 h/cycle, 2.2 cycles/day on average). The VSSF specific surface area in the E.line was 1.9 m^2/PE on average. The automatic valve at the bottom of the bed was closed to maintain saturated conditions in the bottom layers for approximately 6 h/cycle (6-h-phase). During this phase, 5 min of aeration was provided every 30 min in the VSSF CW by means of holed pipes installed at the bottom of the bed. The air compressor capacity is 8Nm^3/h and its operation is controlled by the panel (Figure 32). At the end of the cycle the valve was opened and wastewater was discharged to the HSSF for 4 h (results are shown in Chapter 11).

(a) (b)

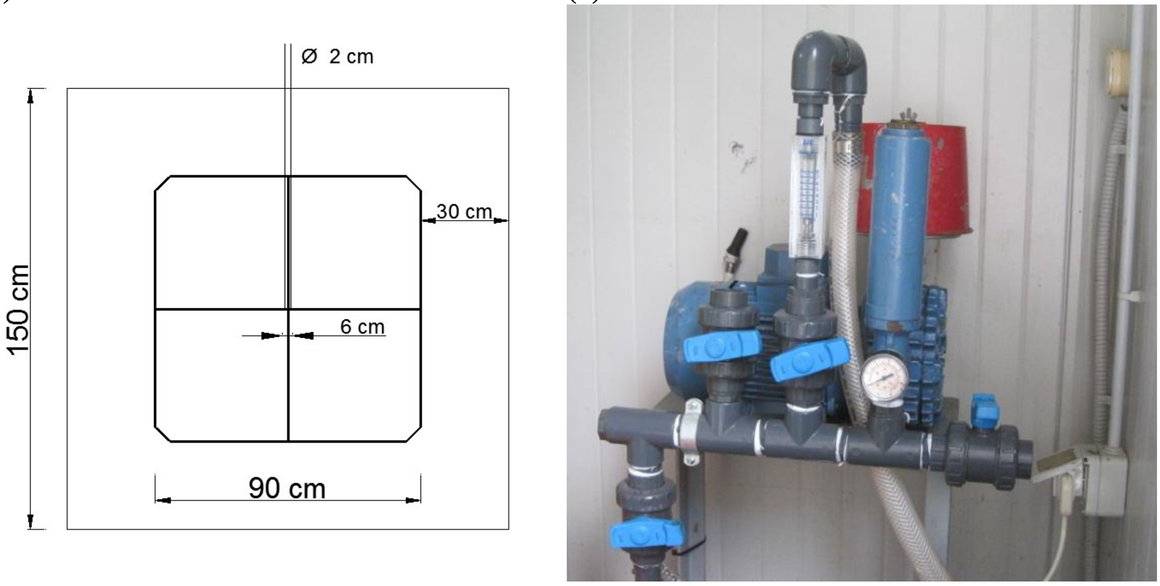


Figure 32 Aeration scheme: (a) scheme of the perforated pipes positioned on the bottom of the VSSF CW and connected with Ø 2 cm pipe to the air compressor; (b) Air compressor and flow meter.

3.2.2.1 Winter operation of the pilot plant

The VSSF run in parallel during the winter operation and results are shown in Chapter 9. C-line (called in Chapter 9 Low-Load VSSF) operated with 70 $Lm^{-2}d^{-1}$ and 25.9 $gCODm^{-2}d^{-1}$ of hydraulic and organic load respectively and E-line (called in Chapter 9 High-Load VSSF) operated with 178 $Lm^{-2}d^{-1}$ and 73.6 $gCODm^{-2}d^{-1}$ of hydraulic and organic load respectively.

The pilot plant was operated during the winter under two different configurations: regular and discontinuous feeding. During the regular operation period (continuous feeding period) the influent wastewater was applied in both VSSF CWs by pumping from the septic tank every 6.5-6.6 h (3.7-3.8 cycles/day on average). In the period with discontinuous feeding influent wastewater was put into the VSSF CW only few times (i.e. every 2-4 weeks) to cause a long-term stress in the system. Figure 33 shows the pilot plant under winter conditions.


Figure 33 View of the pilot plant under winter conditions.

The Ammonia Oxidation Rate (AUR) test was applied in order to estimate the nitrifying activity under different temperatures. The AUR is a test developed to measure the activity of nitrifying bacteria in activated sludge through the determination of maximum specific utilization rates of ammonia in activated sludge. It can also be used to evaluate the inhibition in the nitrifying biomass or the influence of temperature, oxygen and pH on the nitrification activity. The test isusually performed in a batch reactor with few liters of activated sludge (concentration of 3-4 gSST/L). Parameters like pH, temperature, and dissolved oxygen must be carefully monitored due to their influence on the nitrification rate.

The mixture is continuously aerated in order to avoid the oxygen limitation (OD> 4 mgO_2/L), temperature and pH are monitored to keep them around 20°C and 7.5-8.0, respectively. At the beginning of the test a known amount of ammonia nitrogen is added in order to have an initial concentration of 20-30 mgN/L. Samples of activated sludge are taken every 15-30 minutes over a total period of 3-4 hours and, after filtration, they are analyzed to determine the concentration of ammonia, nitrous and nitric acids. The maximum specific nitrification rate (v_N) can be calculated as the slope of the production of NO_2 and NO_3 or the slope of the curve consumption of NH_4.

In order to use the AUR test in the field of CW, the cores used for respirometric test were used for this test. At the end of the typical VSSF cycle, when wastewater was drained by gravity, a little amount of liquid remained in the interparticle voids (pore water content was approximately 5% of the wet sand/gravel weight). Thus the amount of water in the column was 370 mL before the beginning of the AUR test. The modified AUR test was realized with 600 mL of water fed on top of the column with a concentration of 30-50 $mgNH_4$-N/L which drained throughout the column. The water collected on the bottom is put on top of the column again every 15 minutes. The samples collected from the bottom were analyzed for NH_4-N, NO_2-N, NO_3-N, pH and temperature every 30 minutes. The overall test lasted 5 to 8 h until the complete oxidation of the NH_4 added. Each test was run with different temperatures (around 2.0˚C, 6.0˚C, 9.0˚C 12.0˚C, 14.8˚C, 18.0˚C and 20˚C). The maximum specific nitrification rate (v_N, expressed as mgN m^{-2} d^{-1}) was calculated considering the consumption of NH_4-N instead of the production of nitrite and nitrate because denitrification can take place in the porous of the filter material.

Filter material from the pilot plant: AUR tests were also applied to the granular material collected from the VSSF CW pilot plant in order to evaluate the effects on the nitrification activity after three months at low temperatures and discontinuous feeding. Samples were extracted during the winter after a two month period of intermittent feeding and at temperatures inside the CW between 1 and 10°C. Few kilograms of the granular material were collected in the top layers of the VSSF CWs and placed in the column where AUR test was performed at controlled temperature.

Chapter 4

Kinetics of heterotrophic biomass and storage mechanism in wetland cores measured by respirometry[1]

4.1 Introduction

Numerical models of different complexity have been published in recent years to simulate hydraulic behaviour, biochemical transformation and degradation processes for organic matter and/or nitrogen in CW, for example, using mechanistic models describing reactive transport in saturated or variably saturated conditions as reviewed by Langergraber (2008). Although some of these models have been used in several applications and a good match of the measured data has been obtained, the parameters used in the models are often assumed from literature and not always obtained from the specific CW plant. Kinetic and stoichiometric parameters involved in biological processes such as CWs cannot be taken as universal, since they may be influenced by many factors e.g. the influent wastewater composition, properties of gravel/sand bed, operational strategies, etc. Procedures for the direct and experimental measurement of the kinetic parameters of microbial biomass in CWs are still rare or absent today (Langergraber and Šimůnek, 2005). Some authors have highlighted the need for further research to develop experimental methods for estimating model parameters, both kinetic and stoichiometric, with the aim of improving the accuracy of numerical models to be used as a reliable design tool for CWs (Langergraber, 2008).

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[1] This chapter was published as: Ortigara A. R. C., Foladori P., Andreottola G. (2010). Kinetics of heterotrophic biomass and storage mechanism in wetland cores measured by respirometry. Water Science and Technology 64(2), 409-415.

The application of respirometric techniques to investigate carbonaceous substrate oxidation or nitrification using columns that simulate cores of VSSF CW was first proposed by Andreottola et al. (2007). The dynamic of the oxygen uptake rate (OUR) is usually known as respirogram, and batch OUR tests have been widely used in activated sludge processes to measure kinetic and stoichiometric parameters or to characterise wastewater biodegradability. Furthermore, respirometry is often considered as a traditional method in the calibration of activated sludge models (inter aliaVanrolleghemet al., 1999). Although the use of OUR dynamics is still new and not yet fully investigated and understood in the field of CWs, this approach seems promising to obtain kinetic and stoichiometric parameters involved in the oxidation of organic matter, such as the maximum oxidation rate of readily biodegradable COD, endogenous respiration and maximum growth yield.

This chapter shows how respirograms can be interpreted to improve the knowledge of the kinetic and stoichiometric parameters of heterotrophic biomass processes occurring in CW cores, interpreting the respirograms obtained under aerobic respirometric tests carried out with pure substrate permitted the calculation of the maximum growth yield of heterotrophic biomass, while respirometric tests carried out with raw wastewater allowed us to quantify the biodegradable COD of wastewater when applied in a CW system. Particular emphasis was given to the description of the storage mechanisms, which lead to the formation of internal storage products under “feast” conditions and their degradation under “famine” conditions, very similar to the phenomena already observed in activated sludge systems (inter aliaMajoneet al., 1999; Sin et al., 2005).

4.2 Materials and Methods

CW cores of the E-line configuration were tested in this Chapter (more details about the cores can be found in Chapter 3). The columns were differently acclimatised for several months using raw municipal wastewater and applying average COD loads of 40 gCOD m^{-2} d^{-1} (equivalent to 2.8 m^2/PE). All CW cores performed COD removal and nitrification.

During the respirometric tests, CW cores were connected to pipes and to a peristaltic pump with a known flow rate (Q = 18 L/h in our tests). Aeration is supplied continuously on top of the core at aeration rate ranging from 2 to 7 NL/min. The liquid volume (V_L) in the respirometer was 3.2 L, including the porosity of the media (2.20 L) and the water in the pipes, dissolved oxygen (DO) chamber and a small water column on top of the core (0.98 L). Two DO probes are placed at the top and the bottom of the core. CW cores were firstly aerated overnight until endogenous respiration was achieved. Sodium acetate was used as a readily biodegradable carbonaceous substrate and a spike addition correspondent to 187 mgCOD/L was applied in the core.

Alternatively in this chapter, raw municipal wastewater (taken from the wastewater treatment plant of the city of Trento, Italy) with COD concentrations of 324-465 mgCOD/L was used. During the respirometric test with wastewater, the CW core was aerated until endogenous conditions were achieved and then 1.5 L of wastewater was gently added to the water column at the top of the core, replacing 1.5 L of liquid which was extracted from the bottom of the core, being careful to avoid air entrapment. Ammonia in excess was present in all the tests. Allylthiourea was always added to the CW cores to avoid nitrification and to measure only the oxygen consumption of heterotrophic bacteria.

Respirometry of activated sludge – Closed-respirometers consisted of temperature controlled batch reactors where 1.2 L of activated sludge (taken from the oxidation tank of Trento Nord WWTP, Italy) was aerated intermittently between two DO set-points. Aeration and mixing were provided by compressed air and magnetic stirrer. DO was monitored by oximeters (OXI 340, WTW GmbH, Weilheim, Germany) connected to a data acquisition system. OUR was calculated as the slope of DO concentration during a phase without aeration and between the two set-points. In order to inhibit nitrification, allylthiourea was added at the beginning of the respirometric tests.

4.3 Results and Discussion

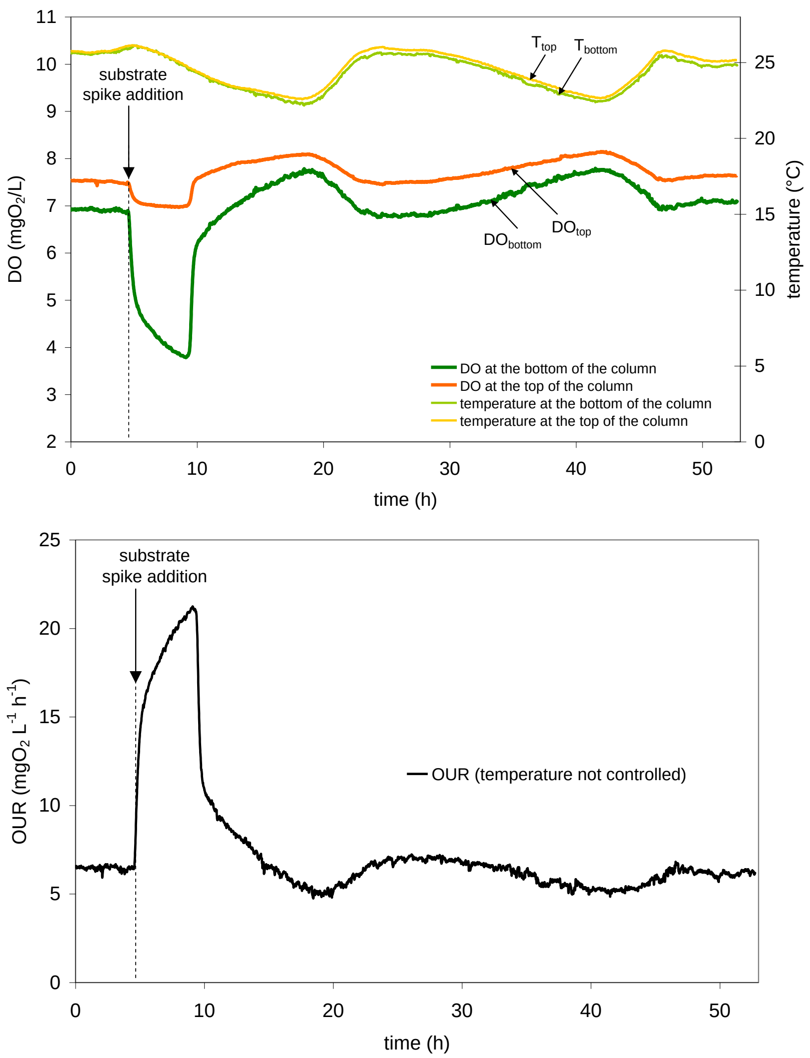
4.3.1 Calculation of the respirogram of the CW cores

A respirometric test starts providing aeration overnight in the CW core in order to achieve endogenous respiration. DO concentrations at the top and the bottom of the column are indicated in the example in Figure 34 (a) and depend on:
- room temperature (ranging from 22.3 to 26.1°C), which causes daily DO variations;
- the substrate spike addition (acetate) which causes an immediate significant decrease of DO concentration at the bottom of the column and a slight decrease at the top.

Using equation 6, the OUR was calculated and indicated in Figure 34 (b). The OUR profile calculated at room temperature is affected by the daily variations of temperature and therefore it is not easy to identify exactly the profile of endogenous respiration. In order to exclude the influence of temperature variations, the respirogram has to be corrected considering a reference temperature of 20°C (however another reference temperature could be considered). The correction of temperature was calculated considering the following form of the Arrhenius equation:

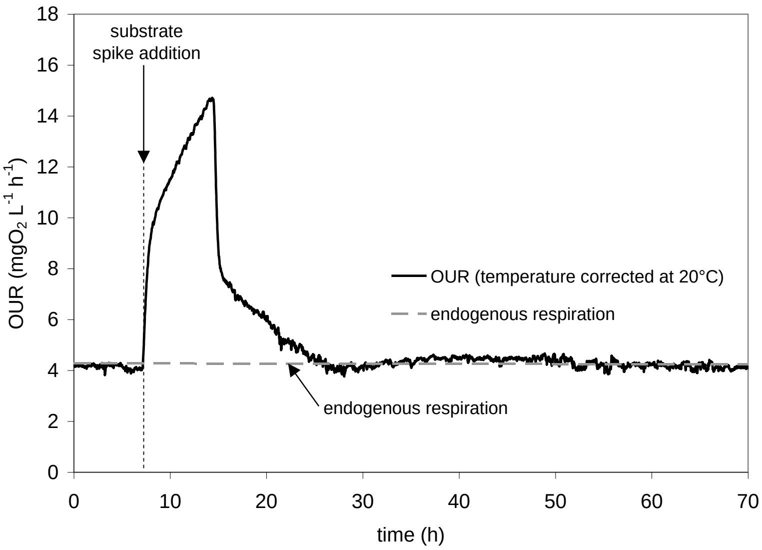
$$OUR_{20°C} = \frac{OUR_t}{\alpha^{(T-20°C)}} \quad (\alpha=1.08) \tag{14}$$

but maintaining the same integral under the respirogram (by applying the trapezium rule and changing the time). The respirogram corrected to 20°C is indicated in Figure 34 (c), where the expected regular profile of the data can be immediately observed, which allows us a better understanding of how the process and the endogenous respiration change over time.



(a)

(b)



(c)

Figure 34 Calculation of the respirogram and correction of temperature: (a) DO and T at the top and bottom of the CW core; (b) OUR at room temperature; (c) OUR after the correction of temperature to 20°C.

4.3.2 Respirometry of CW cores using acetate and storage mechanisms

Respirograms of two different CW cores obtained after the addition of 187 mgCOD/L of acetate (S_S) and after correction to 20°C are indicated in Figure 35. The following phases can be observed:

- *phase 1*: initial endogenous respiration (OUR = 4-5 $mgO_2\ L^{-1}\ h^{-1}$);
- *phase 2*: rapid increase of OUR immediately after the addition of S_S (OUR peak of 14.5-32 $mgO_2\ L^{-1}\ h^{-1}$) and rapid decrease of OUR after S_S depletion;
- *phase 3*: slow decrease of OUR due to the utilization of the stored compounds, until the endogenous respiration is reached;
- *phase 4*: endogenous respiration (OUR = 4-5 $mgO_2\ L^{-1}\ h^{-1}$).

(a)

(b)

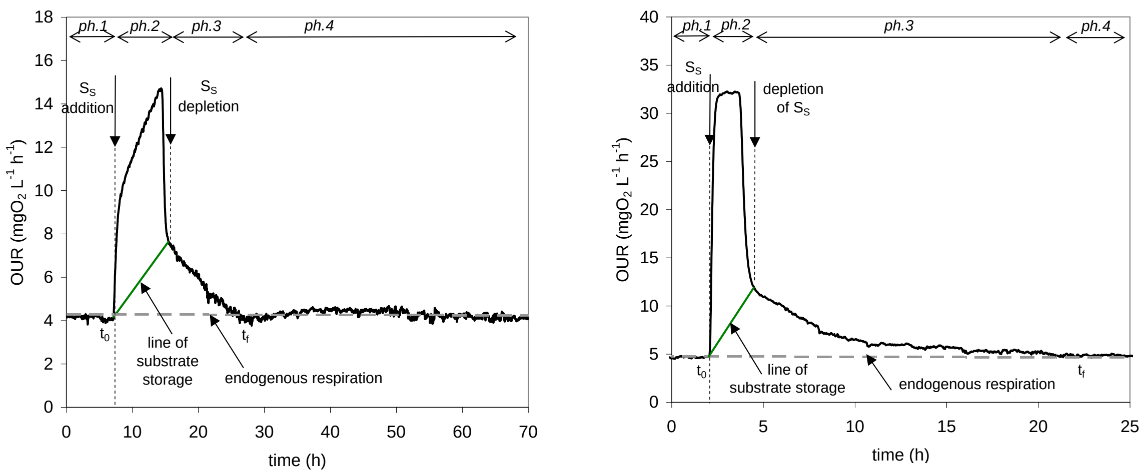


Figure 35 Respirograms of CW cores (a)(b) obtained after the addition of 187.5 mgCOD/L of acetate (S_S)

After the addition of S_S, “feast” conditions were formed in the CW core and the OUR increased rapidly up to a peak of 15 and 32 $mgO_2\ L^{-1}\ h^{-1}$in the CW cores (a) and (b), respectively (phase 2 inFigure 35). Simultaneously, internal storage products were gradually formed (X_{STO}) until all the external S_S was consumed. When S_S was completely removed from the bulk liquid, OUR dropped from the maximum level to a level above the endogenous respiration. During the following phase (phase 3 in Figure 35) OUR decreased more slowly and in general in a non-linear way, to reach the values of endogenous respiration maintained prior to substrate addition. Phase 3 is associated with “famine” conditions, in which the growth of heterotrophic biomass occurs using X_{STO}.

The internal storage products, mainly polysaccharides and lipids, are intermediate products in overall carbonaceous substrate removal, which are formed especially when the biomass is subjected to feast and famine conditions, as widely observed in activated sludge systems (inter alia Majone et al., 1999; Carucciet al., 2001; Dirckset al., 2001). The occurrence of the storage mechanism in CW cores is probably due to the intermittent loads applied to the CW cores, causing transient and highly dynamic load conditions, especially when long feast/famine periods are applied. Dynamic conditions can lead to a storage response, even in the absence of any external limitation for the growth. The storage of substrate available under feast conditions allows microorganisms capable of substrate storage to survive during the subsequent famine conditions when the external substrate is depleted (Karahan-Gül al., 2003; van Loosdrecht et al., 1997). In biological systems with high SRT and low growth rate – CW systems can be consider as belonging to this category – the formation of storage polymers was frequently observed (Sin et al., 2005).

Important stoichiometric parameters in carbonaceous substrate oxidation and storage mechanisms are: the maximum growth yield Y_H which represents the fraction of substrate converted into biomass and the storage yield Y_{STO} which represents the fraction of substrate converted into storage products then utilised for growth. These parameters can be easily determined by respirometry using acetate, also in the case of CW cores, as performed in this research. For the calculation of Y_H the following expression was used:

$$Y_H\left(mgCOD/mgCOD\right)=1-\frac{\Delta O_2}{S_s}=1-\int_{to}^{Tf}\left[OUR(t)-OUR_{end}(t)\right]dt \qquad (15)$$

where the total amount of oxygen (ΔO_2) needed for the oxidation of the external substrate was calculated as the integral between the respirogram and the endogenous respiration calculated from t_0(time of addition of acetate) to t_f(when endogenous respiration is reached again). Y_H for CW cores was 0.56-0.59 (Table 6) which is lower than the typical value of 0.67 expected for conventional activated sludge.

To calculate Y_{STO} (according to the definition in ASM No.3, Gujeret al., 1999, in which it is considered that substrate storage is the preliminary step and the subsequent growth occurs solely on the stored products) from the respirograms of CW cores indicated in Figure 2 we adapted a simplified graphic method which does not require model simulation, as proposed by Karahan-Gület al. (2002). As shown in Figure 35, a straight line was drawn connecting the first OUR point and the final OUR point of phase 2. The area between the respirogram and this straight line is oxygen used for storage (ΔO_{STO}) and so Y_{STO} can be easily calculated using the equation 10 in Chapter 3 (results in Table 6). The values of Y_{STO} for CW cores using acetate were 0.75-0.77 mgCOD/mgCOD, lower than the value of 0.85 suggested in ASM No. 3 for activated sludge, but only slightly lower than the value of 0.78 obtained by Karahan-Gület al. (2003) for activated sludge fed with acetate.

Although the storage mechanism has been extensively investigated and modelled in the field of activated sludge, this phenomenon is new in the field of CWs and models of CW processes have not yet been adapted to include the storage mechanism. In the field of activated sludge, some conceptual modifications have recently been introduced to describe storage mechanisms, such as the concept that biomass growth occurs during both feast and famine phases, using both S_S and storage products (Karahan-Gület al., 2003; Sin et al., 2005), but further research should be done to investigate whether these recent conceptual models are reliable in CW systems.

The main kinetic and stoichiometric parameters for CW cores are summarised inTable 6, where kinetics were calculated to 20°C and expressed per unit of volume and surface of CWs. Considering the duration of the respirograms in Figure 35, a period longer than 24 h is needed to ensure the complete consumption of biodegradable substrate and to reach stable endogenous respiration. Therefore the organic load applied as a spike addition corresponds to a daily organic load of 81 gCOD m^{-3} d^{-1} and 49 gCOD m^{-2} d^{-1}. The kinetics indicated in Table 6 are calculated per hour because the exogenous maximum rates last several hours. The values indicated in Table 6 may appear high when compared to the typical COD removal rates expected in real CWs. The reason is because during respirometric tests conditions are prevalently aerobic and the biodegradation kinetics may be overestimated with respect to the kinetics expected in real CWs, where the oxygen transfer from the atmosphere is limited and not enough to ensure a fully aerobic environment. It is well known that anoxic/anaerobic processes are important in CWs, and anaerobic kinetics are considered slower than aerobic ones. However, the high kinetics found in CW cores suggest the high potential of CW systems in biodegradation, if oxygen were not the limiting factor.

Although the kinetics of CW cores A and B were different, and higher for core B, the parameters Y_H and Y_{STO} were similar. The reason is because the kinetics depend on the amount of bacterial biomass in the core, while the stoichiometric parameters are independent of the biomass amount.

Table 6 Main kinetics at 20°C and stoichiometric parameters of heterotrophic biomass in CW cores A and B estimated from Figure 35.

Parameter	CW core A		CW core B	
	CW volume unit	**CW surface unit**	**CW volume unit**	**CW surface unit**
Endogenous respiration	1.9 $gO_2\ m^{-3}h^{-1}$	1.1 $gO_2\ m^{-2}h^{-1}$	2.0 $gO_2\ m^{-3}h^{-1}$	1.2 $gO_2\ m^{-2}h^{-1}$
Maximum OUR (with acetate)	4.4 $gO_2\ m^{-3}h^{-1}$	2.7 $gO_2\ m^{-2}h^{-1}$	11.8 $gO_2\ m^{-3}h^{-1}$	7.1 $gO_2\ m^{-2}h^{-1}$
Max COD removal rate (with acetate)	10.7 gCOD $m^{-3}h^{-1}$	6.5 gCOD $m^{-2}h^{-1}$	26.8 gCOD $m^{-3}h^{-1}$	16.2 gCOD $m^{-2}h^{-1}$
Maximum OUR (with wastewater)	5.0 $gO_2\ m^{-3}h^{-1}$	3.0 $gO_2\ m^{-2}h^{-1}$	-	-
Y_H	0.59 mgCOD/mgCOD		0.56 mgCOD/mgCOD	
Y_{STO}	0.75 mgCOD/mgCOD		0.77 mgCOD/mgCOD	

4.3.3 Respirometry of CW cores using municipal wastewater and comparison with activated sludge

Respirograms obtained in a CW core and in activated sludge taken from a conventional WWTP (3.5 $kgTSS/m^3$) during the oxidation of the same raw municipal wastewater are compared in Figure 36 (a) and (b) respectively. In both cases, after wastewater addition (at time t_0) the higher OUR values were due to the oxidation of readily biodegradable substrates, while a gradual decrease of OUR was observed successively, due to the consumption of slowly biodegradable compounds limited by hydrolysis. When the biodegradable substrates were completely oxidised the endogenous respiration (at time t_f) was achieved.

The calculation of the integral between the respirogram and endogenous respiration (ΔO_2) allowed us to quantify the biodegradable COD (COD_B) in wastewater, according to the procedures proposed by Spanjers and Vanrolleghem (1995) and Vanrolleghem et al. (1999) for activated sludge, and adapted to the case of CW cores. In particular, the following expression was used for CW cores:

$$COD_B(\text{mgCOD/L}) = \frac{1}{1-Y_H} \cdot \frac{V_{ww}+V_L}{V_{ww}} \int_{t_0}^{t_f} \left[OUR(t) - OUR_{endogenous}(t) \right] dt \tag{16}$$

Where V_L is the liquid volume in the respirometer (3.2 L in this case) and V_{ww} is the wastewater added (1.5 L in this case). Once Y_H is known, as determined above (0.575 on average), the concentration of COD_B in the wastewater was easily calculated.

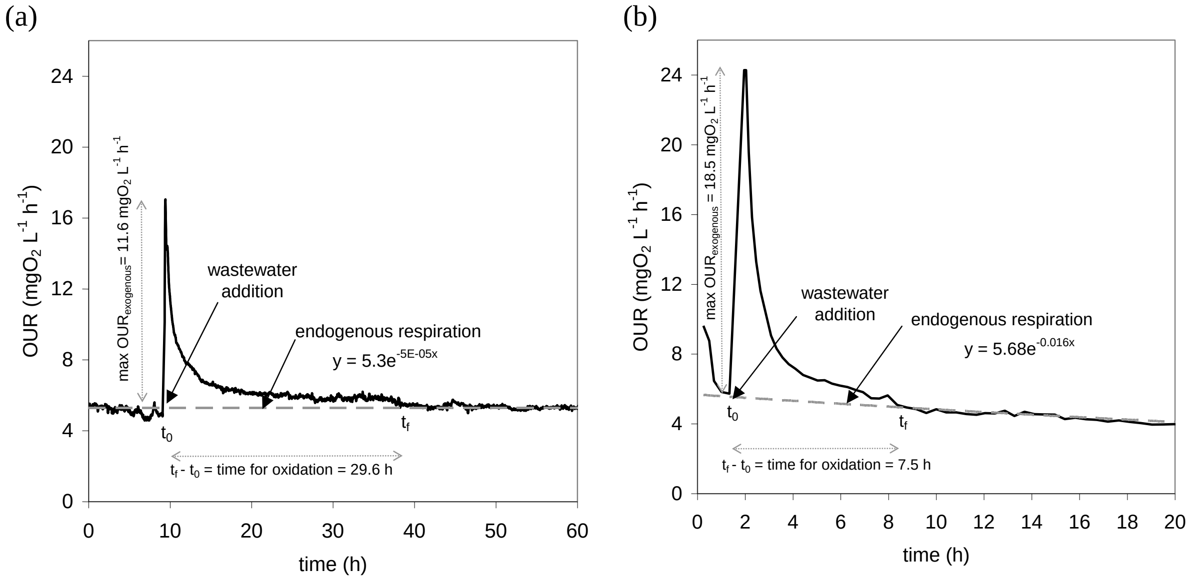


Figure 36 Respirograms for the oxidation of raw municipal wastewater (a) in CW core and (b) in activated sludge.

Comparing the respirograms in Figure 36 some similarities and differences can be highlighted:

- a long time was required for the complete oxidation of biodegradable substrate in the CW core (29.6 h) compared to activated sludge (7.5 h);
- the maximum exogenous OUR obtained for the oxidation of readily biodegradable COD was higher in activated sludge (18.5 mgO_2 L^{-1} h^{-1}) compared to the CW core (11.6 mgO_2 L^{-1} h^{-1});
- Y_H was 0.575 in the CW core and assumed 0.67 in activated sludge;
- COD_B in the wastewater was 182 mgCOD/L when applied in the CW core and 214 mgCOD/L when applied in the activated sludge, corresponding to 39.1% and 46% of total COD in the wastewater respectively.

Comparing the endogenous respiration in Figure 36, it can be observed that in the CW core the decay rate was close to zero, much slower than the value measured for activated sludge in Figure 36 (b). This behaviour of CW cores suggests that the decay of biomass is slow or its variations are negligible within a relatively short period of 60 h.

Chapter 5

Application of off-site liquid respirometric tests for the estimation of kinetic parameters during CW lab core acclimatization

5.1 Introduction

CWs are widely known for their high efficiency in BOD removal after only few days of operation. However, few investigationshave been conducted during the acclimatization phase in order to verify how the estabilishement of the biomass occurs inside the bed (Ramond et al., 2012). Weber and Legge (2011) demonstrated, through the analysis of a CLPP (community-level physiological profiling), that microbial communities reach a steady-state after a period of 75-100 days by the use of (CLPP). Ramond et al. (2012) investigated, through PCR-DGGE (Polymerase chain reaction - denaturing gradient gel electrophoresis), the evolution of the biomass and the time needed to achieve community equilibrium in CW. The authors found that after 89 days of nutrient supplementation of the pilot-scale CW, the microbial biomass community was presenting a highly similar fingerprint in the surface and the deep sediments, and it would take around 100 days to the overall system to reach similar microbial community structures.

CLPP and PCR are tecniques used in CWs for an in-depth analysis of the microbial community structure. Most laboratory studies using CW cores are based on different filter materials or different operational conditions being compared. In the latter case, the use of complex techniques like CLPP and PCR to establish the steady state can be disproportionate to the final scope of the analysis. Nevertheless, when comparing two different operational conditions, it is important to ensure that the system is going to start the operation underthe same biological conditions. In this case, the use of respirometric techniques could be a reliable option to follow the evolution of the biomass and to detect the achievement of steady-state conditions, due to their relative simplicity and limited cost. Liquid respirometry has already been tested with VSSF filter material obtaining consistent and repeatable results (see Chapter 4). However, in Chapter 4 it was used to estimate kinetic and stoichiometric parameter in already acclimatized lab cores and no data were available during the acclimatization phase.

The aim of this Chapter is to investigate the acclimatization period of CW cores by the use of respirometric tests. The evolution of the biomass inside the CW bed and the achievement of steady-state conditions were investigated through analysis of the respirograms and chemical analysis performed during the acclimatization phase.

5.2 Materials and Methods

Four VSSF CW cores at lab-scale were built to evaluate the acclimatization period. The cores presented two different layer configurations, as it is described below starting from the top of the column:

- VSSF-1 and VSSF-2: 0.1 m sand 1/3 mm (p=31%), 0.2 m sand 1/6 mm (p=28%); 0.1 m gravel 7/15 mm (p=30%); 0.2 m gravel 15/30 mm (p=31%)(similar to E-line in the pilot plant – see chapter 3);
- VSSF-3 and VSSF-4: 0.05 m gravel 7/15 mm (p=30%); 0.5 m sand 1/3 mm (p=31%) and 0.05 m gravel 7/15 mm (p=30%)(similar to C-line in the pilot plant – see chapter 3);

CW cores were fed using the same pre-settled municipal wastewater and an average COD load of 28 gCOD m^{-2} d^{-1} (equivalent to 3.86 m^2/PE) was applied in both cores. The feeding was done four times a day (i.e. every 6 hours).

5.2.1 Respirometric tests

The acclimatization of the four lab cores started on the same day (week 0). VSSF CW cores were divided in two groups (group 1: VSSF-1 and VSSF-3; group 2: VSSF-2 and VSSF-4) in order to perform respirometric tests for two cores every week. Table 7 shows the summary of the respirometric tests performed during the acclimatization phase.

Table 7 Summary of the respirometric tests performed during the acclimatization phase.

Respirometric Tests	Cores Tested	Acclimatization period
1st Test	VSSF 1 and VSSF 3	1st week
	VSSF 2 and VSSF 4	2nd week
2nd Test	VSSF 1 and VSSF 3	3rd week
	VSSF 2 and VSSF 4	4th week
3rd Test	VSSF 1 and VSSF 3	5th week
	VSSF 2 and VSSF 4	6th week
4th Test	VSSF 1 and VSSF 3	7th week
	VSSF 2 and VSSF 4	8th week
5th Test	VSSF 1 and VSSF 3	9th week
	VSSF 2 and VSSF 4	10th week

Respirometric tests were done on a weekly basis over a period of 10 weeks. Hence, each core was tested five times. Tests for heterotrophic and autotrophic biomass were done on the same week, with the addition of acetate at the beginning of the week and the ammonia after the core had returned to the endogenous respiration. During all the experimentation period the columns remained under the same feeding conditions and they were tested again two more times: after 120 and 480 days after the beginning of the acclimatization (these are called middle-term respirometric test and long-term respirometric test, respectively).

In order to investigate the behaviour obtained of heterotrophic bacteria in the respirometric test, conventional respirometric tests (Spanjers et al., 1995) were proposed with detached biofilm and with pre-settled municipal wastewater. At the end of the experimentation period, the first 10 centimeters of filter material from the VSSF-cores were gently washed in 1.3 L of water in order to detach the biomass from the gravel. This liquid (water + biomass) was used in a conventional respirometric test for activated sludge (with acetate). Conventional respirometric tests for activated sludge (with acetate) were also done with fresh pre-settled municipal wastewater used to feed the lab cores.

For the respirometric tests done with the addition of NH_4, Ammonia Uptake Rate (AUR) tests were done. AUR test is a test developed to measure the activity of nitrifying bacteria in activated sludge measuring the amount of NH_4, NO_2 and NO_3 through time, with the aim of measuring the maximum specific nitrification rate. In the CW cores, an AUR test was realized with the addition of solution of NH_4 (15 mL of a solution with 2gNH_4/L). Samples were collected from the top using a needle and they were analyzed for NH_4-N, NO_2-N, NO_3-N, pH and temperature. The overall test lasted 8 h until the complete oxidation of the NH_4 added.

5.3 Results and Discussion

COD and NH_4-N removal efficiency are shown in Figure 37. COD removal already reached 60% in the first weeks of operation and this value increase up to 80% after 60 days. NH_4 removal efficiency in the lab cores was around 40% since the first weeks of operation and it increases up to 60% after 2 months. The removal efficiency of NH_4 was related with the biological development inside the core, which was further investigated by respirometric tests. Table 8 shows the result of chemical analysis obtained in the first 5 months of experimentation.

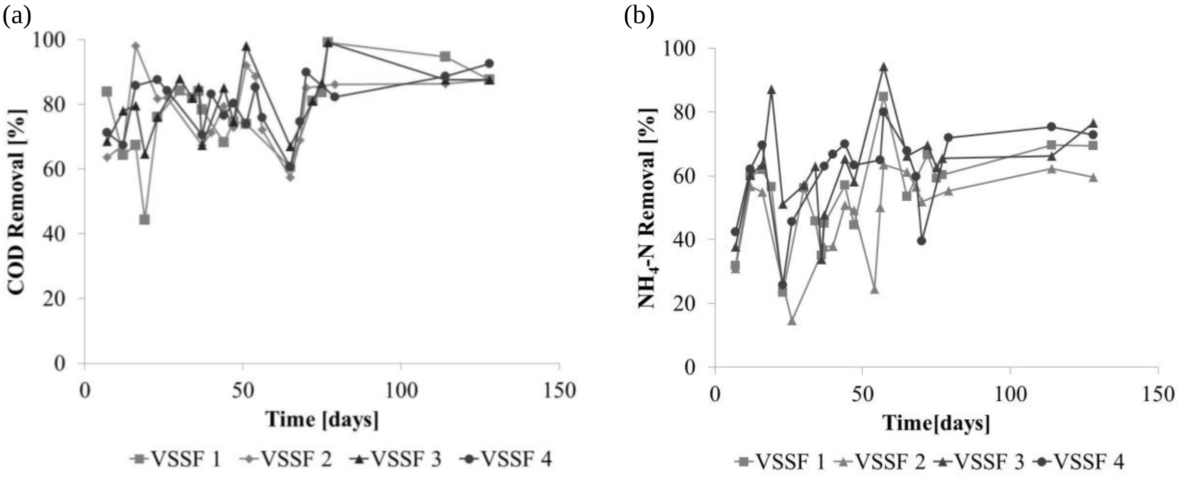


Figure 37 Removal efficiency of VSSF cores during this experimentation: (a) COD (b) NH_4.

Table 8 Results from chemical analysis done during the experimentation.

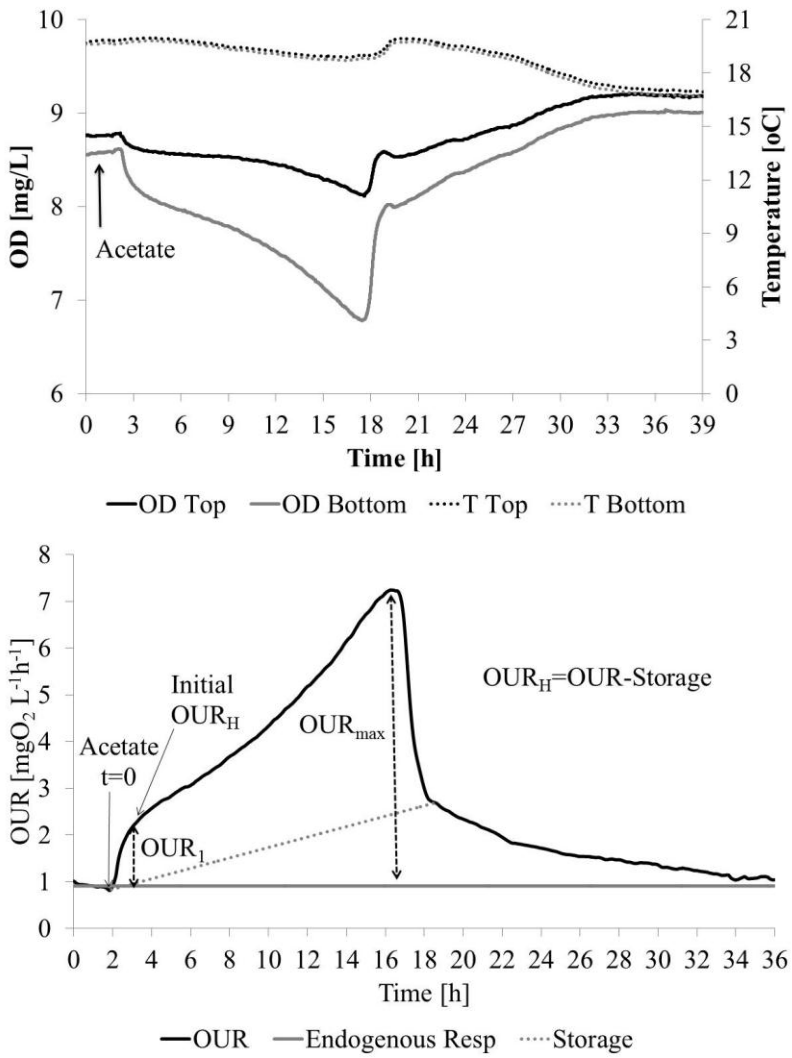
	IN	VSSF 1	VSSF 2	VSSF 3	VSSF 4
COD (mg/L)	224.6±94.9	45.2±26.8	49.2±27.5	40.1±20.4	44.4±25.3
NH_4-N (mg/L)	34±14	15.6±7.2	17.6±6.6	13.4±7.4	14.2±7.6
NO_2^--N (mg/L)	0.3±0.2	1.6±1.1	1.3±0.7	1.4±1.0	1.4±1.0
NO_3^--N (mg/L)	3.6±1.0	17.7±7.8	16.1±6.4	19.7±8.3	19.4±7.5

5.3.1 Respirometric tests during acclimatization – acetate removal

Alongwith the chemical analysis that was conducted to verify the pollutant removal performances, respirometric tests were carried out to investigate the evolution of the biomass until it reaches the steady-state conditions. Respirometric tests conducted in the lab cores during the acclimatization show a different behaviour from the behaviour observed in already colonized lab cores (see Chapter 4, Figure 35).

Figure 38 (a) shows an example the DO concentration in the VSSF 1 of the probe placed above (probe called Top) and the probe located on the bottom (probe called Bottom).

Figure 38 (b) shows the behaviour of the OUR that was typical during the first 10 weeks of respirometric tests. In Figure 38 (b) it is also possible to observe the endogenous respiration line and the effect caused by the acetate addition.



(a)

(b)

Figure 38 Respirogram of acetate consumption for VSSF 1 (a) DO concentration of the probes places in the Top and Bottom and (b) OUR response at the 2nd Test.

OUR behaviour in the cores under acclimatization was divided in OUR_1 and OUR_{max} in order to allow the elaboration of the data from the respirometric test (Figure 38 b). The OUR values do not increase rapidly to the maximum values after the addition of acetate. They increase until a first step called OUR_1 and then increase smoothly until they reach the OUR_{max}. OUR_1 evolution during the acclimatization period can be observed in
Figure 39, where initial values were lower than the values obtained in the last tests, starting from 1 $mgO_2L^{-1}h^{-1}$to 2.5$mgO_2L^{-1}h^{-1}$, except for the core VSSF 3.

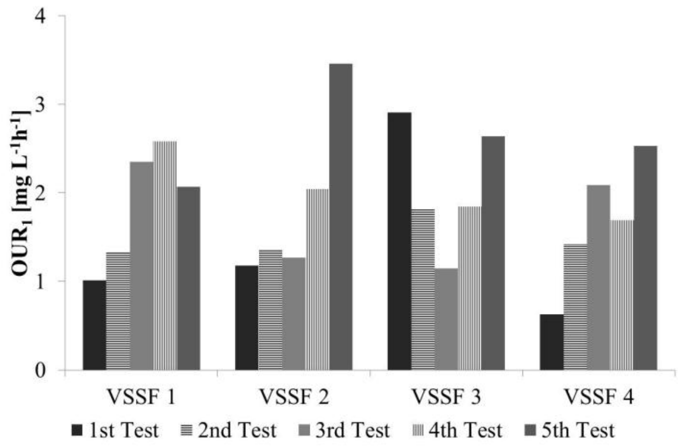


Figure 39 OUR_1 values obtained during the acclimatization phase.

The expected behaviour can be seen in Figure 40, which shows the long-term respirometric tests of VSSF 1 and VSSF 3 at 480 days. However, this behaviour had already been reached 120 days after the start up of the system. After 120 days, the system can be assumed to be working under steady state conditions, showing that respirometric tests can be used as a non expensive tool to estimate the attainment of steady state conditions of CW filter material.

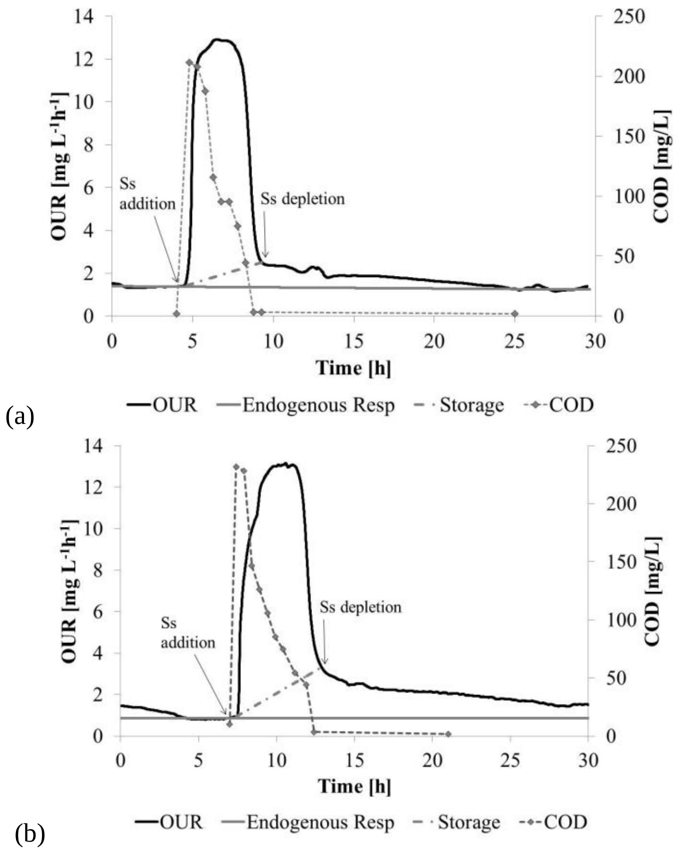


Figure 40 Long term respirometric test (480 days) and COD analysis of the liquid phase done during the test for (a) VSSF 1 (b) VSSF 3.

In Figure 40, it is also possible to observe that internal storage products were gradually formed (X_{STO}) until all the external S_S was consumed. When S_S was completely removed from the bulk liquid, OUR dropped from the maximum level to a level above the endogenous respiration. OUR decreased slowly to reach the values of endogenous respiration maintained prior to substrate addition.

Even though respirometric tests could be used to verify the achievement of steady state conditions, the shape of the respirograms during the acclimatization phase was not explained and further studies are needed to investigate it in greater detail.

The amount of biomass present in the VSSF cores (X_0) during the acclimatization period was estimated using the method of Wentzel et al. (1998), already used by Andreottola et al. (2002), as follows:

$$X_0 = \frac{e^{(intercept)}}{\frac{1-Y_{STO}}{Y_{STO}}.(slope+b)} \tag{17}$$

where Y_{STO} is the storage yield coefficient for heterotrophic biomass obtained in the respirogram, b is the specific decay rate (values between 0.1 and 0.6 d^{-1}) and the intercept is obtained from the linear interpolation of ln(OUR) vs. time inthe exponential growth between OUR_I and OUR_{max} minus storage products (see Figure 38). Values of X_0 for the VSSF are shown in Figure 41. Besides the results obtained with the VSSF 3 core, the cores presented an increasing amount of the biomass.

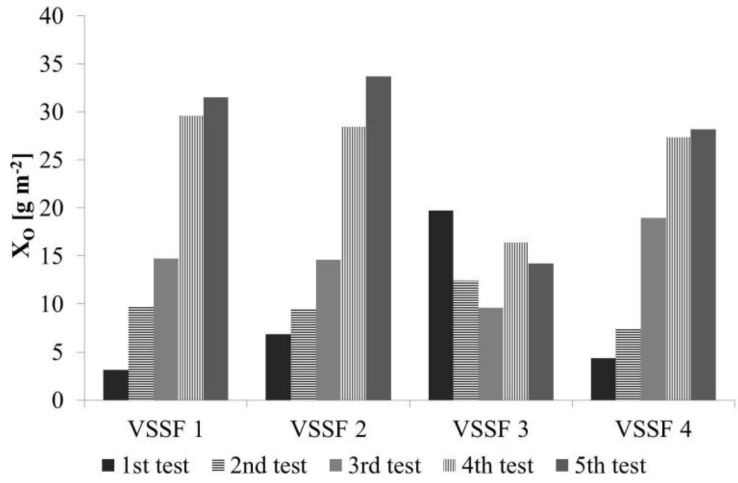


Figure 41 (a) X_0 values for the VSSF during the acclimatization period.

The X_0 estimation was also calculated for the middle and long-term respirometric test. In the case of these respirometric tests, the values of OUR_1 were difficult to detect. This occurs due to the increasing importance of the biofilm in the biomass present in the core, that reduces the exponential growth of the OUR. OUR values grow quickly and directly to the OUR_{max} and plateau values (Figure 40). The correlation between X_0 values and values of COD retained inside the core(that was considered as being 60% of the total COD) during the evaluated period was studied. A linear relation was found between the biodegradable COD (60% of the total COD) that was trapped in the bed and the amount of biomass computed by respirograms (Figure 42).

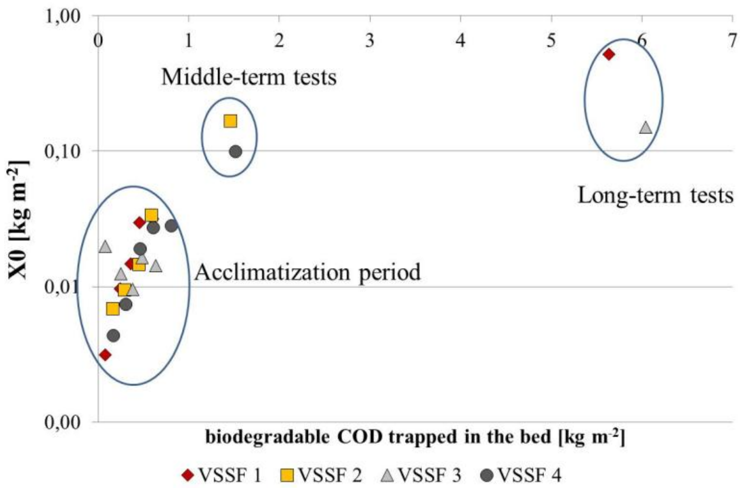


Figure 42 Correlation between the biodegradable trapped COD and X_0 values obtained from the respirograms

Table 9 shows the average results for all the VSSF cores obtained from the respirometric tests, during the acclimatization period, middle and long-term respirometric tests.

Table 9 Average values of kinetic parameters for heterotrophic biomass for all VSSF cores.

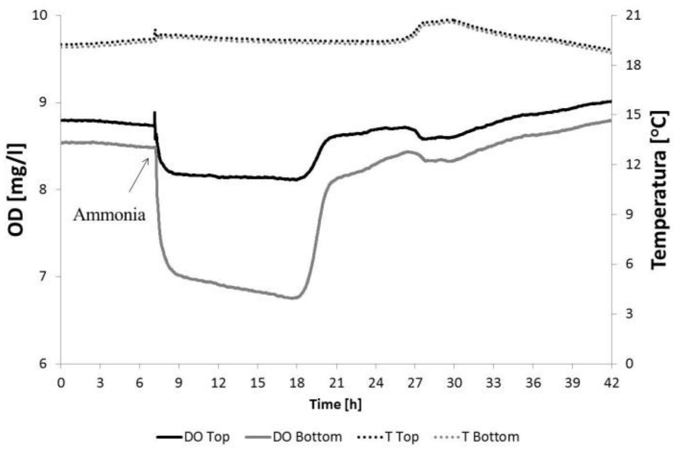
Parameter	**Acclimatization period**		**Middle term test**		**Long term test**	
	CW liquid unit ($mgO_2\ L^{-1}\ h^{-1}$)	CW surface unit ($gO_2\ m^{-2}d^{-1}$)– h^{-1}	CW liquid unit ($mgO_2\ L^{-1}h^{-1}$)	CW surface unit ($gO_2\ m^{-2}\ d^{-1}$) – h^{-1}	CW liquid unit ($mgO_2\ L^{-1}\ h^{-1}$)	CW surface unit ($gO_2\ m^{-2}d^{-1}$) – h^{-1}
Endogenous respiration	1.17	8.22 / 0.34	2.61	18.38/ 0.77	1.08	13.04/ 0.54
OUR_{max}	6.46	45.47 / 1.89	10.06	70.83/ 2.95	11.96	84.17/ 3.51
$v_{COD,max}$	16.70	117.56 / 4.90	23.25	163.69/ 6.82	34.06	239.76/ 9.99
X_0 (g/m^{-2})	8.5-26.9 g m^{-2} (1^{st}-5^{th} test)		133.0 gm^{-2}		335.3 gm^{-2}	
Y_H(mgCOD/mgCOD)	0.61 mgCOD/mgCOD		0.57 mgCOD/mgCOD		0.64 mgCOD/mgCOD	
Y_{STO}(mgCOD/mgCOD)	0.74 mgCOD/mgCOD		0.85 mgCOD/mgCOD		0.93 mgCOD/mgCOD	

The values obtained in this research can be compared with values obtained in Ortigara et al. (2011) (Chapter 4) with a good agreement between the results of those acclimatized cores and the middle-term tests, except for the values of the endogenous respiration and Y_{sto}.

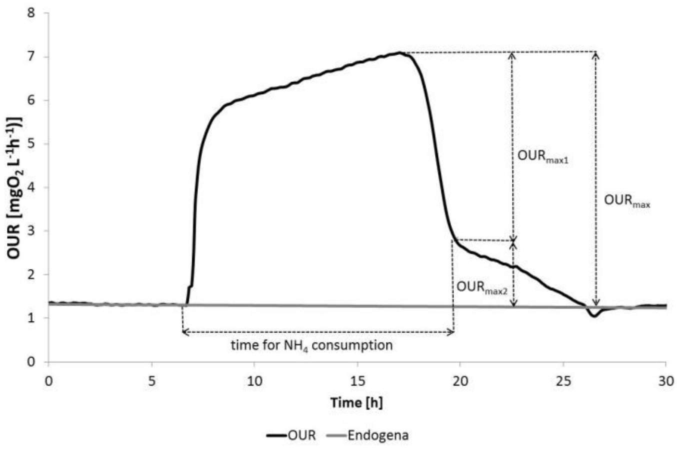
Storage is a complex phenomenon which involves the accumulation of readily biodegradable COD as internal storage products that are consumed after the COD depletion in the liquid phase. Even if the Y_{sto} values in this research were higher than those obtained in Chapter 4 (Table 6), the storage can be observed inFigure 40, where the concentration of acetate in the liquid phase was around zero but the OUR values were still above the endogenous respiration line. The maximum COD removal rates obtained were around 18-22mgO_2 $L^{-1}h^{-1}$for the chemical analysis against values of 16-19 mgO_2 $L^{-1}h^{-1}$ in the respirometric test for the same test with different cores. Andreottola et al. (2007) had also applied liquid respirometry to CW lab cores but the results presented for $v_{COD,max}$were lower than those obtained in this research (5.8 mgO_2 L^{-1} h^{-1}). However, the values of the endogenous respiration are higher (6-10 mgO_2 L^{-1} h^{-1}) and such difference can be due to the use of different materials and different feeding conditions.

5.3.2 Respirometric tests durin acclimatization – NH_4 removal

After reaching the endogenous phase, an ammonia solution of (30 mL of 2g/L) was added in the core. The behaviour of NH_4-N consumption in the VSSF cores did not show any evolution in time as opposed to what observed for the tests with acetate. The behaviour observed in Figure 43 has the same pattern from the second week of respirometric tests (i.e. third week after the acclimatization had started). It can also be observed in Figure 44, where the values of maximum ammonia removal rate did not show an increasing pattern during 10 weeks. Thus, for the ammonia removal no middle and long term tests were done. Instead AUR tests were done for comparison with the results from the respirometric tests (Figure 45).



(a)



(b)

Figure 43 Respirogram of ammonia consumption of VSSF 2 (a) DO concentration of the probes places in the Top and Bottom probe and (b) typical behavior of the OUR during 10 weeks of respirometric tests.

From the respirogram, the $v_{N,max}$ was calculated and its values are shown in Figure 44. Observing Figure 43, it is also possible to verify the nitrite accumulation characterized by the slope above the endogenous respiration after the ammonia consumption (signed by the end of the "time for NH_4 consumption"). In this case, the consumption rate of Ammonia Oxidizing Bacteria (AOB – $v_{N,AOB}$) and Nitrite Oxidizing Bacteria (NOB – $v_{N,NOB}$) can be calculated using the equation 12 and 13.

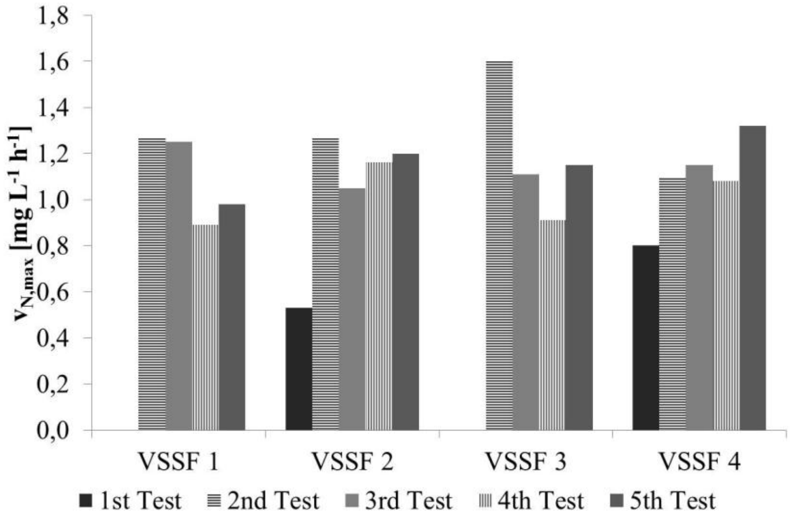


Figure 44Maximum ammonia removal rate ($v_{N,max}$) for the VSSF during the acclimatization period.

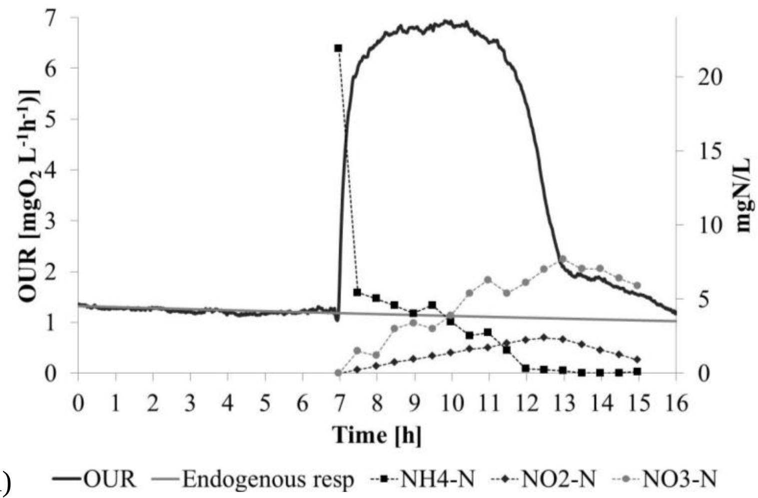
Kinetic parameters obtained from the respirograms of NH_4 consumption are shown in Table 10.

Table 10 Average values of kinetic parameters for autotrophic biomass for all VSSF cores obtained by liquid respirometry

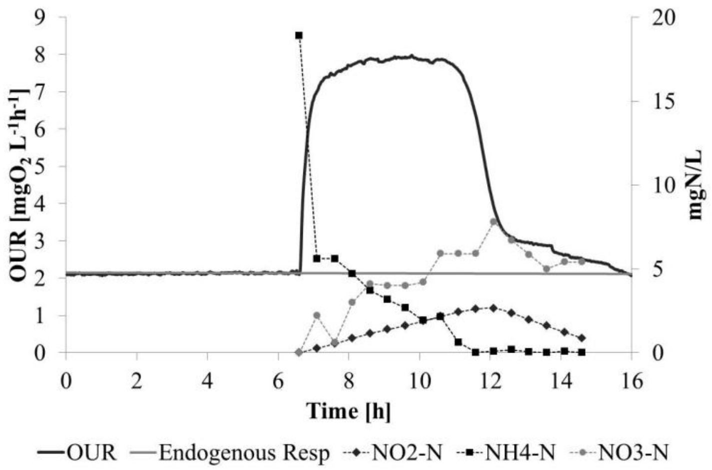
Parameter	**Acclimatization period**	
	CW liquid unit ($mgO_2\ L^{-1}\ h^{-1}$)	CW surface unit ($gO_2\ m^{-2}d^{-1}$) –h^{-1}
Endogenous respiration	1.48	10.38/ 0.43
$OUR_{max,N}$	4.98	35.05/ 1.46
$v_{N,max}$	1.10	7.75/ 0.32
$v_{N,AOB}$	1.13	7.99/ 0.33
$v_{N,NOB}$	1.29	9.05/ 0.38

AUR tests were done by adding half of an ammonia solution that is usually added in respirometric tests (concentration inside the core around 10 $mgNH_4/L$ instead of 20 $mgNH_4/L$) in order to reduce the duration of the test to 8 hours. Ammonia consumption and NO_x formation can be observed in Figure 45. The maximum specific nitrification rate (v_{AUR}) was calculated considering the consumption of NH_4-N rather than the production of nitrite and nitrate because denitrification might have taken place inside the biomass. The values obtained for v_{AUR}were 1.14 $mgO_2\ L^{-1}\ h^{-1}$ whereas the $v_{N,\ max}$ calculated from the respirogram were 1.08 $mgO_2\ L^{-1}\ h^{-1}$ considering the same tests.

As opposed to the results obtained for the COD consumption, the $v_{N,\ max}$ values obtained in this research were similar to the values obtained by Andreottola et al. (2007) (4.2 $mgO_2\ L^{-1}\ h^{-1}$) and lower than those obtained by and Morvannou et al. (2011) (32-50 $mgO_2\ L^{-1}\ h^{-1}$). It is important to highlight that the tests by Morvannou et al. (2011) were performed under conditions different from those considered in this research, which make a comparison of the results difficult. The main difference is in the phase in which oxygen consumption took place: air in Morvannou's test and in liquid in our study.



(a)



(b)

Figure 45 Respirometric test and AUR test for the (a)VSSF 2 (b)VSSF 4.

Liquid respirometric testsproved to be a reliable tool for the estimation of kinetic and stoichiometric parameters of CW material at the lab scale. Moreover, they could also be used to determine wheter a filter material is operating under steady-state conditions. Knowing that the biomass presentin a CW filter material operates under steady state conditions it is important to start experiments that aim to compare different operational conditions. For example, before starting any experiment aimedat comparingthe influence of a certain contaminantin one out of two systems, it is important to know that the biomass present in both has the same kinetic and stoichiometric parameters.Some limitations were found on the use of liquid respirometric tests. The main question that can arise iswhether the use of a saturated test, such as liquid respirometry, can be used to evaluate the unsaturated biomass present in the VSSF. Further investigations are needed to compare respirometric tests under unsaturated conditions (as the tests performed by Morvannou et al., 2010) and liquid respirometry, for the same VSSF material and for similar test conditions in order to establish the conditions under which one is preferable to the other.

In order not to change the conditions in which the filter material is operating in the field, there is also the possibility of using the off-gas technique to assess the kinetic and stoichiometric parameter conditions whitout the need to transport the material to the lab. Chapter 6 will discuss the preliminary results of the application of the off- gas technique for CW filter material. The off-gas technique is widely used for activated sludge and in this research a preliminary investigation was done at the lab scale to evaluate the reliability of the test in CWs.

Chapter 6

Preliminary applications of the off-gas technique in aerated CWs for obtaining kinetic parameters

6.1 Introduction

Addressing the problem of large land area requirement is a key step towards the application of CWs in areas where the availability of land is limited, due to environmental as well as socio-economic conditions. Some of the biological reactions occurring in CW are oxygen demanding, mainly in the case of VSSF CW. The oxygen required can be supplied naturally during the feeding in non-saturated beds, but large land areas are necessary to reach higher removal efficiency. The aeration of CWs could be a viable strategy to increase the efficiency per unit area, because it enables a higher amount of wastewater to be treated within the same surface (see Chapter 11).The amount of oxygen required in both cases to degrade the organic matter could be assessed by respirometric tests.

Respirometric tests conducted with material from VSSF CWs need such material to be transferred to a lab for analysis. During the test proposed by Andreottola et al. (2007) and applied in Chapters 4 and 5, the material passes from an unsaturated condition (in the field) to a saturated one (during the respirometric test), which is not desirable in case of unsaturated CWs because tests would be performed under conditions different from those of the field. Despite this drawback, the technique allows the estimation of stoichiometric and kinetic parameters. OUR measurement under unsaturated conditions has been performed by Morvannou et al. (2011) adapting a methodology used for computing the Dynamic Respiration Index (DRI) of urban waste and made it applicable to constructed wetland materials. The authors used bulking material during the test in order to maintain the humidity of the material, modifying the initial conditions that the material has on the field.

The off-gas analysis is a reliable method to measure the Oxygen Transfer Efficiency (OTE) and obtain precise data for the design of aeration systems on activated sludge (Redmon et al., 1983). In particular, this methodology would allow onsite oxygen consumption measurements in CWs. Estimating the maximum oxygen consumption on aerated CW plants can give an indication on the estimation of oxygen demand of these systemsand on the kinetic parameters of their biomass. This chapter presents some preliminary results from an application of the off-gas technique to aerated CWs at lab scale.

6.1.1 Basics of off-gas technique

The off-gas technique was developed by Redmon et al. (1983) to measure the oxygen transfer capacity of different aeration systems in the liquid phase and therefore gain insights into the efficiency of such systems. By increasing the efficiency of the aeration systems, it would be possible to get a better trade-off between energy consumption and the removal of pollutants.

The oxygen capability of a submerged air device may be estimated by means of a gas phase mass balance over aerated volume (Redmon et al., 1983). Since its first applications, some assumptions were made in order to simplify the analysis, such as: inert gases are conservative (nitrogen is included in this category), the air flow rate to the basin is constant during the test, etc. The gas mass balance over the liquid volume, described by Redmon et al (1983), may be written as follows:

$$V\rho' \frac{dC}{dt} = \rho q_i Y_R - \rho q_o Y_{og} = K_L a\left(C^* - C\right)V \tag{18}$$

Where:

- ρ': density of liquid at temperature and pressure at which gas flow is expressed (M/L^3),
- ρ: density of oxygen at temperature and pressure at which gas flow is expressed (M/L^3),
- q_i,q_o: total gas volume flow rates of inlet and outlet gases (L_3/t),
- Y_R, Y_{og}: mole fractions (or volumetric fractions) of oxygen gas in inlet and outlet gases,
- K_La = the oxygen mass transfer coefficient (1/t)
- C^*: saturation concentration of oxygen in test liquid in equilibrium with exit gas (M/L^3),
- C: equilibrium concentration of oxygen in test liquid (M/L^3),
- *V*: test cell volume (L^3).

Considering that the volume of CO_2, produced to the gas stream is just equals that of oxygen absorbed, and that nitrogen is conservative, K_La can be calculated as follows.

$$K_L a = \frac{\rho}{V} q \frac{(Y_R - Y_{og})}{(C^* - C)} \tag{19}$$

In the equation 18 $q=q_i=q_o$. Measurements are made of Y_R and Y_{og} (the inlet and outlet mole fractions of oxygen), q (the total gas flow rate) and C, and an estimation must be made of C^* under test conditions. The oxygen transfer can also be reported by the calculation of the OTE (Oxygen Transfer Efficiency, expressed as a fraction) with no estimation of C^* and assuming that CO_2 evolution is equivalent to oxygen absorption:

$$\mathrm{OTE} = \frac{Y_R - Y_{og}}{Y_R} \tag{20}$$

Where:

$$Y_R = 0.2095(1 - Y_{W(R)}) \tag{21}$$

$$Y_{ogR} = \left(\frac{MV_{(og)}}{MV_{(R)}}\right) Y_R \tag{22}$$

$Y_{W(og)}$, $Y_{W(R)}$: mole fraction of water vapor in the inlet gas (R) or off-gas (og).
$MV_{(og)}$, $MV_{(R)}$: millivolts output readings of the partial pressure of oxygen.

According to Leu et al (2010), the off-gas flow rate or flux and total air flow can also be determined using air flow rate captured by the hood, hood area and tank surface area. The Oxygen Transfer Rate (OTR, KgO_2h^{-1}) is the product of OTE and off-gas flow rate. The OTR is calculated as follows:

$$OTR = Q_g^{Hood} \frac{O_2^{IN} - O_2^{OUT}}{O_2^{IN}} x \frac{Area^{Tank}}{Area^{Hood}} \tag{23}$$

According to the experiment carried out by Harris et al. (1996) in biofilters, the off-gas analysis shows an increase of OTE when the air flow decreases and the volumetric loading rate increases. The OTE influences the oxygenation capacity (OC - amount the oxygen transferred per unit of time- kgO_2/h): the OC quickly rises from the lowest superficial air velocity used up to 7m/h, and after that the OC increase is gradual.

Above these air flow values the authors believe that oxygen near the biofilm is partially depleted causing a better use of the available oxygen (Harris et al., 1996). OTE is also increased during periods with high organic load, because biological activity is higher and also associated oxygen demand (Lee and Stensel 1986 apud Harris et al., 1996).

6.1.2 KLa and OTE determination

Chemical and physical methods can be used for K_La determination. Chemical methods were the first ones to become widely accepted for oxygen transfer estimation, but physical methods are the most used ones. Physical methods are based on the measurement of dissolved oxygen concentration in the liquid during the absorption or desorption of oxygen in the solution (Garcia-Ochoa and Gomez, 2009).

The dynamic method is based on the measurement of dissolved oxygen concentration in the medium by absorption or desorption of oxygen. The dynamic technique of absorption involves the elimination of oxygen in the liquid phase, for example by means of bubbling nitrogen or by the addition of sodium sulfite, until the oxygen concentration is equal to zero. After the depletion, aeration is turned on again and the oxygen concentration is measured by an oxygen probe. The increase in dissolved oxygen concentration over time can be used to calculate kLa (Garcia-Ochoa and Gomez, 2009). In the absence of biomass, when biochemical reactions do not take place, OUR is equal to zero. In this case, the basic equation of oxygen transfer is:

$$dc/dt = K_L a(C_S - C) \qquad (24)$$

Figure 46 shows the expected conditions in the gas and liquid phase when this kind of K_La estimation is performed. It is possible to observe that the oxygen is depleted after the Na_2SO_3 addition and until Na_2SO_3 has been completely consumed, the values obtained are stable. The oxygen values obtained during the re-aeration phase are used for the estimation of K_La.

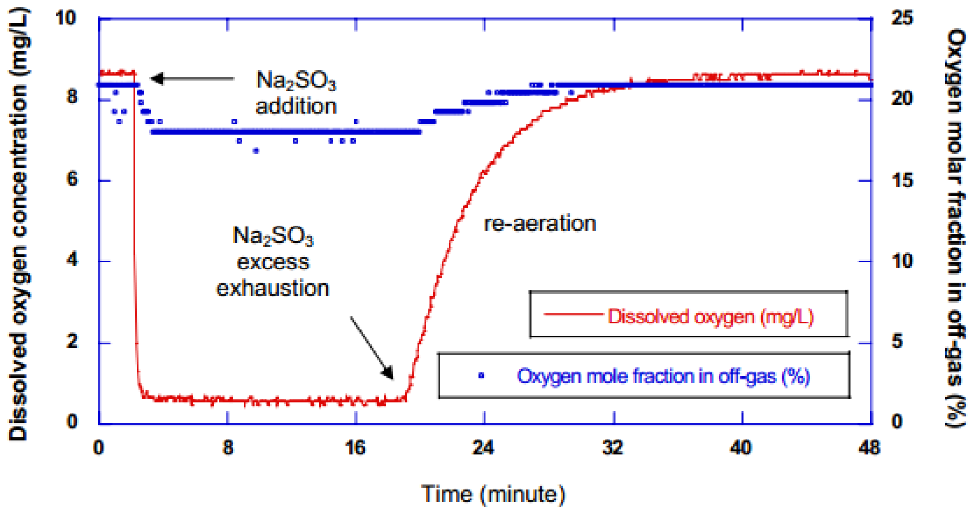


Figure 46 DO concentration and Oxygen mole fraction in the off-gas during a clean water re-aeration test (from Stenstrom et al., 2006).

Nonlinear regression is used to estimate K_La and C_S. Values are adjusted to standard temperatures by using the Arrhenius equation. The Standard Oxygen Transfer Rate (SOTR in $gO_2\ h^{-1}$) is obtained as the average of the products of the adjusted K_La values (h^{-1}), which corresponds to the adjusted C_S (in g/m^3) value and the tank volume (V in m^3).

$$SOTR = K_La \cdot (C_S) \cdot V \qquad (25)$$

Oxygen Transfer Efficiency (OTE) is the fraction of oxygen in an injected air stream dissolved into the liquid under given conditions. The Standard Oxygen Transfer Efficiency (SOTE) is the oxygen transfer efficiency under standard conditions of temperature and pressure. It can be computed through the following equation:

$$SOTE = \frac{SOTR}{W_{O2}} \qquad (26)$$

W_{O2} is the mass flow rate in the air stream (kg/h). It can be calculated by multiplying the air flow rate (m^3/h) by air density (≈ 1250 g/m^3) and fraction weight/weight for oxygen in the atmospheric air (0,232).

The objective of this chapter was to determine the K_La, through which the capacity of YSI oxygen probe in reading/ measuring oxygen consumption in CW lab cores might be estimated and if this tool can be used for measuring kinetic and stoichiometric parameters in CW.

6.2 Materials and Methods

6.2.1 Lab cores

The acclimatized cores (height = 0.60 m; diameter = 0.125 m, volume = 7.4 L) VSSF 2 and VSSF 4 (showed in chapter 3) were used in this research. The filling media has the following characteristics from the bottom to the top of the column:

- VSSF-2: 0.1 m of sand with size of 1-3 mm (p=31%), 0.2 m of sand with size 1-6 mm (p=28%); 0.1 m of gravel with size of 7-15 mm (p=30%); 0.2 m of gravel with size of 15/30 mm (p=31%) (E-line)*;*
- VSSF-4: 0.05 m of gravel with size of 7-15 mm (p=30%); 0.5 m of sand with size of 1/3 mm (p=31%) and 0.05 m of gravel with size of 7/15 mm (p=30%) (C-line).

6.2.2 Off-gas apparatus of Lab cores

The apparatus used in the off-gas technique is shown in Figure 47.

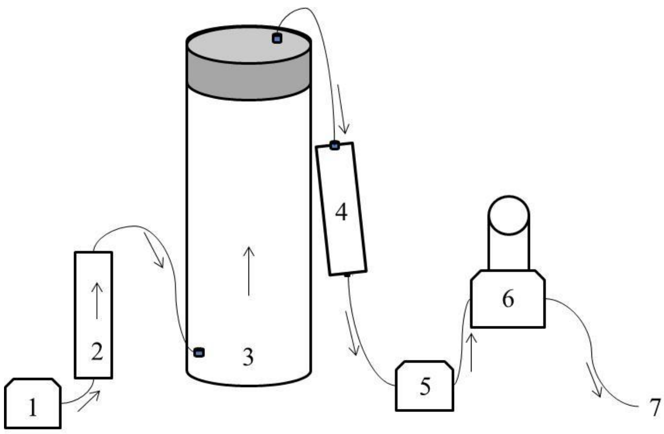


Figure 47 Scheme of the off-gas used at lab scale. (1)Air pump; (2) Air Flowmeter; (3)Pump CW core sample; (4) Dehumidification with silica; (5) Micropump; (6) WTW O2 Probe (7) Air oulet.

The system is composed by an air pump (n° 3 in Figure 47) that introduces air inside the CW core (n° 3 in Figure 47). The air flow is measured in a Flowmeter Rota with a capacity for 40-740 L/h of air (n° 2 in Figure 47). After passing through the CW core, a micro pump (0.75L/min - n° 5 in Figure 47) captures the air from the lab core and transfers it to a silica container (for dehumidification - n° 4 in Figure 47) and subsequently introduces the air into the cell where the oxygen is measured (SondaWTW - n° 6 in Figure 47). After the reading the air is released to the atmosphere.

6.2.3 Test with OD probes

The OD probe used in this research (n° 6 in Figure 47) is a WTW model Trioxomatic 701-7 (WTW), provided with a special 25 micron thick membrane to maximize resolution and response time, and an NTC probe for temperature measurement (range: 0.0 ÷ 200.0 %, response time lower than 30 seconds, resolution below 0.1 %, temperature range from -5°C to +50°C). For oxygen values above 20%, results were given with a 0.1% interval, while for values below 20% results were given with a 0.01% interval (precision ±1% of measured value). There are two probes with the same characteristics: Probe 1 and Probe 2. Most of the experiments were performed with Probe 1.Whenever Probe 2 was used, it is specified in the text.

Probe 1 was connected to a datalogger (Pico green) that supplies a signal in mA (00.000). This signal was converted to % for oxygen and ºC for temperature, by plotting the result given by the datalogger in mA (x) and the result given in % by the probe (y). From this result a calibration line was drawn, and the equation given by the linear equation was used to convert the results in % and ºC. Data obtained from the calibration line had a precision of three decimals. Figure 48 shows the calibration line obtained and the equation used to transform the data.

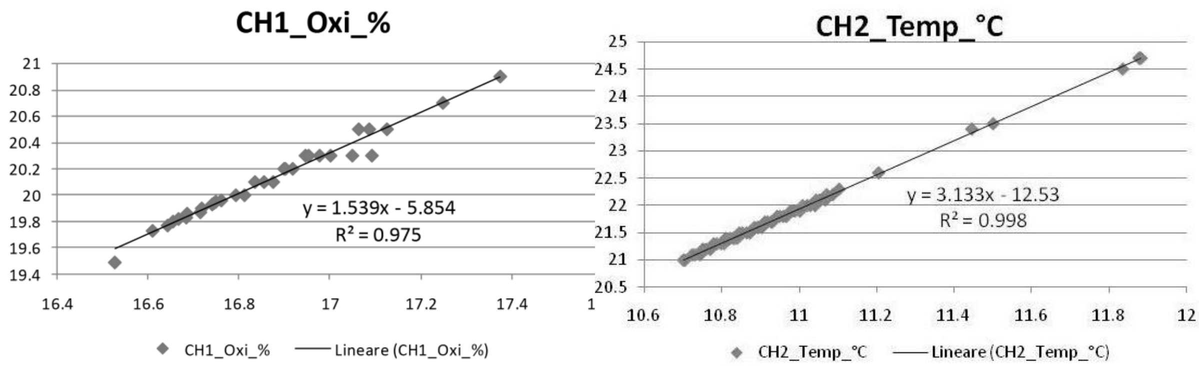


Figure 48 Calibration line obtained from the data logger and probe readings.

In order to verify the reliability of the O_2 probes two tests were done:

- Test 1: The Probe was calibrated and left in the lab in a vertical position for about 40 hours, reading the O_2 concentration in the air. Initial slope: 1.04. Final slope: 1.03.
- Test 2: The Probe was put inside the oxygen measurement circuit and data were collected for about 80 hours. A CW core without biomass was used and the aeration was turned on at the bottom of the core in order to simulate the normal conditions proposed in the test.

6.2.4 K_La and OTE determination in lab cores

Tests 3, 4 and 5 were conducted using a new CW core with a VSSF 4 configuration in order to determine the k_La (the filter material was new and washed to performe the test). Oxygen measurements were performed with two different probes: Probe 1 (the same O_2 oxygen probe used so far) was used in Tests 3 and 5 and Probe 2 in Test 4.

Measurements in the air and in the liquid phase were done before the test in order to assess probes' stability. During the test values were collected once a minute in the case of air measurement and once every 30 seconds in the case of water measurements. Air measurements were done after dehumidification and the CO_2 stripping.

A solution with 5 g/L of NaSO (3.6 liters) was put into the core from the bottom. Recirculation was done for about 10 minutes in order to ensure that all the pores had been filled with the solution. After 10 minutes, recirculation was stopped. Initial concentrations of OD, inside the core and before aeration, were around 0.10 mg/L. The aeration was turned on (air tube was on the bottom of the column with a flow rate of 40 nL/h) and the re-aeration phase started after 6 hours of aeration (when the reaction with NaSO has finished).

6.2.5 Application of the Off-gas technique

The off-gas technique was applied for saturated cores, with the liquid respirometric test running in parallel. Tests were given numbers 6, 7 and 8 and were all of then the response of off-gas technique and liquid respirometer to the addition of a spike of 60 mL of acetate solution (10g/L).

VSSF 2 and 4 were tested in the liquid respirometer (Andreottola et al., 2007; Ortigara et al., 2010) and with the off-gas test. The respirometric test implies that aeration is performed on top of the core, the liquid is recirculated from the bottom to the top and two oxygen dissolved probes measure the amount of oxygen consumed inside the core (for a thorough explanation of this procedure refer to Chapter 2). The inflow oxygen was measured before the column using an air flow meter (40 L/h and 100L/h were used). The off-gas apparatus (Figure 47) was positioned on top of the lab core. Tests lasted about 20 hours. The core is filled with water (3.6 L), the recirculation starts along with off-gas measurements. Acetate (Volume 60 mL of a solution with concentration of 10g/L) was added after the core reach endogenous conditions and the measurements were done until it reaches the endogenous phase again.

The off-gas was estimated by considering the difference between inlet and outlet oxygen concentration: O_2 inlet concentration in the airflow is assumed to be 20.9% and the outlet concentration is measured by a probe (% of O_2 concentration in the air). The system is assumed to be working at atmospheric pressure (1atm). To transform the oxygen concentration from % to mg/h, the air was considered an ideal gas (Gay Lussac law):

$$\rho \cdot V = n \cdot R \cdot T \tag{27}$$

$$n_{O2} = \cdot \frac{\rho \cdot V \cdot \%O_2}{R \cdot T} \cdot 32 \cdot 1000 \tag{28}$$

Where nO_2 is the oxygen concentration (mg/h);ρ is the pressure (in the test a 1 atm pressure was assumed);V is the air flow rate from the aeration system (L/h);$\%O_2$ is the amount of oxygen estimated in the air (inlet) or measured (outlet);R is a constant (0.0821 L atm $K^{-1}mol^{-1}$);T is the temperature in °K (the probe reads the temperature in the cell in °C, the values were converted in Kelvin).

The difference between the inlet and outlet concentration (ΔO_2) was considered as the oxygen consumed by the biomass inside the core. This value was used in the estimation of kinetic parameters.

6.3 Results and Discussion

6.3.1 Test of the OD probes

The oxygen measurement inside the cell was performed under two different conditions: with dehumidification and without dehumidification. Figure 49 shows the results obtained with the O_2 probe in the air (Tests 1 and 2). It is possible to observe a high influence of the temperature and fluctuations in the oxygen measurement. The influence might be due to probe fluctuations and/or humidity of the air content. Figure 50 shows the readings obtained inside the oxygen measurement cells after the humidity stripping using a silica tube (n° 4 in Figure 47).

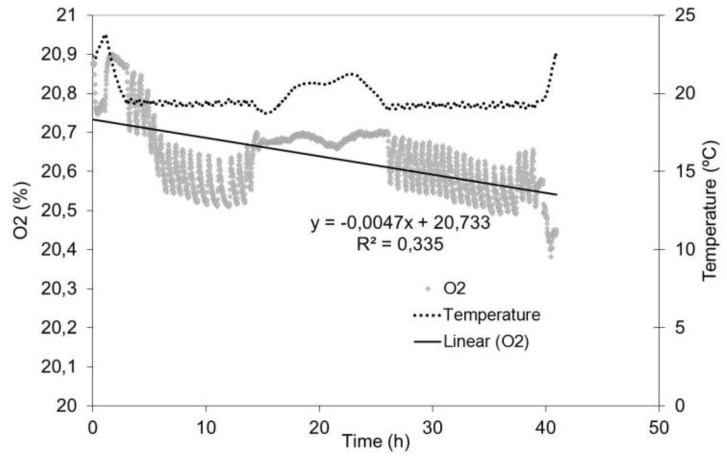


Figure 49 O_2 measurements in air (Test 1).

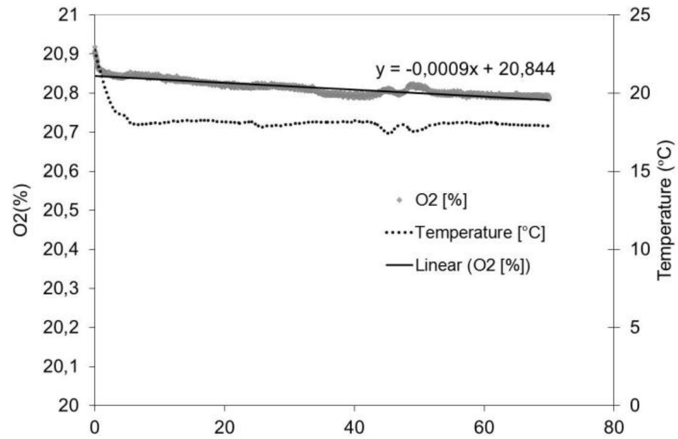


Figure 50 O_2 and temperature measurement inside the oxygen measurement circuit after the humidity stripping using a silica tube (Test 2).

The importance of humidity stripping before the oxygen measurement (Figure 50) was confirmed and the following tests were done using the silica tube.

6.3.2 K_La and OTE determination in lab cores

K_La was determined for a new CW core in order to verify the response of the O_2 probe during the reaeration phase when the oxygen concentration in the liquid phase is zero. The determination of K_La in the liquid phase was done with an unsteady state aeration test, in which the addition of sodium sulfite (without the addition of cobalt) makes the oxygen concentration in the water drop to values around zero. When values around zero are reached the aeration starts.

Oxygen measurements were done with two different probes: Probe 1 (the same O_2 oxygen probe used so far) was used in Tests 3 and 5 and probe 2 in Test 4. Figure 51 shows the results obtained in the Tests 1 and 2.

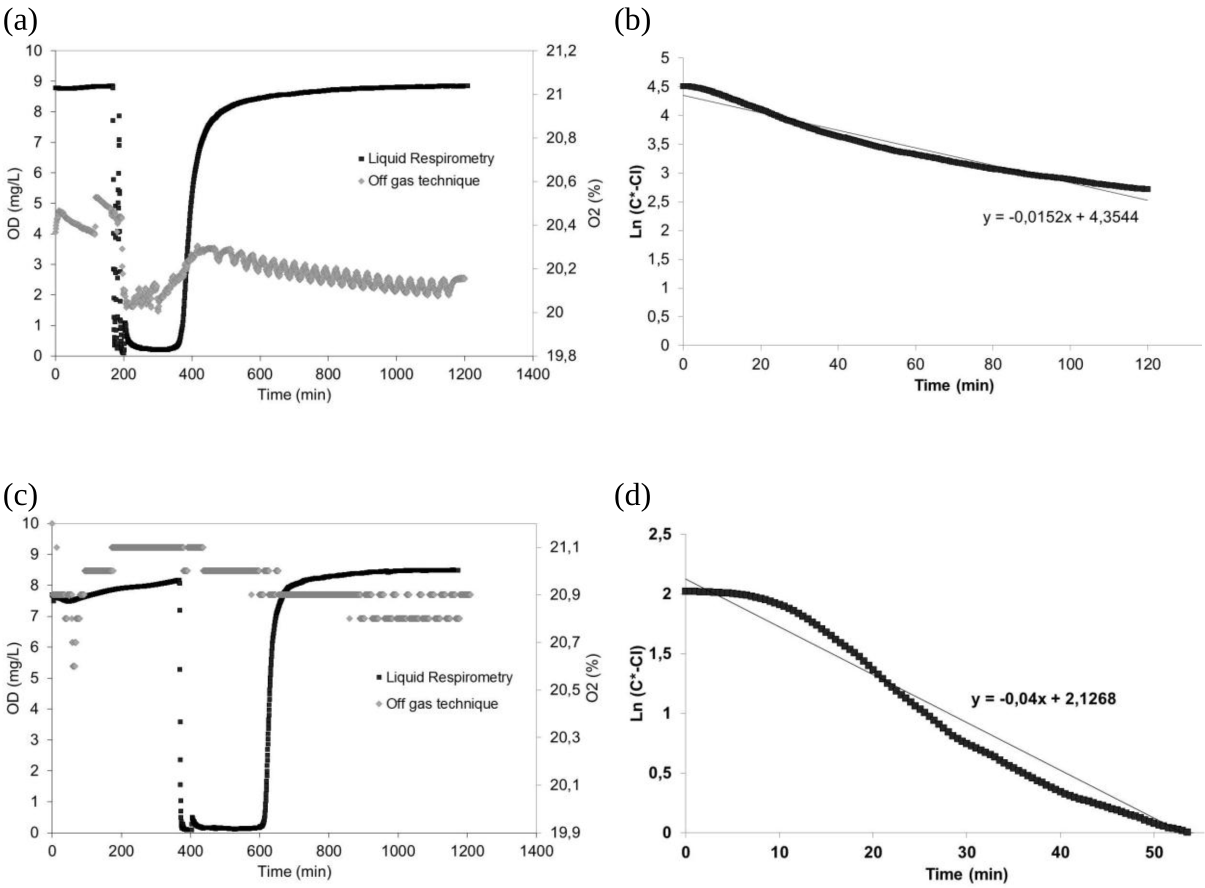


Figure 51 Oxygen concentration in the liquid phase (mg/L) and air phase (%) from Test 3(a and b) and 4 (c and d).

From Figure 51 it is possible to observe that both probes present problems in reading the off-gas concentrations. Probe 1 allows a small variation in the oxygen concentration to be detected. However, the probe has a trend in the values: data always show a decreasing pattern. Probe 2 is connected with a data acquisition system and values above 20% are recorded with intervals of 0.05 %. This interval is not enough to capture the difference we had in the gas phase during the test to determine K_La.

Test 3 presents lower values of K_La when compared with subsequent tests. This was assumed to be an effect of the first filling of the VSSF core that might have influenced the performance of aeration, with short cuts that do not allow the bubbles hold-up. Tests 4 and 5 give similar results, even if using different probes (Probe 1 and Probe 2) as shown in Table 11.

Table 11 Parameters estimated from the re-aeration test with sodium sulfide

	K_La (h^{-1})	Cs (mg/L)	C(mg/L)	SOTR (gO_2/h)	SOTE (%)
Test 3	0.894	9.33	1.69	0.030	0.258
Test 4	2.412	7.80	-1.45	0.068	0.583
Test 5	2.385	7.83	2.456	0.067	0.579

An OTE of 0.6% means that only 0.6% of the oxygen introduced in the core will be transferred. Considering an initial oxygen concentration of 21%, it means that the probe should be able to read differences around 0.126% (≈0.13%). The difference of 0.13% in the oxygen values might be measured by a probe that supplies results with at least two decimals even for values above 20%. This precision in the measurements is not achievable with our current probes (problems and limitations will be discussed on Chapter 12).

6.3.3 Application of off-gas technique compared with liquid respirometry

Figure 52, Figure 54 and Figure 56 show the response of O_2 concentration and temperature during the Test 6, 7 and 8 respectively. Figure 53, Figure 55 and Figure 57show the results of tests 6, 7 and 8 after correction of temperature and transformation from % of oxygen to mg of O_2. It is possible to observe the response of both probes after the addition of acetate. The results obtained are promising, even though some problems with the OD probes were encountered. In particular, the probe seems to detect a deviation that does not take place, even if it had been calibrated before the tests.

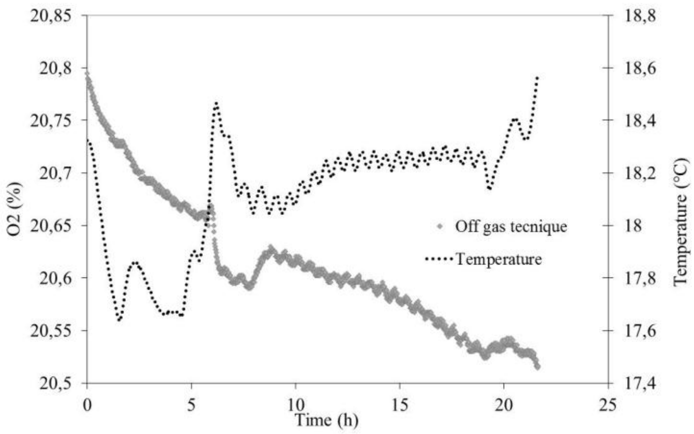


Figure 52 O_2 concentration and temperature during the Test 6

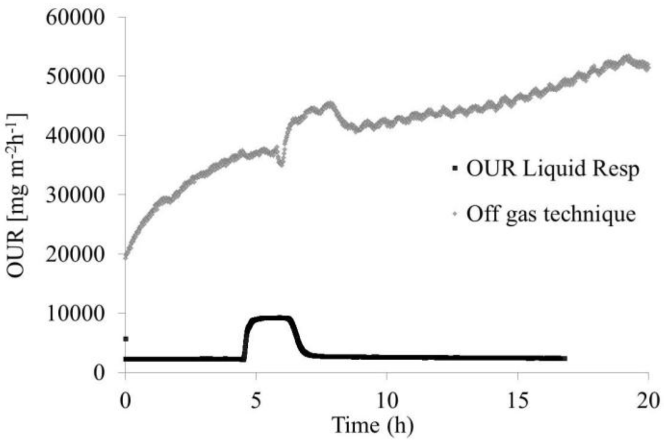


Figure 53 Results from Liquid respirometry and Off-gas application obtained in Test 6 using the core VSSF 4.

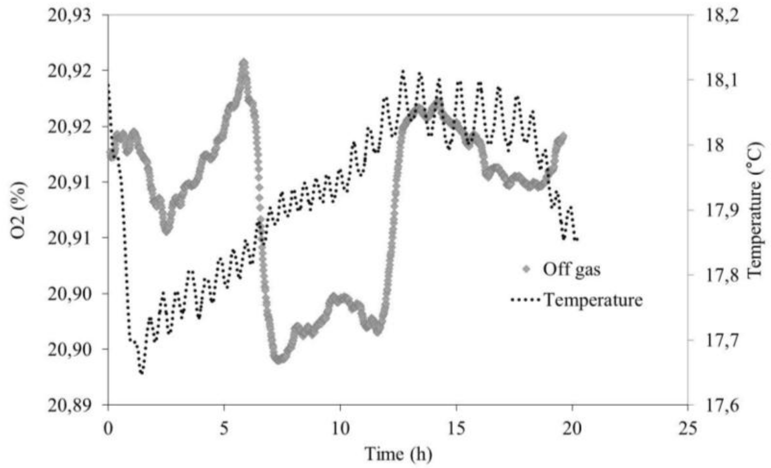


Figure 54 O_2 concentration and temperature during the Test 7.

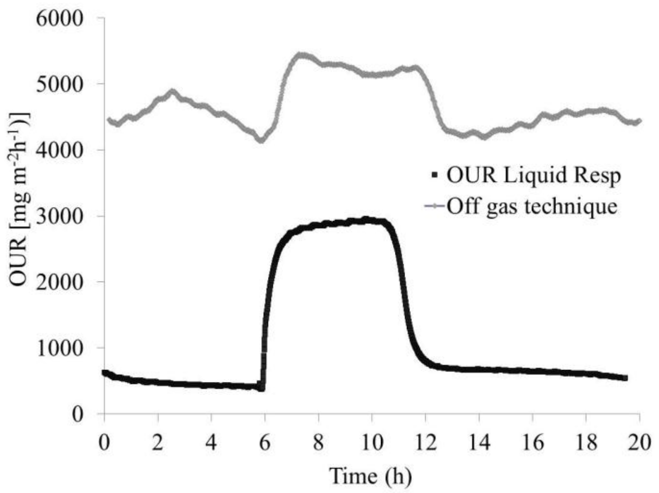


Figure 55 Results from Liquid respirometry and Off-gas application obtained in Test 7 using the core VSSF 2.

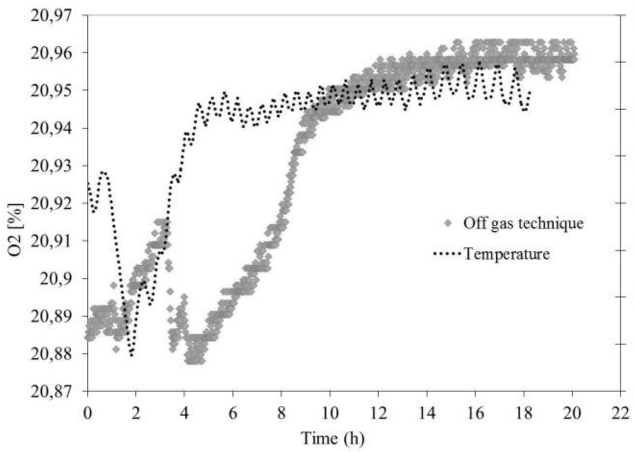


Figure 56 O_2 concentration and temperature during the Test 8.

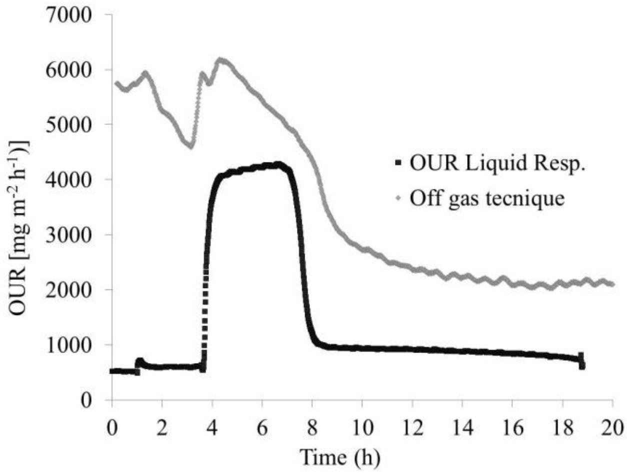


Figure 57 Results from Liquid respirometry and Off-gas application obtained in Test 8 using the core VSSF 2.

Liquid respirometry provides reliable and repeatable results in all the tests. Results of the application of the Off-gas technique showed the consumption in the air phase, though values obtained from the O_2 probe were not reliable due to the above-mentioned problems. Probe has its own deviation even if calibration was conducted before its use and the slope was in the range recommended by the WTW (from 0.60 to 1.20 which means that the probe is working well). Moreover, small variations in the O_2 initial concentration can drive to higher differences in the ΔO_2 value. For example, varying the concentration of initial oxygen from 21% (Figure 54) to 20.93% (Figure 58), the endogenous phase drops from a value around 4g m^2h^{-1} to 0.5 g m^2h^{-1}, and in this case the values of the endogenous phase are similar between the liquid respirometry and the off-gas analysis.

The comparison between the kinetic parameters estimated using the same amount of readily biodegradable COD (acetate) and initial O_2 concentration of 21% is shown in Table 12. Data were corrected for 20°C of temperature, using the Arrhenius equation (Θ=1.08).

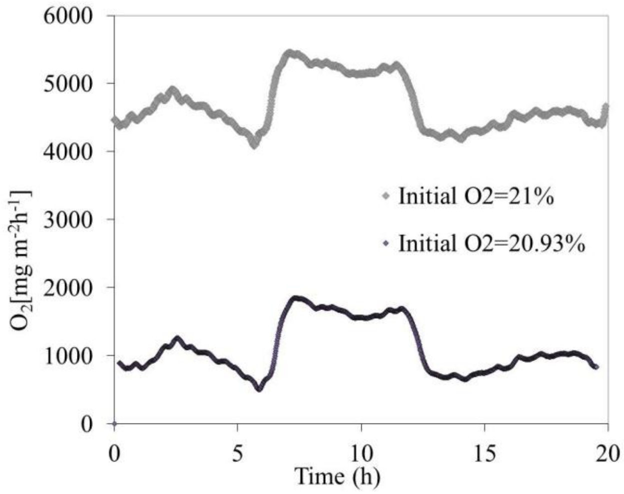


Figure 58 Test 7 results when changing the initial concentration of oxygen from 21% to 20.92%.

Table 12 Results obtained from Step 1 (comparison between liquid respirometry and off-gas analysis):

	Core	Air flow rate (L/h)	Liquid Respirometry			Off-gas analysis		
			ΔO_2 [gO_2m^{-2}]	Y_H[mgCOD/mgCOD]	Max Removal Rate [g m^2h^{-1})]	ΔO_2 [gO_2m^{-2}]	Y_H[mgCOD/mgCOD]	Max Removal Rate [g $m^{-2}h^{-1}$)]
Test 6	VSSF 4	100	14.96	0.69	22.59	14.52	0.70	14.15
Test 7	VSSF 2	40	13.12	0.73	9.26	6.21	0.87	0.59
Test 8	VSSF 2	100	15.73	0.68	11.29	7.69	0.84	2.48

Results obtained during Test 6 were satisfactory, and consistency was found between liquid respirometry and off-gas analysis (Y_H and ΔO_2 value). However, the results were not as good in Tests7 and 8, where values of ΔO_2 were higher in the liquid respirometry, and $Y_h$was slower in the off-gas test.

The off gas technique seems a promising one for the measurement of kinetic parameters in CWs, though various limitations emerged from the analyses that we have conducted. Hence, further studies are needed in order to tackle the problems observed during this research. In particular, probes guaranteeing higher precision standards must be usedand oxygen transfer efficiency has to be improved. Even though the off-gas technique was applied here as a proposal for the measurement of oxygen consumption on aerated VSSF (that are going to be presented in Chapter 11), the test could be a viable solution to evaluate the oxygen consumption (and also the efficiency of oxygen transfer) in aerated HSSF CW, as already proposed for wastewater treatment by Nivala et al. (2007); Ouellet-Plamondon et al. (2006); Zhang et al. (2010) among others.

Chapter 7

Comparison of two different configurations of VSSF in terms of efficiency and cost

7.1 Introduction

CWs are widely applied to decentralized wastewater treatment plants, single home projects and rural communities. Among the problems associated with the decentralization of wastewater treatments is that of investment. The fact that a treatment unit receives wastewater from few households results in considerable costs being paid by few people. According to Rousseau et al. (2004), the ‘economy of scale’ also applies to the design of VSSF CW: the investment cost per PE decreases as the design size of the constructed wetland increases. The main investment costs in CWs are due to: (1) use of land depending on the CW surface and the applied load, (2) sealing (waterproofing) of the CW bottom, (3) digging activity and depth of the bed, (4) filter material such as gravel and sand. As demonstrated by Chen et al. (2008) for the case of HSSF CW, the main expenditure for the construction of CWs is due to the substrate (41.2%), followed by the construction engineering fee (30.1%), the plants (14.3%), the membrane (10.1%), pumps, pipes and other facilities (4.3%). Hence, a significant reduction in the cost of CWs can primarily be obtained by reducing the depth of the substrate.

In the case of VSSF CWs, a technology that is widely applied to obtain nitrification and organic matter oxidation, national or regional guidelines indicate the specific layers of gravel and sand to be used, the size of the filter material and the recommended depth (Table 13). The main filter media, which plays a major role in biological and physico-chemical removal processes, is generally the layer of sand.

Table 13Parameters for the design of a VSSF CW as obtained from the guidelines of various countries.

Reference		Surface Area Requirements	Depth (all layers included)	Organic load
Germany	ATV, 1998	2.5 m^2/PE	0,8 m	20 -25 g $BOD_5 m^{-2}$
Austria	Önorm, 2008	4 m^2/PE	0,7 - 0,8 m	-
Czech Republic	Vymazal, 1998	2^{nd} treatment: 1° stage: 0.8- 2 m^2/PE 2° stage: 2 - 5 m^2/PE 3^{rd} treatment: 2 - 5 m^2/PE	0,6 m	-
United Kingdom	Cooper, 1996	1 -2 m^2/PE<100 PE: 1° stage: 3.5 x $PE^{0.35}$ +0.6 x PE 2° stage: 50% of 1° stage	1 m	23 -25 g $BOD_5 m^{-2}$
France	Cemagref-EC, 2001	1° stage: 1.2 - 1.5 m^2/PE 2° stage:0.8 m^2/PE	0.6 - 0.8 m	24 -25 g $BOD_5 m^{-2}$
Denmark	Brix and Arias, 2005	3 m^2/PE	1 m	20 g $BOD_5 m^{-2}$
Italy	APAT, 2005	2-5 m^2/p.e for discharge in surface water 4-6 m^2/p.e for discharge in surface water in sensible areas	0.55 m	-

For VSSF CWs located in regions characterized by low temperatures (e.g. the Alps, where this research was carried out), Austrian and German guidelines can be considered: the Önorm B2505 (2008) indicates the use of a 0.5 m sand layer with size 0/4 mm, while the DWA-A 262 (2006) indicates the use of sand with uniformity coefficient $U=d_{60}/d_{10}<5$, permeability $K_f = 10^{-3}$-10^{-4} m/s and d_{10} in the range 0.2-0.4 mm.

The comparison between the two VSSF CWs was carried out on the basis of conventional parameters (COD, N forms, P, TSS) and more in-depth investigations such as respirometric tests. In this research, respirometric tests were used to investigate the biodegradable COD fractions in the effluents from VSSF CWs at various times of the typical cycle, evaluating the fate of readily and slowly biodegradable COD and inert COD fractions.

This research aims to assess how a reduced filter depth (and therefore a cheaper construction) affects the removal efficiency. This was achieved by considering two VSSF CWs with the same applied loads. One of them was designed according to local guidelines (PAT n. 902/2002) similar to the Austrian Önorm guidelines, while the other had a lower depth and a modified sand composition in order to test the removal efficiency differences from these two configurations.The comparison between the two VSSF CWs was carried out on the basis of (1) conventional and innovative parameters (COD, N forms, P, TSS and COD fractionation, respirometric tests) to assess the removal efficiency and (2) costs involved in the construction of VSSF CW, including those associated with the transportation of the filling material from the quarry to the construction site.

7.2 Materials and Methods

The two VSSF CWs of the pilot plant were operated in parallel. The main difference between them is the filter materials as follows:

1) C-line: this configuration was based on a single main layer of sand and layers of gravel at the top and at the bottom, according to local guidelines (PAT n. 902/2002) and similar to the indications of Austrian Önorm B2505 (2008), described as follows starting from the top: 0.1 m gravel 7-15 mm; 0.5 m sand 1-3 mm (porosity (p) = 31%, d_{60}/d_{10}=1.6); 0.05 m gravel 7-15 mm (p= 30%); 0.2 m gravel 15-30 mm (p= 31%); this VSSF CW is called afterward “0.5m-VSSF”;
2) E-line: this configuration was based on 2 main layers of fine and coarse sand and gravel at the bottom, described as follows starting from the top: 0.1 m sand 1-3 mm (p= 31%, d_{60}/d_{10}=1.6); 0.2 m sand 1-6 mm (p= 28%, d_{60}/d_{10}=2.6); 0.1 m gravel 7-15 mm (p= 30%); 0.2 m gravel 15-30 mm (porosity p= 31%). The coarse sand (fine gravel) had a commercially available size different from fine sand recommended by guidelines (0/4 mm) (Austrian Önorm B2505, German DWA-A 262) but similar hydraulic conductivity (10^{-3}-10^{-4} m/s); this VSSF CW is called afterward “0.3m-VSSF”.

Washed sand was used in both VSSF CWs, in order to limit the presence of silt which may decrease significantly the hydraulic conductivity of the bed even if present in a very small fraction. The saturated hydraulic conductivity (K_f) of sand layers was very similar between the two VSSF configurations as indicated in
Table 14. Even if the presence of plants would create a complex environment much more similar with the full scale CWs operating in Italy, both VSSF CWs were completely unplanted. This decision is acceptable for pilot plants and consistent with the purpose of comparing the influence of different filter material, thus eliminating any possible influence of plants on the performance of the configurations to be compared.

Table 14 Physical characteristics of sands recommended in guidelines and used in this study.

Description	**Depth of main filter material**	**Size of the main filter material**	**Porosity (-)**	**d_{10} (mm)**	**d_{60} (mm)**	**d_{60}/d_{10}**	**Hydraulic conductivity (m/s)**
Austrian Önorm B2505 (2008)	0.5 m	0/4 mm	-	-	-	-	-
German DWA-A 262 (2006)	≥0.5 m	-	-	0.2-0.4	-	<5	10^{-3}-10^{-4}
“0.5m-VSSF” (C-line)	0.5 m	1/3 mm	0.31	1.0	1.5	1.6	$6.3x10^{-4}$
“0.3m-VSSF” (E-line)	0.1 m	1/3 mm	0.31	1.0	1.5	1.6	$6.3x10^{-4}$
	0.2 m	1/6 mm	0.28	1.0	2.6	2.6	$5.8x10^{-4}$

The distribution of wastewater is enabled by four pipes ending with a plate located few cm above the gravel surface for the 0.5m-VSSF and the 0.3m-VSSF. Drainage occurs through one hole on the lower side of the wetland. While this is not really appropriate, it can be accepted considering the small size of the plant. Hydraulic and organic loads applied to the VSSF CWs are shown in the Table 15.

Table 15 Mean values of the hydraulic and organic loads applied to the VSSF CWs.

Parameter	Units	0.5m-VSSF (C-line)	0.3m-VSSF (E-line)
Feeds	Times per day	3.6	3.5
Resting period (between feeds)	hours	6.6	6.5
Hydraulic load	L m^{-2} d^{-1} [mm/d]	56	60
Superficial organic load	gCOD m^{-2} d^{-1}	33.8	36. 7
Area/person equivalent	m^2/PE*	3.5	3.2

*one person equivalent (PE) is assumed to be the organic biodegradable load having a chemical oxygen demand (COD) of 110 g per day.

In this research, the data shown are related just to the period in which both systems operated with the same organic and hydraulic loads. However is important to say that the 0.5m-VSSF is still operating with the same organic load (3 years of operation) without presentingany problem with clogging or decrease in removal efficiency. In the case of the 0.3m VSSF, the system was tested with this organic and hydraulic load for just a two month period, after that the system was operated with higher organic loads (E-line in the Chapters 8,10 and 11).

7.2.1 Wastewater analyses

Wastewater analyses of COD, 0.45-µm-filtered COD, Total Suspended Solids (TSS), NH_4-N, NO_2-N, NO_3-N, TKN, total P were carried out twice a week over a two month period (16 samples) in the influent and effluents from the two VSSF CWs. Temperature data for the whole period were collected from the nearest meteorological station, located 2 km far from the pilot plant (S. Massenza, Province of Trento, Italy). The weather temperature during this period varied from 13ºC to 22.6ºC, while the average wastewater temperature was 17.8°C (influent), 19.3°C (0.5m-VSSF) and 19.7°C (0.3m-VSSF). Particulate COD was calculated as difference between total COD and filtered COD. Analyses were performed according to Standard Methods (APHA, 2005).

The quality standards for the effluent are those suggested by the Italian Decree 152/2006, that is 125, 25 and 35 mg/L for COD, BOD_5 and TSS, respectively. In the case of CWs the values of TSS may be greater than or equal to 150 mg/L. Nutrient standards are imposed only on sensitive areas where the annual average should not be higher than 15 and 2 mg/L for total N and total P, respectively. For small wastewater treatment plants (less than 2000 PE) an appropriate treatment may be chosen in order to meet the relevant quality objectives of receiving waters according to EU Directives.

7.2.2 Cost Evaluation

Beyond their treatment performance, VSSF CWs were evaluated on the basis of their costs. These were computed by considering hypothetical plants built to serve a community of 500 inhabitants, and following the local guideline that recommends 4m^2/PE (i.e. total area of 2000 m^2) (Table 16).

The cost comparison is maybe a bit simplistic, asonly one design option (i.e. VSSF) was considered, while hybrid systems (e.g. HSSF+VSSF or VSSF+VSSF) should also be considered. However, we focused on just the VSSF because the aim of the study was to verify how a difference of 20 cm in the filter material may affect the total cost of a pilot plant,given a basic area of the system.

In accordance with rules proposed by ONORM, a size of 400 m^2 was chosen for beds, as the optimal trade off between expenditure and maintenance. The analysis accounted for the main costs (e.g. digging activity, filling material, transportation of material, which depends on the distance between the quarry and the plant site), while disregarded others that are either difficult to compute or equal for both configurations (e.g. sieving, piping, pumps, etc.). In particular, we did not consider the cost of land acquisition, which is ultimately an opportunity cost related to the potential alternative uses of that portion of land.

Table 16 Amount of material used in the VSSF CWs.

Layer	**0.5m-VSSF (C-line)**		**0.3m-VSSF (E-line)**	
	M	m^3	m	m^3
7/15	0.10	200	0.10	200
1/3	0.50	1000	0.20	400
1/6	-	-	0.10	200
15/30	0.20	400	0.20	400
Total depth (material to dig)	0.80	1600	0.60	1200

The cost of all items was derived from the official price list provided by the local administration for the 2012 period (however one should consider that costs may change from one region to another, and that they may be affected by additional factors that are not taken into account here, such as the purchase of plants, pipes, pumps, and the acquisition of land). Masi et al. (2003), found that the cost of the primary treatment and the acquisition of land is about 8 €/m^2, but this is not considered in this research. The two configurations were compared on the basis of their removal performance per unit of investment (kg of COD removed per Euro per year, kg of TKN removed per Euro per year), considering an expected lifetime of 20 years, and the investment per inhabitant.

7.3 Results and Discussion

7.3.1 Overall performances of the VSSF CWs

The rapid feeding of influent wastewater in the VSSF CWs causes a hydraulic short-circuit in the first minutes immediately after feeding (Figure 59). Fifty percent of the volume of influent wastewater passed through the bed just during the first 30 minutes, 75% passed in the first 2 h and 90% passed in 4 h in similar manner in the 0.5m-VSSF and in the 0.3m-VSSF. The similar hydraulic behaviour between the two VSSF CWs was the result of the similar hydraulic conductivity of the filter materials (indicated in
Table 14).

The two VSSF CWs were operated at the same hydraulic and organic loads (Table 15) with a slight difference of 8.5% due to the use of different pumps. The slight difference was also evident between the applied COD loads (on average 33.8 and 36.7 gCOD m^{-2} d^{-1} in the 0.5m-VSSF and 0.3m-VSSF, respectively) and the removed COD loads (28.6 and 29.2 gCOD m^{-2} d^{-1} in the 0.5m-VSSF and 0.3m-VSSF respectively), while effluent COD concentration was lower in the 0.5m-VSSF (63 mg/L on average compared to 87 mg/L in the 0.3m-VSSF effluent) probably due to the higher depth of the filter material. The mean values of the chemical parameters in the influent and effluents are shown in Table 17.

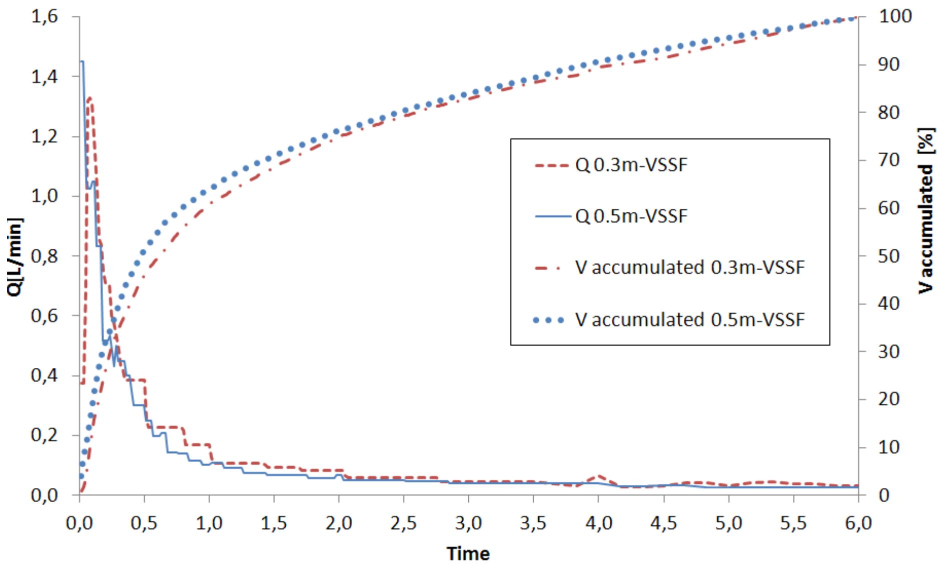


Figure 59 Profiles of effluent flow rates and cumulated wastewater volumes during a typical cycle of the VSSF systems.

The filtered COD/total COD ratio increased from 56% in the influent wastewater to 66% and 73% in the effluents from the 0.3m-VSSF and 0.5m-VSSF, due to the retention of the particulate COD in the filter material where the slowly biodegradable organic matter undergoes subsequent biological oxidation during the resting period (Giraldo and Zarate, 2001). Accordingly, particulate solids measured as TSS were reduced by 87% in the 0.5m-VSSF and 80% in the 0.3m-VSSF, showing that the 0.5m-VSSF has a higher efficiency in TSS entrapment due to the higher depth and the lower sand size which enhances the filtration effect.

Table 17 Characterisation of influent and effluent wastewater (mean ± standard deviation).

Parameter	Units	Influent wastewater	Effluent from the 0.5m-VSSF	Effluent from the 0.3m-VSSF
COD	mg/L	574 ± 110	63 ± 28	87 ± 34
0.45-µm-filtered COD	mg/L	334 ± 79	44 ± 10	51 ± 14
TSS	mg/L	187 ± 29	25 ± 23	37 ± 29
TKN	mg/L	72.3 ± 5.0	12.8 ± 11.5	18.3 ± 8.1
NH_4-N	mg/L	56.4 ± 7.6	11.7 ± 7.4	17.0 ± 5.0
NO_2-N	mg/L	0.05 ± 0.04	1.50 ± 0.90	0.82 ± 1.2
NO_3-N	mg/L	3.0 ± 0.7	41.4 ± 15.7	38.6 ± 19.8
Total N	mg/L	75.5 ± 6.1	57.5 ± 14.5	57.7 ± 19.8
Total P	mg/L	7.9 ± 0.7	3.3 ± 0.6	4.0 ± 0.5
Temperature	°C	17.8°C	19.5°C	19.5°C

A very high TKN concentration in the range 61.4-77.2 mgN/L was observed in the influent wastewater (Table 17), which is not unusual in wastewater coming from very small villages and pre-settled in anaerobic septic tanks. As a consequence of the prevalent aerobic conditions in the VSSF systems, a stable nitrification occurred in both VSSF CWs. The influent TKN (72.4 and 74.2 mgN/L in the 0.5m-VSSF and 0.3m-VSSF, respectively) was discharged partially in the effluent (11.7 mgN/L in the 0.5m-VSSF and 17 mgN/L in the 0.3m-VSSF), part was utilized for biomass synthesis and the main part was nitrified. The average TKN loads applied and converted into NO_3 were 4.1 and 3.4 gTKN m^{-2} d^{-1} respectively in the 0.5m-VSSF and 4.4 and 3.3 gTKN m^{-2} d^{-1} in the 0.3m-VSSF. Total N concentration in the effluents was exactly the same in both the two VSSF CWs (57.5 mgN/L and 57.7 mgN/L in the 0.5m-VSSF and 0.3m-VSSF respectively) and the removal of 18 mgTN/L in both systems was due mainly to synthesis and filtration of particulate N while denitrification was minimal.

A higher NO_2^--N concentration was observed in the effluent from the 0.5m-VSSF (1.50 mgNO_2^--N/L on average) compared to the 0.3m-VSSF (0.82 mgNO_2^--N/L on average), probably due to the formation of local not fully aerobic microzones in the deeper bed of the 0.5m-VSSF, which may cause local limiting conditions for nitratation and an accumulation of nitrite.

With regards to total P, the removal efficiency in the deeper 0.5m-VSSF (57.7%) was better than in the0.3m-VSSF (49.6%). In this case, the major efficiency might be related to a larger quantity of filter material in the 0.5m-VSSF, where the P removal occurred by adsorption on the sand.

7.3.2 Cost evaluation

In Table 18 and Table 19 the values of filter material needed for VSSF CWs and digging activity in the construction of VSSF CW are shown. In Table 20, the total fixed costs are summarised.

Table 18 Values of the filter material used in the VSSF CW construction .

Material/activity	Typical price	unit	0.5m-VSSF (C-line)			0.3m-VSSF (E-line)		
			m^3	unit	€		unit	€
Gravel 7/15	20	€/m^3	200	m^3	4,000	200	m^3	4,000
Sand 1/3	30	€/m^3	1,000	m^3	30,000	400	m^3	12,000
Sand 1/6	35	€/m^3	-	-		200	m^3	7000
Gravel 15/30	20	€/m^3	400	m^3	8,000	400	m^3	8,000
Total	-	-	-	-	42,000	-	-	31,000

Table 19 Values of the material in waterproofing impermeabilization and in the construction of VSSF CW.

Material/activity	Typical price	Unit	0.5m-VSSF (C-line)			0.3m-VSSF (E-line)		
				Unit			unit	
Impermeabilization with HDPE 2 mm	15	€/m^2	2,754	m^2	41,306	2,672	m^2	40,076
Mechanical excavation	9	€/m^3	1,600	m^3	14,400	1,200	m3	10,800
Regularization of the floor and ramps	0.65	€/m^2	2,328	m^2	1,513	2,246	m^2	1,460
Total	-	-			57,219			52,335

*Hypothetically 5 beds of 400 m^2 each (ÖNORM 2505, 2008).

Table 20 Fixed costs in the VSSF CWs construction.

Fixed costs	0.5m-VSSF (C-line)	%	0.3m-VSSF (E-line)	%
Filter Material	42,000	42	31,000	37
Impermeabilization and Digging Activity	57,219	58	52,335	63
Total*	99,219€		83,335€	

*These costs do not include plants, pipes, pumps, and land acquisition.

Given the hydraulic load applied, the amounts of COD removed were about 20.890 kg.yr^{-1} with the 0.5m-VSSF and 21.331 kg.yr^{-1} with the 0.3m-VSSF (Figure 60). The amounts of TKN removed were about 2.432 kg.yr^{-1} and 2.365 kg.yr^{-1}, respectively. These values were used to calculate the removal-investment ratio (Figure 61).

While the 0.5m-VSSF performs slightly better than the 0.3m-VSSF in terms of treatment, the outcome is inverted when considering the removal-investment ratio (Figure 60): the better performance of the 0.5m-VSSF may compensate its higher monetary costs. Figure 6 shows that, for increasing transportation distances of the filling material, the above-mentioned ratio keeps constant between the two configurations. This suggests that, given the design characteristics of the two configurations, varying costs due to transportation are not likely to modify the difference between their “removal efficiency” return on investments. On the other hand, the investment per inhabitant and the investment per m^2 emphasise an increasing economic difference between the two proposed configurations for increasing transportation costs (Figure 61).

In the Flanders database, the design size of the VSSF CW varies from 4 up to 2000 PE with an average surface area of 3.8 m^2/PE and an average investment cost of 507 €/PE (Rousseau et al., 2004). In our study, the investment per person (without considering the acquisition of land, pipes, pumps, etc.) ranges from 229 to 352 €/PE for the 0.5m-VSSF and from 190 to 282 €/PE for the 0.3m-VSSF when the distance (one way) between the quarry and the site passes from 10 km to 50 km. The investment per unit area ranges from 57 to 88

€/m^2 in the case of the 0.5m-VSSF and from 47 to 70 €/m^2 in the case of the 0.3m-VSSF (without considering the acquisition of land, pipes, pumps, etc.). These values are comparable to those obtained by Masi et al (2003) in their study on 34 SSFCW in Italy, they observed that the price changes according to the area, the land morphology and the design criteria. When considering CWs larger than 2000 m^2 values are about 100 Euros/m^2 and 500-600 Euros/PE (Masi et al., 2005).

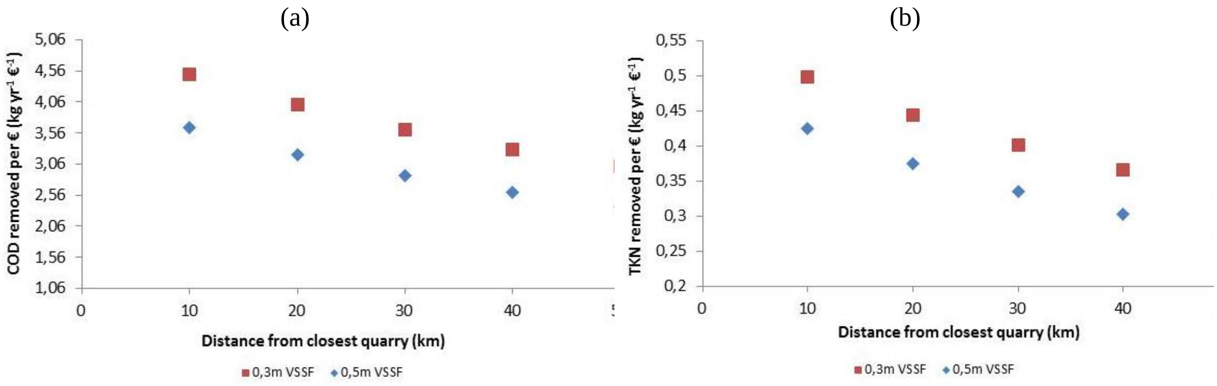


Figure 60(a) COD removed per euro invested for increasing transportation distances of the filling material. (b) TKN removed per euro invested for increasing transportation distances of the filling material (these costs do not include plants, pipes, pumps, and land acquisition.)

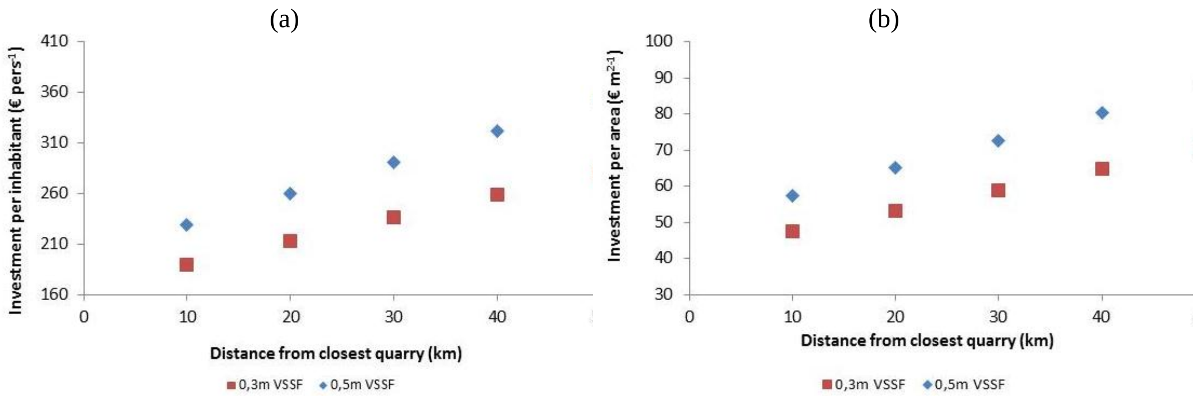


Figure 61(a) Investment in euro per person (500 inhabitant) for increasing transportation distances of the filling material. (b) Investment in euro per m^2 for increasing transportation distances of the filling material.

Figure 62 shows the cost calculated on a 50 km transport distance per inhabitant considering just the filter material, impermeabilization and digging activity. It was assumed that two beds of 100m^2 each are used for 50 PE, two beds of 200 m^{2}each are used for 100 PE and that, for increasing population, one bed of 400 m^2 is added every 100 PE, respecting the maximum area recommended by the Onorm (2008). According with Chen et al. (2008), the cost of substrate, membrane and construction engineering fee (digging activity in the present paper) corresponds to 81,4% of the total costs of a constructed wetland (without the price of the land).

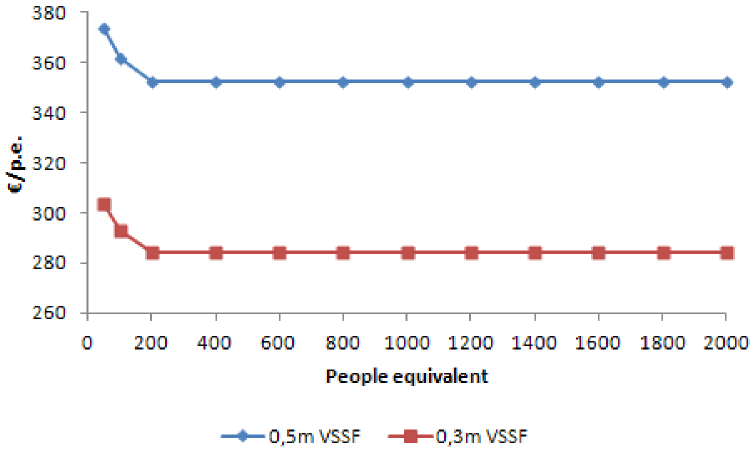


Figure 62 Cost per inhabitant on a 50 km transport distance.

Although the economic evaluation was performed on a specific case study and the use of different data could have generated different results, it may provide some insights about how to choose the best technology when money does matter. In particular, it stresses the importance of considering the transportation of the filling material as a significant variable cost. While it is evident that the 0.5m VSSF will always cost more (and in this case it perform worse in terms of economic efficiency) than the 0.3m VSSF (Figure 60) no matter the transportation distance, differences can vary when considering the investment per inhabitant and the investment per area (Figure 61). In this case, increasing transportation distances may result in an increasing difference between the two configurations, with possible consequences on the decision of whether to apply one configuration or the other. The selection of the suitable configuration is indeed a complex task where multiple and often conflicting objectives must be achieved. This study showed, as expected, that the 0.5m-VSSF performs slightly better from the point of view of the removal efficiency, but this may not be enough when the filling material comes from faraway. This is a step when the judgment of decision-makers will play a fundamental role and the different importance given to the environmental and economic issues will drive the final selection.

Chapter 8

Influence of high organic loads during the summer period on the performance of Hybrid Constructed Wetlands (VSSF+HSSF) treating domestic wastewater in the Alps region[2]

8.1 Introduction

In many mountain areas in the Alps (excluding ski areas) the tourism is mostly concentrated in a 2 and a half month period (from mid June through the end of August). Therefore the population increases significantly during the summer due to the presence of large floating population compared to the resident population who live there for the whole year. In the Province of Trento (north-eastern Italy) the amount of resident population and total population (resident + floating in the 2-month tourist period) was monitored for 31 small tourist alpine villages (with less than 1,000 resident population and not collected to a centralised WWTP) and results are shown in Figure 1.

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[2]This chapter was published as: Foladori P., Ortigara A. R. C., Ruaben J., Andreottola G. (2012). Influence of high organic loads during the summer period on the performance of Hybrid Constructed Wetlands (VSSF+HSSF) treating domestic wastewater in the Alps region. Water Science and Technology 65(5), 890-897.

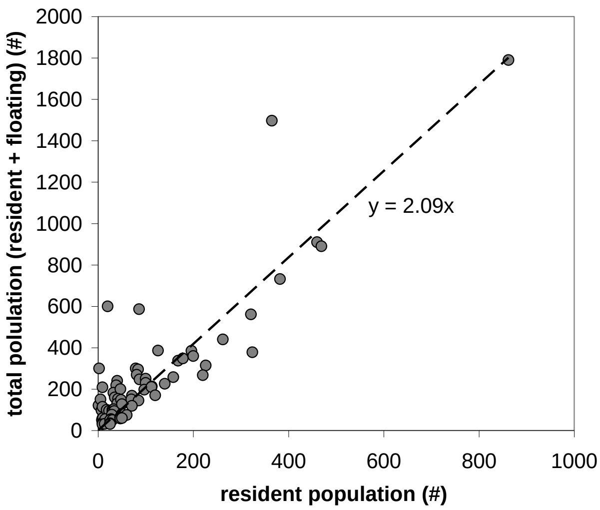

Figure 63 Correlation between resident population and total population (resident + floating) in 31 small tourist villages and fractions (Province of Trento, Italy) during 2-month summer period.

Because the total population is twice the resident population for just two months throughout the year (Figure 64), it may be very expensive to design a CW plant for wastewater treatment based on the high organic load discharged during the summer period. For example, a small tourist village with a resident population of 600 and a tourist population of 600 would require a hybrid CW plant for 1200 PE. When considering a specific surface of approximately 4 m^2/PE, 4.800 m^2 of contiguous land surface would be needed, which represents an amount of land hardly found in a mountain region due to slope characteristics and the importance to conserve wildness.

This chapter aimed to evaluate whether a hybrid CW plant designed on the basis of the resident population only, can treat the additional nitrogen and organic load produced by the floating population during the 2-month tourist period, without drastic decrease of efficiency in organic matter removal and nitrification and without clogging problems. If the performance of the CW plant during this overloaded period were maintained at acceptable levels, a significant reduction of the land area for the hybrid CW system would be obtained.

8.2 Materials and Methods

The E-line (VSSF+HSSF) was evaluated in this Chapter. The influent wastewater was applied in the VSSF unit discontinuously (3.6 cycles/day on average) and pumping of influent wastewater took few minutes. The VSSF effluent drained and flowed by gravity into the HSSF unit. Two operation periods were tested in this study as showed in Table 21.

Table 21 Main operational parameters of the VSSF and HSSF systems during the 1st low-load period and the 2nd high-load period.

	Parameter	Units	1st low-load period (May-June 2010)	2nd high-load period (July-August 2010)
VSSF	Influent flow rate (hydraulic load)	L/d	124	276
	Specific hydraulic load	$L\ m^{-2}\ d^{-1}$	55	123
	Surface organic load	$gCODm^{-2}d^{-1}$	37	87
	Specific area	m^2/PE	3.2	1.3
	Cycles per day (feeds per day)	#/d	3.6	3.6
	Resting period (between feeds)	H	6.6	6.6
HSSF	Specific hydraulic load*	$L\ m^{-2}\ d^{-1}$	27	61
	Specific area*	m^2/PE	6.4	2.6

* in this chapter only two sections of the HSSF were considered: H1 and H2.

8.2.1 Chemical analyses

Samples of influent and effluents from VSSF and HSSF systems were collected twice a week. Intensive monitoring campaigns were conducted during the VSSF normal operation cycle to obtain the concentration time-profiles of wastewater effluent from the VSSF over 8 different time frames (track-studies): 0-5 minutes, 5-10 minutes, 10-20 minutes, 20-30 minutes, 30 minutes-1 hour, 1-2 hours,2-4 hours and 4-6 hours. Samples along the HSSF unit were taken from taps to obtain the longitudinal profile of concentrations in the bed (2 sample points – H1 and H2). Track-studies were performed 3 times when steady-state conditions in VSSF and HSSF systems were reached.

Concentrations of COD, TKN, NH_4-N, NO_2-N, NO_3-N, PO_4-P and total P were analyzed according to Standard Methods (APHA, 2005). Soluble COD was measured after filtration of the sample on 0.45-µm-membrane and Biodegradable COD was estimated in influent and effluent wastewater taken from the CW units as described in Chapter 3. The respirogram obtained for a conventional activated sludge used as reference was compared to the respirogram obtained after the addition of a known amount of wastewater to the activated sludge. The comparison allows calculation of the amount of biodegradable COD in the tested wastewater on the basis of the oxygen consumed for its oxidation.

8.3 Results and Discussion

In the 1st low-load period temperature was 19.7°C and 19.2°C on average in VSSF and HSSF units respectively, while in the 2nd high-load period (summer) temperature was 22.4°C and 22.3°C respectively. The period with tourist population and with high loads in the hybrid CW system coincides exactly with the most favourable temperatures for the biological kinetics and the plants are in the period of maximum growth.

In the 1st period the typical hydraulic and organic loads were applied in the hybrid CW units (55 L m^{-2} d^{-1} and 37 gCOD m^{-2} d^{-1} on average in the VSSF) in order to represent the typical loads usually considered in the conventional design of hybrid CW systems. This organic load corresponded to 3.2 m^2/PE in the VSSF system and to about 6 m^2/PE in the HSSF system.

In the 2nd period, which lasted 2 months from the beginning of July to the end of August 2010, higher hydraulic and organic loads were applied to simulate the additional presence of the tourist floating population, according to the ratio of Figure 63. In this case the hydraulic and organic loads in the VSSF reached 123 L m^{-2} d^{-1} and 87 gCOD m^{-2} d^{-1} on average, correspondent to the use of 1.3 m^2/PE in the VSSF system (2.6 m^2/PE in the subsequent HSSF system until H2). The specific area used in the VSSF unit during the 2nd high-load period (1.3 m^2/PE) can be considered significantly low and not so common in the design of VSSF systems. Clogging problems were not observed during the 2-month period at high load although the progressive accumulation of the suspended solids in the VSSF (settleable solids were always lower than 6 mL/L in the pre-settled wastewater) and the growth of microorganisms, which will undergo mineralization during the rest 10-month period operating at low load.

Table 22 Characterisation of the influent and effluent wastewater during the 1st low-load period and the 2nd high-load period. *COD_B = biodegradable COD measured by respirometry

Parameter (mg/L)	1st low-load period (May-June 2010)			2nd high-load period (July-August 2010)		
	Influent	Effluent VSSF	Effluent hybrid CW (VSSF+HSSF)	Influent	Effluent VSSF	Effluent hybrid CW (VSSF+HSSF)
Total COD	572	105	36	692	179	82
Soluble COD	325	61	-	360	102	38
COD_B/total COD*	0.71	0.45	0.53	0.78	0.56	0.21
TKN	72.3	18.3	14.6	79.8	30.2	22.6
NH_4-N	57.5	17.0	11.4	64.7	26.3	20.2
NO_2-N	0.046	0.82	0.57	0.02	1.94	0.05
NO_3-N	3.0	38.6	22.5	2.9	17.9	2.8
Total N	75.5	57.7	16.8	82.6	49.6	20.9
Total P	7.9	4.0	0.19	9.6	6.1	3.5
pH (-)	8.3	8.1	8.1	7.8	7.7	7.6
ORP (mV)	-103	105	70	-190	115	87
Temp. (°C)	17.8	19.7	19.2	21.1	22.4	22.3

The characterisation of the influent and effluent wastewater in the 1st low-load and the 2nd high-load periods are indicated in Table 22. Due to the dependence of the profile of COD and nitrogen on time during a VSSF cycle (as described more in depth in the following paragraphs), flow-weighted composite samples were collected and analysed to assess the average value indicated in Table 22. During the 2nd high-load period the concentrations of COD, nitrogen forms and phosphorus in the influent wastewater increased compared to those observed in the 1st period, as expected when the tourist floating population is present. Furthermore, occasional high TKN peaks appeared in the influent during the 2nd high-load period, reaching values higher than 90 mgTKN/L.

Due to the configuration used in this Hybrid CW system, the major role in the removal of COD and nitrogen forms was played by the VSSF unit, while the HSSF unit completed the treatment by providing a "polishing/finishing function", mainly in the 2nd high-load period, when the COD concentration effluent from the VSSF was higher (179 mgCOD/L). In the VSSF unit the nitrification process occurred in both periods as demonstrated by the significant decrease of TKN and NH_4-N concentration in the effluent from the VSSF unit. The organic and nitrogen loads applied and removed in the VSSF unit during the 1st low-load period and the 2nd high-load period are shown in Table 23.

Table 23 Applied and removed loads in the VSSF system and removal efficiency.

	Parameter	**1st period (low organic load) May-June 2010**	**2nd period (high organic load) July-August 2010**
COD	Applied COD load in VSSF [gCOD m^{-2} d^{-1}]	36.7	86.9
	Removed COD load in VSSF [gCOD m^{-2} d^{-1}]	29.9	64.3
	COD removal efficiency in VSSF (%)	82%	74%
	COD removal efficiency in VSSF +HSSF (%)	94%	88%
TKN	Applied TKN load in VSSF [gTKN m^{-2} d^{-1}]	4.4	10.3
	Removed TKN load in VSSF [gTKN m^{-2} d^{-1}]	3.3	6.6
	TKN removal efficiency in VSSF (%)	75%	62%
	TKN removal efficiency in VSSF +HSSF (%)	80%	72%
Total N	Applied total N load in VSSF [gN m^{-2} d^{-1}]	4.5	10.6
	Removed total N load in VSSF [gN m^{-2} d^{-1}]	1.6	4.8
	Total N removal efficiency in VSSF (%)	24%	40%
	Total N removal efficiency in VSSF +HSSF (%)	78%	75%

8.3.1 Comparison of COD removal during low-load and high-load conditions

Due to the discontinuous feeding in the VSSF unit, the effluent is drained and flowed by gravity with a flow rate variable during the time. During the 1st low-load period, when the hydraulic load applied was low, the COD removal efficiency was 81% even in the first 30 minutes after feeding with no significant differences until the end of the cycle (see time-profile in Figure 64). In the 2nd high-load period the hydraulic load was doubled, which caused an immediate effluent peak from the VSSF unit (hydraulic short-circuit caused by the higher volume applied): about 50% of the applied wastewater volume passed through the VSSF unit in the first 5 minutes in which COD removal was about 48% and COD concentration in the VSSF effluent was 360 mgCOD/L (see time-profile in Figure 64).
However, COD concentration decreased progressively during the cycle and after 1 h from the feeding it reached 135 mgCOD/L, while after 6h it reached the minimum value of 60 mgCOD/L (Figure 64). The significant removal of COD observed in the first hour for both

periods (Figure 64) may be due to two phenomena: (1) physical retention of COD, especially in particulate form, by sedimentation and filtration; (2) a dilution effect due to the mixing of the influent wastewater with the pore water content during itsrapid passage throughout the VSSF bed. When the VSSF was drained by gravity a little amount of liquid is retained in the interparticle voids. Considering that the pore water content at the end of the typical VSSF cycle was approximately 5% of the gravel weight (corresponding to 120 L in the whole bed), a partial dilution of the influent wastewater can occur. As confirmed by Giraldo and Zarate (2001), when the hydraulic retention time inside the bed is of only a few minutes, the physical retention into the bed is the major mechanism for the removal of organic matter, while the biological oxidation takes place for a longer time until the next feeding and therefore low concentrations are expected in the pore water at the end of the cycle.

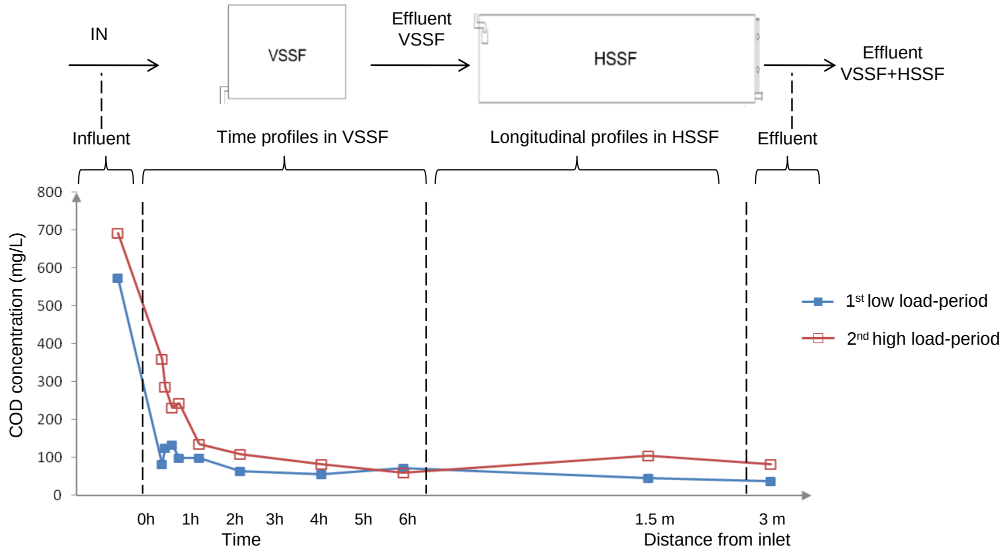

Figure 64Profiles of COD in the hybrid CW system: time-profiles in the VSSF unit, longitudinal profiles in the HSSF unit.

The COD load removed in the VSSF during the 2nd high-load period (Table 22) was 64.3 gCOD m^{-2} d^{-1} more than double the load removed during the 1st low-load period (29.9 gCOD m^{-2} d^{-1}). Although this high organic load applied in the VSSF unit, COD removal during the 2nd high-load period remained acceptable, around 74%, compared to the removal efficiency of 82% in the 1st low-load period. The increase of COD concentration in the VSSF effluent during the 2nd high-load period, especially at the beginning of the cycle, was compensated by a further reduction of COD concentration in the subsequent HSSF system, whose effluent concentration was lower than 82 mgCOD/L in both periods (see longitudinal profile inFigure 64). Despite the double organic load applied during the 2nd high-load period, the overall COD removal efficiency in the hybrid CW system (VSSF +HSSF) did not change significantly and it was 94% in the 1st low-load period and 88% in the 2nd high-load period. That is because the peaks of COD concentration effluent from the VSSF unit at the beginning of the cycle were removed in the subsequent HSSF, which contributed to remove 14% of the influent COD during the 2nd high-load period compared to 12% in the 1st low-load period.

8.3.2 Comparison of nitrogen removal during low-load and high-load conditions

In the VSSF unit the nitrification process was stable during both the 1st low-load period and the 2nd high-load period with similar removed concentrations. In fact in the VSSF unit, the mean TKN concentration decreased from 72.3 mgTKN/L to 18.3 mgTKN/L in the 1st low-load period (reduction of -54 mgTKN/L), while it decreased from 79.8 mgTKN/L to 30.2 mgTKN/L in the 2nd high-load period (reduction of -49.6 mgTKN/L) (see Table 22). However the applied and removed TKN loads were significantly different. The average applied and removed TKN loads in the VSSF unit during the 1st low-load period were 4.4 and 3.3 gTKN m^{-2} d^{-1}respectively, while in the 2nd high-load period the applied and removed loads were higher (applied load 10.3 gTKN m^{-2} d^{-1}; removed load 6.6 gTKN m^{-2} d^{-1}). The increased TKN load resulted in a decrease of the removal efficiency in the VSSF unit from 75% to 62%.

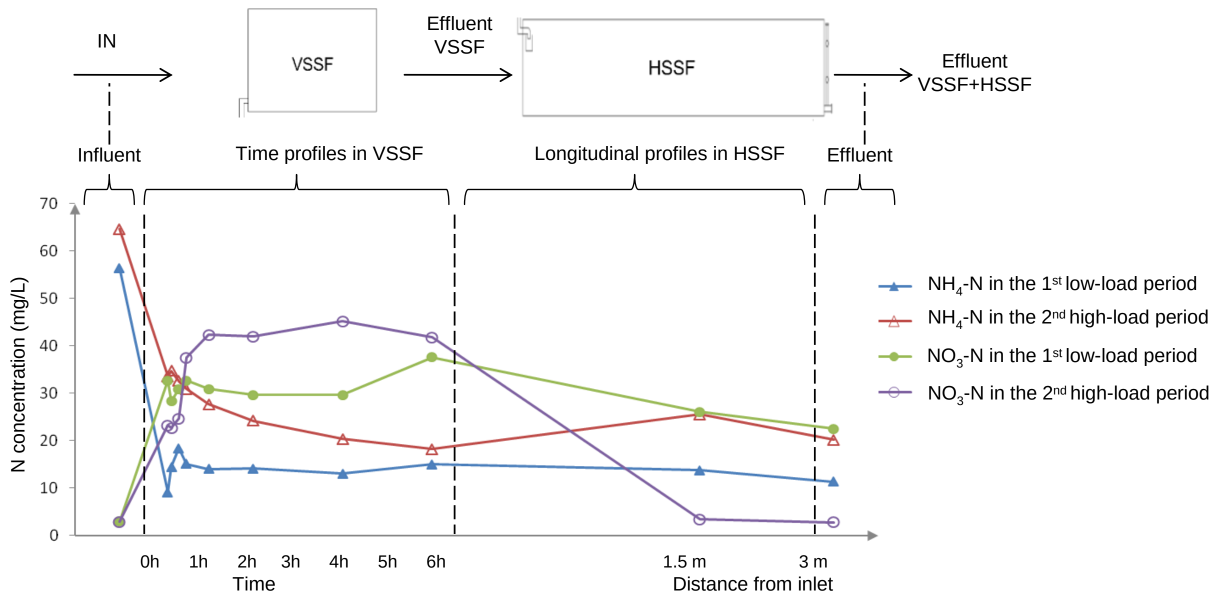

Figure 65 Profiles of NH_4-N and NO_3-N in the hybrid CW system: time-profiles in the VSSF unit, longitudinal profiles in the HSSF unit.

Observing the time-profile during the 1st low-load period (Figure 65), it can be observed that NH_4-N concentration dropped from 56.4 mgN/L in the influent wastewater to 15.1 mgN/L in the first 30 minutes and successively the NH_4-N concentration remained quite constant until the end of the cycle. Conversely, when a high load was applied during the 2nd period, the removal efficiency in the first 10 minutes from the feeding is modest (about 46-58%), compared to a removal of 68-72% between 2 and 6 hours. The NH_4-N concentration effluent from the VSSF unit during the 2nd high-load period was 26.3 mgNH_4-N/L compared to 17 mgNH_4-N/L in the 1st low-load period. This higher concentration of NH_4-N remained also along the longitudinal profile in the HSSF unit (Figure 65) and a decrease of 28-35 mV in ORP was observed along the HSSF bed (Table 22).

Considering the difference between NH_4-N in the influent and in the effluent from the VSSF unit, it was possible to estimate the specific nitrification rate in the VSSF, which resulted 2.4 gNH_4-N m^{-2} d^{-1} in the 1st low-load period and 4.7 gNH_4-N m^{-2} d^{-1} in the 2nd high-load period. Although the high organic load applied, we did not observe a decline in nitrification rate during the 2-month research period. The amount of nitrifying biomass developed in the previous low-load period and the favourable temperatures during the summer allowed the activity of the nitrifying biomass and a significant nitrification rate.

Due to nitrification in the VSSF unit, the NO_3-N concentration increased of 35.6 mgNO_3-N/L in the 1st low-load period, while the increase was lower in the 2nd high-load period. The dynamic of NO_3-N production in the two periods was different due to the different influence of simultaneous denitrification. In the 1st low-load period the NO_3-N concentration was quite constant during the entire cycle, from the first minutes until the end of the cycle after about 6 h (see time-profiles in Figure 65). Conversely, in the 2nd high-load period, NO_3-N concentration was lower at the beginning of the cycle (during the first 30 minutes after the feeding) and increased drastically after 30 minutes (Figure 65). VSSF unit adsorbs a huge amount of COD immediately after feeding, the oxygen in the bed decreases rapidly (for the rapid oxidation of readily biodegradable COD), the water content in the bed increases and oxygen transfer is limited and these are suitable conditions for the occurrence of the simultaneous denitrification. After 0.5-1 h from the feeding, 80% of the water volume was drained and aerobic conditions are restored, causing a progressive increase of NO_3-N concentration (Figure 65).

The HSSF unit played an important role in denitrification, especially during the 2nd high-load period, when a higher COD concentration was discharged from the VSSF unit to the HSSF unit (Figure 65). The biodegradable COD measured by respirometry in the VSSF effluent was 55% of total COD: this high presence of biodegradable compounds (mainly due to the hydraulic short-circuit in the first minutes after the feeding) supported the denitrification in the HSSF unit. In the HSSF effluent the biodegradable COD was 21% of total COD, indicating its consumption by denitrification. Contextually the NO_3-N concentration dropped significantly, as indicated in the longitudinal profile of Figure 65.

During the 2nd high-load period the specific denitrification rate was 0.9-1.9 gNO_3-N m^{-2} d^{-1}, higher than the value of 1.0 gNO_3-N m^{-2} d^{-1} estimated in the 1st low-load period.

In the 2nd high-load period the presence of nitrification and a significant simultaneous denitrification in the VSSF unit caused an appreciable increase of NO_2-N, which passed from 0.8 $mgNO_2$-N/L on average during the 1st low-load period to 1.9 $mgNO_2$-N/L during the 2nd high-load period. However, this increase of NO_2-N was not a problem for the effluent discharged from the plant because the VSSF effluent passed through the HSSF unit. In the HSSF system the concentration of NO_2-N dropped rapidly reaching a final effluent concentration of about 0.5 $mgNO_2$-N/L.

The phosphorus removal in the VSSF unit was 49.3% in the 1st low-load period and 36.5% in the 2nd high-load period. P removal was completed when wastewater passed through the HSSF unit in which removal efficiency was 27-48% and this behaviour did not change significantly in the two periods.

Chapter 9

Constructed wetlands for mountain regions: investigation on the effect of discontinuous loads and low temperatures[3]

9.1 Introduction

As previously demonstrated, VSSF can effectively treat wastewater with high hydraulic and organic loads during few months in summer. Nevertheless, doubts remain on whether VSSF CWs are applicable and efficient in winter with low temperatures and discontinuous loads, that is the conditions found in winter months with the presence of tourists.

Another constraint to the application of CWs in mountain areas is related to the large area required. Geomorphologic conditions – only small extensions of flat land – constitute a primary limiting factor to the installation of CWs. In Europe, the design of CWs is mostly done by using simple scaling factors (e.g. each country specifies the minimal area required for construction). Nevertheless, this is done only where a significant competences is availablein the same weather/environmental conditions. In other cases, the design is still done by the use of sizing models based on first-order kinetics rather than the use of advanced mathematical models. First-order kinetics models use the following equation for

[3] This chapter was presented at SIDISA 2012: Ortigara A. R. C., Foladori P., Ruaben J. and Andreottola G. (2012). Constructed wetlands for mountain regions: investigation on the effect of discontinuous loads and low temperatures. Proceedings of the 9th SIDISA – Sustainable Technology for Environmental Protection. Milan, Italy, 2012. ISBN: 978-88-9035572-1.

the estimation of the area of a VSSF CW. Among the parameters necessary to the first order kinetics models is the estimation of k_T is rather important during the design phase.

The biological activity in subsurface CWs is also correlated with temperature: a decrease of temperature results in a lower bacterial growth and metabolic rates are reduced as well. A modified van't Hoff-Arrhenius equation is used to estimate temperature effects on the biological reaction rates (equation 2). Even though studies have shown that seasonal temperature variations do not always significantly affect COD and BOD removal in CWs (Kadlec and Reddy, 2001), the activity of nitrifying bacteria is strongly limited below 10˚C and denitrification activity is detected only above 5˚C (Brodrick et al., 1988; Herskowitz et al., 1987; Werker et al., 2002). The temperature coefficient θ is 1.11-1.37 for nitrification at T below 10°C (Kadlec and Reddy, 2001). Temperature coefficient strongly affects the design procedure, especially in regions where the average temperature in winter is below 10°C. The use of the wrong coefficient can lead to an overestimation of the system's area, which eventually make designers and administrators prefer other technologies.

The estimation of the temperature coefficient can be done using the removal rates obtained from real or lab scale applications of the CW or by the use of specific lab tests. In the case of the nitrification rate, it can be estimated on the basis of the consumption of ammonia or oxygen concentrations for a period of few hours. Ammonia Uptake Rate (AUR) is a test developed to measure the activity of nitrifying bacteria in activated sludge measuring the amount of NH_4, NO_2 and NO_3 through time, with the aim to measure the maximum specific nitrification rate. AUR tests can also be used to evaluate the inhibition of the nitrifying biomass or the influence of temperature, oxygen and pH in the nitrification process. In this Chapter an AUR test opportunely modified for the application to VSSF CWs cores at the lab scale or collected from full-scale CW plants was proposed and applied.

This Chapter focuses on the monitoring of COD and nitrogen removal performance in the pilot plant described in the Chapter 3 under the following conditions: (1) low temperatures of 2-10°C; (2) discontinuous feeding causing long idle periods (which may reduce the active biomass within the CW bed) and low temperatures. AUR tests carried out on VSSF cores and on the granular material collected from the VSSF pilot plant were applied to evaluate the influence of temperature and discontinuous loads on the nitrification rate.

9.2 Materials and Methods

Both VSSFconfigurations were operated under winter conditions: C-line will be called Low-Load VSSF and E-line will be called High-Load VSSF in this Chapter.Samples of influent and effluents from the VSSF were collected during two winter periods (2010/2011 and 2011/2012). In the period with continuous feeding (winter months in 2011/12) the temperature of the VSSF effluent dropped from 10 to 2ºC, while the air temperature decreased to 0.6°C. In the period with discontinuous feeding, sampling campaigns were done with intervals from 13 to 30 days, to simulate the tourist presence during the winter holidays.

9.2.1 AUR Tests onVSSF lab cores

VSSF cores at lab-scale were used for evaluating the influence of temperatures on the nitrification. VSSF-1 and VSSF-2 were used (see Chapter 3 – Materials and Methods for more details on the cores' composition). At the end of the typical VSSF cycle, when wastewater was drained by gravity, a little amount of liquid remained in the interparticle voids (pore water content was approximately 5% of the wet sand/gravel weight). Thus the amount of water in the column was 370 mL before the beginning of the AUR test. The AUR test was realized with 600 mL of water fed on the top of the column with a concentration of 30-50 mgNH_4/L which drained throughout the column. The water collected at the bottom was fed again on the top of the column every 15 min. The samples collected from the bottom were analyzed for NH_4-N, NO_2-N, NO_3-N, pH and temperature. The overall test lasted 5 to 8 h until the complete oxidation of the NH_4 added. The maximum specific nitrification rate (v_N, expressed as mgN m^{-2} d^{-1}) was calculated considering the consumption of NH_4-N instead of the production of nitrite and nitrate because denitrification can take place.

AUR tests were also performed on the granular material collected from the VSSF pilot plant in order to evaluate the effects on the nitrification activity after three months at low temperatures and discontinuous feeding. Few kilograms of the granular material were collected in the top layers of the VSSFand placed in the column where AUR test was performed at controlled temperature.

9.3 Results and Discussion

9.3.1 Performances of the VSSF CWs during the regular operation period at low temperatures

During the regular operation period, air temperatures decreased to 0.6°C, while the temperature of the VSSF effluent (considered similar to the temperature inside the VSSF bed) was in the range 2.4-8.9°C in the Low-Load VSSF and 3.7-9.7°C in the High-Load VSSF. The influent and effluent concentrations and the specific loads applied in this period are indicated in Table 24.

Table 24 Average influent and effluent concentrations in the VSSF CWs and specific loads applied in the regular operation period at low temperatures.

Parameters	Units	Low-Load VSSF (C-line)		High-Load VSSF (E-line)	
		Influent	VSSF effluent	Influent	VSSF effluent
Temperature	°C	12.8	2.4-8.9 (mean 6.8)	12.3	3.7-9.7 (mean 6.9)
Total COD	mg/L	371	71	410	63
Soluble COD (S)	mg/L	146	42	166	40
Total N	mg/L	71.9	52.1	70.4	38.9
TKN	mg/L	70.3	20.1	69.0	31.6
NH_4-N	mg/L	58.5	17.0	56.2	26.8
NO_2-N	mg/L	0.0	0.6	0.0	0.1
NO_3-N	mg/L	1.6	31.5	1.6	9.4
Total P	mg/L	8.2	3.9	8.2	6.5
SST	mg/L	137	38	140	21
pH	-	7.2	7.3	7.2	7.2
Specific hydraulic load	L m^{-2} d^{-1}	70		178	
Specific organic load	gCOD m^{-2} d^{-1}	25.9		73.6	
Specific surface	m^2/PE	4.3		1.7	

Although the average applied organic load in the High-Load VSSF (73.6 gCOD m^{-2} d^{-1}) was higher than in the Low-Load VSSF (25.9 gCOD m^{-2} d^{-1}), the COD removal efficiency was similar (80% and 81% on average, respectively). A very slight influence of temperatures in the range 2-10°C on the COD removal efficiency was observed in both the VSSF CWs as shown by the coincident regression lines indicated in Figure 66 (a). Even at 2-4°C, COD removal efficiency was around 70%.

A significant TKN removal was observed even at 2-10°C (Figure 1B). On average, the TKN loads applied and removed in the High-Load VSSF were 11.9 gTKN m^{-2} d^{-1} and 6.4 gTKN m^{-2} d^{-1} respectively, while the loads applied and removed in the Low-Load VSSF were 4.9 gTKN m^{-2} d^{-1} and 3.5 gTKN m^{-2} d^{-1} respectively (Figure 66 (b)). The higher organic load reduced the TKN removal efficiency from 71% on average in the Low-Load VSSF to 54% in the High-Load VSSF (Figure 66 (b)). The result was a higher TKN concentration in the effluent from the High-Load VSSF (31.6 mg/L on average) compared to the Low-Load VSSF (20.1 mg/L on average).

For comparison, in the field of activated sludge, it is well known that the activity of nitrifying biomass is strongly reduced at temperatures below 10°C and it can disappear at temperatures below 5°C. However, in the VSSF CWs tested in this research the nitrification efficiency decreased when temperature dropped to 2°C but not disappeared, and the mean removal efficiency in the Low-Load VSSF at 2-3°C was 59% for TKN and 58% for NH_4-N.

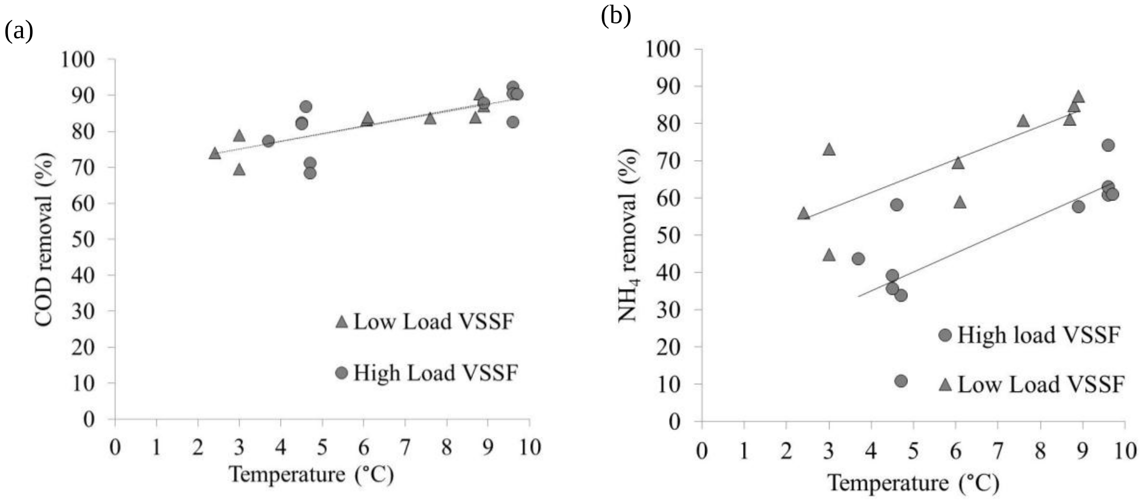

Figure 66 Removal efficiency of COD (a) and TKN (b) in the Low-Load VSSF (C-line) and High-Load VSSF CWs (E-line) as a function of the temperature.

9.3.2 VSSF CWs performance during the period with discontinuous feeding at low temperatures

The VSSF CWs were fed discontinuously (1-2 feedings per month) in order to simulate the discontinuous flows discharged by the occasional presence of tourists during weekends and holiday in winter in not skiing locations. The monitoring period lasted 3 months (December 2010-February 2011) with air temperatures below 0°C for about one month (minimum of -5°C). The aim was to evaluate the performances of VSSF CWs after a long-term stress due to discontinuous feeding coupled with temperatures of VSSF effluent wastewater in the range 1.2-3.6°C for both lines. The influent and effluent concentrations in the VSSF CWs are indicated in Table 25. Due to the feeding of influent wastewater occurring once or twice per month, it was not possible to calculate the average specific hydraulic and organic loads in the systems during the entire period. Thus, the specific loads indicated in Table 25are referred to a single cycle instead of per day.

The COD removal efficiency was 65% in the Low-Load VSSF (specific surface of 3.2 m^2/PE) and 55% in the High-Load VSSF (specific surface of 1.5 m^2/PE). The appreciable COD removal even at low water temperatures (around 2°C) was due to physical retention of particulate COD by sedimentation, filtration and the dilution associated with the mixing of the influent wastewater with the pore water content in the VSSF CW. Although the feeding occurred 1-2 fold per month, resulting in the total absence of feeding in the VSSF CWs for some weeks, an immediate recovery of performances was observed when the influent wastewater was applied in the bed.

Table 25 Average influent and effluent concentrations in the VSSF CWs in the period with discontinuous feeding and low temperatures. Specific loads were calculated per cycle.

Parameters	**Units**	**Low-load VSSF (C-line)**		**High-load VSSF (E-line)**	
		Influent	VSSF effluent	Influent	VSSF effluent
Temperature	°C	6.2	1.3-3.6 (mean 2.2)	5.9	1.2-3.5 (mean 2.1)
Total COD	mg/L	520	181	524	228
Soluble COD	mg/L	239	88	220	79
Total N	mg/L	109.5	82.6	75.5	87.5
TKN	mg/L	104	44.1	72	59.5
NH_4-N	mg/L	64.5	42.1	56.6	45.3
NO_2-N	mg/L	0.1	2.9	0	3
NO_3-N	mg/L	4.4	35.7	3.4	25
Total P	mg/L	9.8	6.2	8.2	7.5
SST	mg/L	159	74	139	90
pH	-	7.7	7.5	6.8	7.1
Specific hydraulic load	L m^{-2} $cycle^{-1}$	17.8		17.8-35.6	
Specific organic load	gCOD m^{-2} $cycle^{-1}$	8.8		14.8	
Specific surface	m^2/PE	3.2		1.5	

Applied and removed TKN loads during the discontinuous period in the Low-Load and High-Load VSSF are indicated in Figure 67 compared to the TKN loads in the regular operation period. During the period with absence of feeding and air temperatures below zero degrees, the average TKN removal in the Low-Load VSSF was 57%, while in the High-Load VSSF the TKN removal decreased from 55% in December to 0-18% in January-February. This decrease in the TKN removal efficiency in the High-Load VSSF was affected also by the higher organic load applied in the system during the previous summer and autumn periods. Conversely, in the Low-Load VSSF, the TKN removal remained always beyond 42% even after the 3 months with discontinuous feeding. In the TKN removal, the dilution of influent wastewater may be important, because the pore water content at the end of the VSSF cycle was approximately 5% of the wet sand weight.

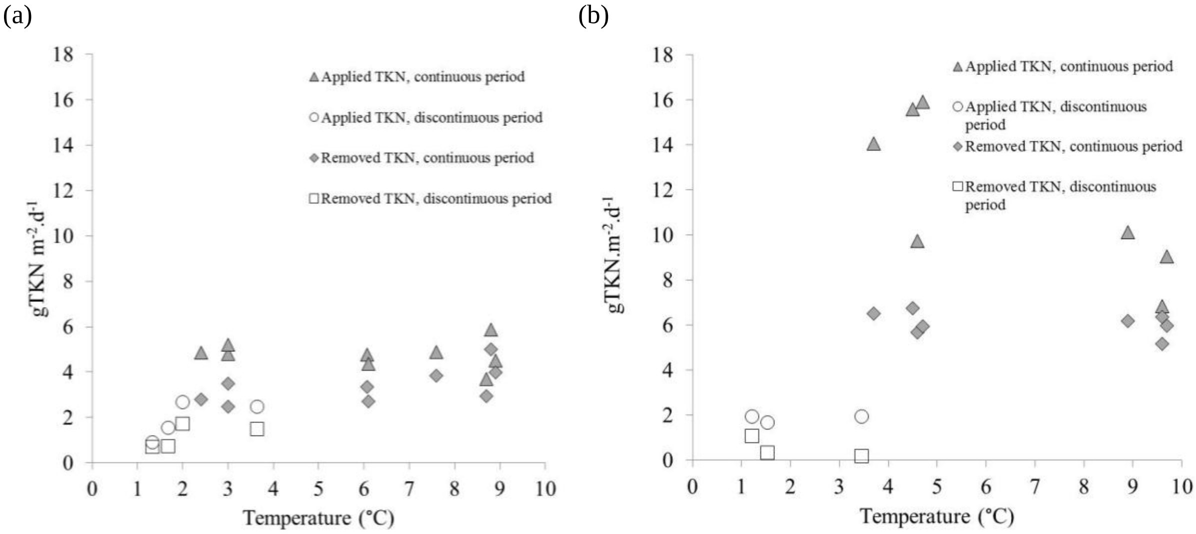

Figure 67 Applied and removed TKN loads in the continuous and discontinuous feeding for (a) Low-Load VSSF (C-line) and (b) High-Load VSSF (E-line)as a function of the temperature.

9.3.3 Influence of temperature on nitrification rate in lab VSSF cores measured by AUR tests

The influence of temperature of 2-18ºC on the maximum specific nitrification rate (v_N, expressed in gNH_4-N m^{-2} d^{-1}) was investigated in two lab cores (VSSF-1 and VSSF-2) using AUR tests. In particular, v_N was estimated using the slope of NH_4-N consumption instead of the slope of NO_3+NO_2 production, because denitrification may occur inside the granular material in pores not completely reached by oxygen and in the biofilm. Values of v_N as a function of temperature are indicated in Figure 68.

Similar values of v_N were found for the two types of VSSF cores used, considering the same organic loads applied in the cores. However, the slight difference in the v_N values may be due to the depth of the main sand layer which was 0.5 m in the VSSF-1 and 0.3 m in the VSSF-2.

Strong temperature dependence was observed in both cores, with v_N values ranging from 14.5-16.2 gNH_4-N m^{-2} d^{-1} at 18°C to 2.8-2.9 gNH_4-N m^{-2} d^{-1} at 2°C. Considering an Arrhenius-type temperature dependence ($v_{N,T} = v_{N,20°C} \cdot \theta^{(T-20)}$), the parameters $v_{N,20°C}$ and θ were estimated from the data of Figure 3 using the least square method and indicated in Table 26. The Arrhenius-type curves obtained for the two VSSFare indicated graphically in Figure 68.

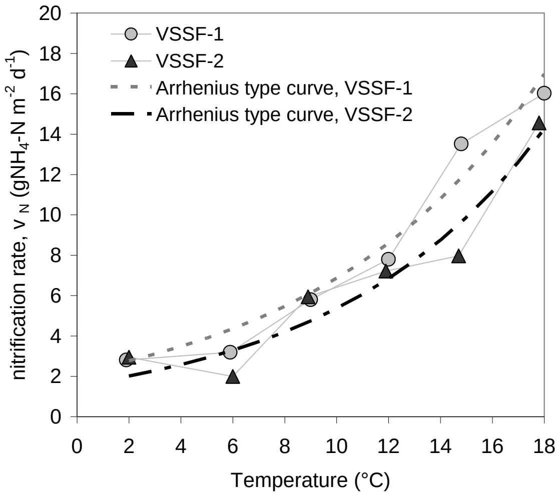

Figure 68 Influence of temperatures on v_N measured in lab VSSF cores with AUR tests.

Table 26 Estimation of $v_{N,20°C}$ and θ according to the Arrhenius-type temperature dependence from the AUR tests.

Parameter	Units	VSSF-1	VSSF-2
$v_{N,20°C}$	g m^{-2} d^{-1}	21.3	18.2
θ	-	1.12	1.13

The values obtained for θ fall within the range indicated in the literature: 1.11-1.37 for temperatures of 5-10ºC, 1.08-1.16 for temperatures of 10-15ºC and 1.06-1.12 for temperatures of 15-20ºC (Kadlec and Reddy, 2001).The values obtained for θ (1.12 – 1.13) can be used in equation 2 in order to obtain a more precise estimation of the k_T. The estimation of this parameter for the design of a VSSF CW configuration tested in this research (using the same material granulometry, organic and hydraulic loads) would be helpful to avoid an overestimation of the superficial area of these systems. On other hand, the AUR test shown to be a reliable tool that can be used for the estimation of this parameter also in other CW configurations.

9.3.4 Comparison between nitrification rates measured in lab VSSF cores and in the VSSF pilot plant at low temperatures

In lab cores the nitrification rate followed an Arrhenius-type law with a strong decrease of v_N at low temperatures. The same influence was observed in the VSSF pilot plant, where the influence of the feeding periods can be observed as well (Figure 69). For the High-Load VSSF during the regular operation period with continuous feeding, the values of v_N were consistent with the data estimated for lab cores on the basis of the Arrhenius-type law (from AUR data). Conversely, during the regular operation period in the Low-Load VSSF, the v_N values were lower than those measured by AUR in lab cores. At 3.5ºC the value of v_N was

3.2 gNH_4-N m^{-2} d^{-1} in lab cores and 1.47 gNH_4-N m^{-2} d^{-1} in the pilot plant. At 12ºC the value of v_N was 8.9 gNH_4-N m^{-2} d^{-1} in lab cores and 4.09 gNH_4-N m^{-2} d^{-1} in the pilot plant.

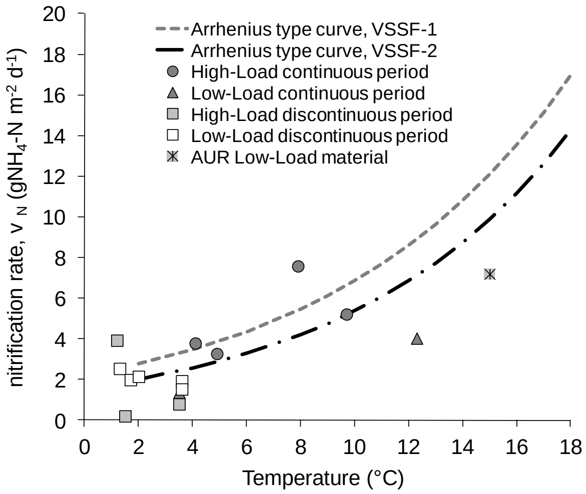

Figure 69 Nitrification rate in the VSSF pilot plants compared with the Arrhenius-type curves measured by lab AUR tests

During the winter period with discontinuous feeding it was observed that the long-time stress caused by long idle periods without feeding and low temperatures caused a strong reduction of the nitrification rate, mainly in the High-Load VSSF. In the High-Load VSSF, the nitrification rate at 1.2°C was 3.93 gNH_4-N m^{-2} d^{-1}, but it decreased strongly after a month operating with discontinuous feeding, confirming the simultaneous effects of long-time stress and low temperatures (TKN removal was reduced to about 18%). Conversely, in the Low-Load VSSF, v_N values were very similar to those obtained in the lab cores and interpreted with Arrhenius-type law.

The granular material collected from the Low-Load VSSF with discontinuous feeding underwent a lab AUR test at 15°C and a recovery in NH_4 removal efficiency was observed. The $v_{N,15}$ was 7.2 gNH_4-N m^{-2} d^{-1}, slightly lower than the $v_{N,15}$ expected from lab cores and Arrhenius-type law (Figure 69). After 3 months with discontinuous feeding, both the VSSF CWs showed a fast recovery (few days) of TKN removal efficiency with the spring arrival.Therefore, the AUR method developed for application on the VSSF material is useful to quantify the nitrification rate of real systems at different temperatures and eventually to predict the removal efficiency throughout the year.

Chapter 10

Recirculation in VSSF –CWs: a new configuration tested to reduce land area requirements[4]

10.1 Introduction

The use of Constructed Wetlands has been spreading around the world since its first application in Europe at the beginning of the 60's. However, a true widespread application has been partly limited by a critical issue, namely the large surface required to ensure high removal rates and subsequently meet the standards specified in national and international guidelines. The PAT (2002), for example, suggests a surface per inhabitant equivalent of up to 4 m^2/PE for the VSSF and 6 m^2/PEfor the HSSF.

In order to reduce the surface required by these systems, different approaches have been proposed. Some of them are based on the improvement of HSSF's removal rates, by means of artificial aeration, the use of alternative feeding periods, the modification of the filter material's thickness, the recirculation of a fraction of the HSSF outlet wastewater to the VSSF inlet(Tunçsiper, 2009; Ayaz et al., 2012), etc. Most of these approaches are used in order to increase the ammonia removal in HSSF, because it is normally limited by the prevalent anoxic conditions in the bed.

Approaches found in the literaturethat are aimed at improving removal rates per unit area in the VSSF are based on changing or alternating feeding periods, recirculating the outlet

[4] This chapter is based on: Foladori P., Ruaben J., Ortigara A. R. C., Andreottola G. (2012). Comparison of innovative Constructed wetland configurations aimed to area reduction. Proceedings of the 9th SIDISA – Sustainable Technology for Environmental Protection. Milan, Italy, 2012. ISBN: 978-88-9035572-1.

wastewater to the septic tank or to the first stage bed in the case of hybrid systems (Brix and Johansen, 2004; Brix and Arias, 2005; ÖNORM B 2505, 2008). Another recirculated configuration was developed to treat domestic wastewater produced from single houses: wastewater is treated in a vegetated VSSF CW and the effluent is collected in a storage tank and then recirculated at the top of the VSSF to prolong the contact time in the CW (Gross et al., 2007; Sklarz et al., 2009). VSSF CWs are widely known for maintaining aerobic conditions inside the bed due to the intermittent feeding conditions. The prevalence of aerobic conditions makes these systems more efficient in ammonia removal than the HSSF.

Fonder and Headley (2011) divided VSSF in 3 different categories: Down Flow (DF) CW (traditional VSSF with free-draining, open outlet, without surface flooding and with intermittent feeding), Up Flow (UP) CW (permanently flooded surface, sometimesreferred to as Anaerobic Bed) and Fill and Drain (FaD) CW (mixed flow direction, normally alternating between up and down flow with saturated and unsaturated conditions resulting from the filling and draining sequences, without surface flooding). According to Austin (2003), cyclical flood and drain steps applied within the wetland bed promotes the occurrence of nitrification and denitrification: NH_{4+} adsorbed to the filter material biofilms during the flood stage are rapidly nitrify when exposed to atmospheric oxygen in the drain stage and NO^{3-} desorbed into bulk water in the next flood stage and are used as terminal electron acceptors in bacterial respiration when a carbon source is available.

The use of saturated and unsaturated conditions in VSSF may be another possibility for increasing the removal performance per unit area, coupling the nitrification and denitrification inside the same system, in order to reduce the area of the system and area of the post treatment. Nitrification capacity inside the VSSF can be limited by the amount of oxygen transferred during the feeding phase, mainly when high organic loads are applied. During the feeding, the air pressure increases inside the bed, because the air is trapped between the two layers of water (on the top and on the bottom), it increases the dissolution of oxygen in the water. After the feeding, the water drainage within the filter materials creates a negative pressure condition that sucks air inside the bed. The theoretical computation of the amount of air that can be trapped in during the feeding was already proposed by Platzer (1999) and the amount of oxygen transferred during a feeding step is limited by the volume of liquid fed.

This chapter proposes a new configuration for VSSF CWs that allows a reduction of the land surface required by these systems. The proposed configuration is based on the recirculation of wastewater inside of the VSSF and it couples the benefits provided by natural aeration being performed during the feeding and the benefits of having saturated and unsaturated conditions inside the bed (as FaD CW). One of the advantages of this system is that the recirculation of the wastewater inside the bed does not just support the re-aeration of the bed, but it also improves the contact between wastewater and the biomass attached to filter material in the upper layers, thus improving the efficiency in COD removal, nitrification and denitrification.

10.2 Materials and Methods

C-line and E-line were evaluated in this chapter (VSSF + HSSF) while operating in parallel, but with different configurations (both lines are described in Chapter 3). The C-line was operated as a free drainage CW, following the guidelines provided by the Province of Trento (around 67 L m^{-2} d^{-1} and 31.7 gCOD m^{-2} d^{-1} on average in the VSSF, 6.36 hours between each feeding) and the automatic control was limited to the start of the feeding pump.

E-line was operated as a Recirculated VSSF. Even if just the VSSF was recirculated, the E-line will be called in this chapter Recirculated E-line. The Recirculated E-line has three main phases of operation were automatically controlled:

1) Feeding: the automatic valve is closed at the bottom of the VSSF and the feeding starts. The wastewater is pumped over the bed with intervals of 15 minutes until the water reaches a probe that controls the level inside the bed. The level inside the bed increases during the experiments, in order to test the limits of the system.
2) Recirculation: a pump starts to recirculate the water from the bottom to the top of the VSSF just after the end of the feeding phase. The duration of the recirculation is 5 minutes and about 105 litres of water are recirculated. After the first recirculation, the bed remains idle for one hour, when a new recirculation starts. This phase lasts 6 hours during which 6 recirculation steps are performed. Samples were taken from the recirculated water.
3) Drainage: after 6 recirculation steps, the automatic valve opens and the wastewater is drained to the HSSF. The bed rests for 4 hours before a new feed. The average cycle duration was 10.5 hours.

The amount of wastewater treated in the VSSF of the Recirculated E-line was 169 L $m^{-2}d^{-1}$, higher than using the C-line (67 L m^{-2} d^{-1}). The Control-line (C-line) was operated with aspecific surface area equivalent to 3.6 m^2/PE, and the Recirculated E-line was operated with 1.5 m^2/PE. The Recirculated E-line was operated with recirculated VSSF for about 10.5 months, though not continuously (operations were interrupted during winter and summer when other experiments where run in the pilot plant). The level of wastewater inside the 0.6 m deep filter material varied from 0.24 m,when all the volume was fed into the bed, toa theoretical 0.9 m, when all the volume were in the recirculation phase.

After passing through the VSSF, the wastewater flows by gravity to the HSSF. The HSSF is equipped with a group of three taps at 1,5 m from each other that allow the effluent to be sampled at 3 different elevations: 0.2 m, 0.4 m and 0.6 from the bottom. It also allows to sample along the side of the HSSF, and to simulate the outlet at different distances from the inlet, for example: collecting in the first group of taps the HSSF would represent a bed with 2.25 m^2, in the second tap it would be 5.50 m^2 and in the third, 7.75 m^2. When collecting a sample at the outlet, al the area of the HSSF will be use, and the HSSF would have 9m^2. Table 27 summarizes the main operational parameters of the VSSF and HSSF used in this chapter.

Table 27 Main operational parameters of the VSSF and HSSF systems in the C-line and Recirculated E-line

	Parameter	Units	C-line	Recirculated E-line
VSSF	Influent flow rate (hydraulic load)	L/d	150	380
	Specific hydraulic load	L m^{-2} d^{-1}	67	169
	Surface organic load	gCOD$m^{-2}d^{-1}$	32	82
	Specific area	m^2/PE	3.6	1.5
	Cycles per day (feeds per day)	#/d	3.6	2.2
	Resting period (between feeds)	H	6.6	10.8
HSSF	Specific hydraulic load	L m^{-2} d^{-1}	17	42
	Specific area (all the bed=9m^2)	m^2/PE	14.7	5.8

10.2.1 Chemical analyses

Samples of influent and effluents from VSSF and HSSF systems were collected once a week. Intensive monitoring campaigns were conducted during the VSSF Recirculated E-line operation to obtain the concentration of wastewater effluent after each recirculation. The samples were called R1 for the first recycle, R2 for the second and so on. For the HSSF, the monitoring campaigns consisted in sampling the taps over the longitudinal profile. Concentrations of COD, TKN, NH_4-N, NO_2-N, NO_3-N, PO_4-P and total P were analysed according to Standard Methods (APHA, 2005). Soluble COD was measured after filtration of the sample on 0.45-μm-membrane and Biodegradable COD was estimated as described in Chapter 3.

10.3 Results and Discussion

10.3.1 Performance of the overall systems (C-line and Recirculated E-line - VSSF+HSSF)

The average results obtained in the weekly analysis for the inlet and outlet VSSF+HSSF for C-line and Recirculated E- line are shown in Table 28. The wastewater temperature varied between 0.5 and 25°C, while the average value was around 15°C. This variation seems not to have an influence on the COD removal of the whole system (VSSF+HSSF), which was always lower than 70 mg/L after passing the VSSF+HSSF.

When comparing the C-line and Recirculated E-line (VSSF recirculated), the COD removal efficiency of the VSSF only was 79% and 86%, respectively. Considering the whole system (VSSF+HSSF), a higher efficiency was observed in the C-line, but the values obtained were similar (95.7% and 93.5% for the C-line and Recirculated E-line, respectively). Even though the removal efficiencies are not very different, the organic loads applied and removed are higher in the Recirculated E-line.

Table 28 Characterization of influent and effluent wastewater (mean ± standard deviation).

Parameter	Units	Influent wastewater	VSSF C-line	Recirculated E-line (VSSF)	HSSF C-line	Recirculated E-line (HSSF)
Temperature	°C	16.2±3.1	15.5±3.2	16.7±6.6	14.6±6.9	15.4±7.4
COD	mg/L	498±113	100±31	71±30	18±3	26±19
Soluble COD	mg/L	227±74	52±13	39±14	17±3	20±7
BOD	mg/L	159	21	12	2	3
TSS	mg/L	162±28	48±20	33±19	1.3±0.6	2.4±3.3
TKN	mg/L	73.7±10.0	19.7±6.3	22.5±10.0	3.2±2.5	8.6±8.3
NH_4-N	mg/L	58.5±9.0	14.9±5.6	17.4±9.0	2.4±2.6	6.4±7.5
NO_2-N	mg/L	0.03±0.07	0.74±0.53	0.32±0.49	0.06±0.04	0.07±0.06
NO_3-N	mg/L	2.0±1.0	34.6±10.15	21.4±11.3	17.1±8.4	11.1±8.2
Total N	mg/L	75.4±10.0	55±10.9	41.7±7.1	20.2±9.0	19.3±11.5
Total P	mg/L	8.7±1.0	6.2±0.6	6.7±0.8	2.7±1.0	3.5±1.7
pH	-	7.3±0.2	7.6±0.3	7.4±0.3	7.5±0.2	7.4±0.2
ORP	mV	-203±87	93.8±46.1	115±44	106±58	115±41

The average value of organic load applied in the Recirculated E-line was 81.5 gCODm^{-2}d^{-1}, though it reached peak values around 100 gCOD m^{-2} d^{-1}, while the values applied in the C-line were 31.7 gCOD m^{-2} d^{-1}. The organic load removed in the Recirculated E-line was 69.7 gCOD m^{-2} d^{-1} as opposed to a mere 24.9 gCOD m^{-2} d^{-1} in the C-line. This means that the recirculated VSSF in the Recirculated E-line is able to treat higher volumes of wastewater without a significant decrease in efficiency. The application of higher organic loads in the VSSF of the Recirculated E-line is not recommended when the system is operating under low temperature due to the poor efficiency, as well as the clogging problems that are normally associated with a prolonged overload.

The wastewater treated by the VSSF flows by gravity to the HSSF where further removal occurs. The applied and removed organic loads are 1.68 and 1.38 gCOD m^{-2} d^{-1} respectively for the HSSF C-line and 3.8 and 2.2 gCOD $m^{-2}d^{-1}$respectively for the HSSF on the Recirculated E-line. When considering the overall system (VSSF+HSSF), the applied and removed organic loads are 6.3 and 6.1 gCOD m^{-2} d^{-1} for the C-line and 16.3 and 15.5 gCOD m^{-2} d^{-1} for the Recirculated E-line, respectively. Figure 70 shows the applied and removed organic loads in the VSSF of C-line and Recirculated E-line (a) and VSSF+HSSF of C-line and Recirculated E-line (b).

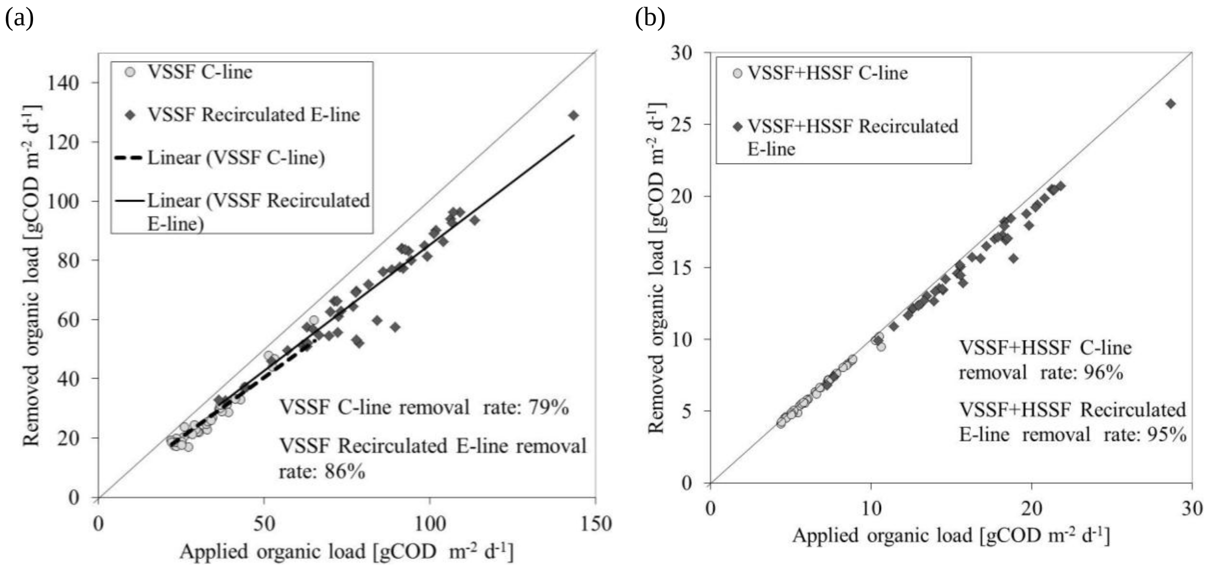

Figure 70 Applied and removed organic loads in the (a) VSSF of C-line and Recirculated E-line and (b) VSSF+HSSF of C-line and Recirculated E-line.

The VSSF on the Recirculated E-line was conceived in an attempt to foster the removal of nitrogen compounds, due to the re-aeration provided by the wastewater recirculation and the alternation of saturated and unsaturated periods. The NH_4 present in the influent wastewater was partially removed in both VSSF lines (average outlet concentrations were 15 mgNH_4/L in the C-line and 20 mgNH_4/L in the Recirculated E-line). The nitrification process in the C-line generated 33.7 mgN/L of NO_2-N + NO_3-N in the effluent of the VSSF, while allowing44.7 mg/L of NH_4 to be removed. When analysing the VSSF effluent of the Recirculated E-line, the values of NO_2-N + NO_3-N were lower (16.8 mgN/L) and the amount of NH_4 removed was 39.6mg/L. It confirms the occurrence of nitrification/denitrification inside the recirculated VSSF. The NO_2-N + NO_3-N present in the effluent of both VSSF were further removed in the HSSF where the final effluent concentration was lower than 20 mgN/L on average (Table 28).

TKN average concentrations in the inlet were 73.7 mgN/L. The maximum values in the outlet effluent were found in the coldest month, with wastewater temperatures around 5°C (16.5 mgN/L and 45 mgN/L for the C-line and Recirculated E-line respectively, and considering VSSF+HSSF). The average TKN removal efficiency was around 96% during the whole year for the C-line considering VSSF+HSSF. In the Recirculated E-line, similar removal rates were obtained during the summer period, where the biological activity where fostered by the temperature (around 93% with average temperature of 17°C). However, during the period with overload (where the TKN load was higher than 20 gTKNm^{-2}d^{-1}) or when the temperature values were around 5°C, the removal efficiency decreased (TKN average removal 66% in the Recirculated E-line). The average TKN applied and removed loads were 4.9 and 3.5 gTKN m^{-2} d^{-1} for the VSSF-C-line and 12.6 and 8.3 gTKN m^{-2} d^{-1} for the VSSF in the Recirculated E-line (Figure 71a). The TKN loads applied and removed considering the overall system (VSSF+HSSF) were 0.98 and 0.94gTKN m^{-2} d^{-1}for the C-line and 2.9 and 2.3 gTKN m^{-2} d^{-1}for the Recirculated E-line (Figure 71b).

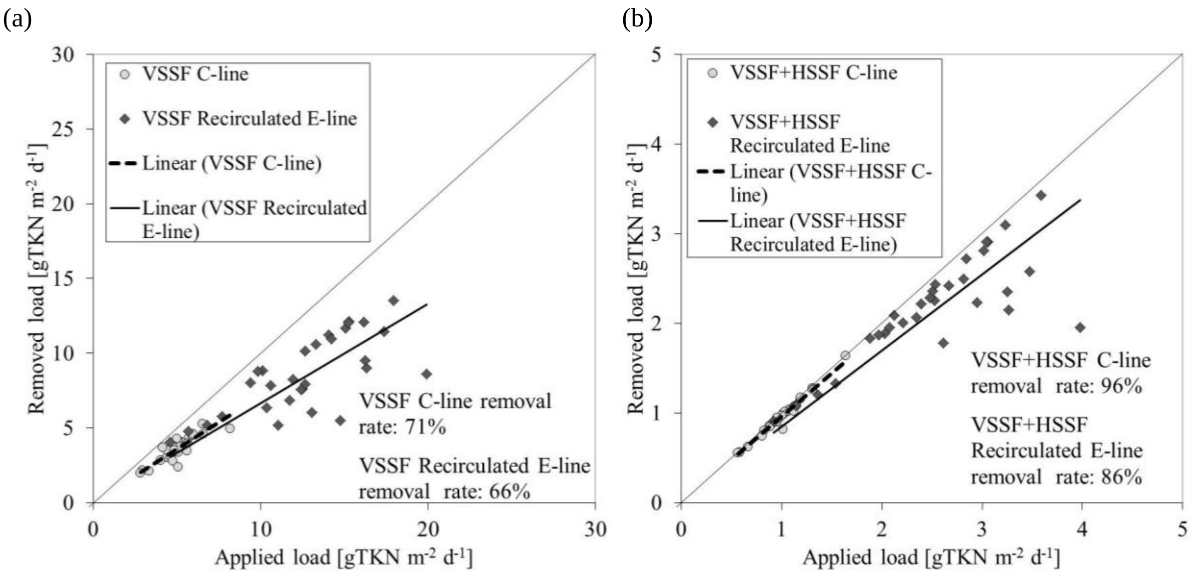

Figure 71 TKN applied and removed loads in the (a) VSSF of C-line and Recirculated E-line and (b) VSSF+HSSF of C-line and Recirculated E-line.

The P removal was higher in the Recirculated E-line due to the higher volume of wastewater treated. Regarding Total P removal, the decrease in the removal efficiency that was observed during this study was assumed to be an effect of the limited capacity of the filter material to retain P. Table 29 shows the applied and removed loads in the C-line and Recirculated E-line and the removal efficiency achieved in these tests.

Table 29 Applied and removed loads in the C-line and Recirculated E-line and removal efficiency.

	Parameter	C-line	Recirculated E-line
COD	Applied COD load in VSSF [gCOD m^{-2} d^{-1}]	31.7	81.5
	Removed COD load in VSSF [gCOD m^{-2} d^{-1}]	24.9	69.7
	COD removal efficiency in VSSF (%)	79%	86%
	COD removal efficiency in VSSF +HSSF (%)	96%	95%
TKN	Applied TKN load in VSSF [gTKN m^{-2} d^{-1}]	4.9	12.6
	Removed TKN load in VSSF [gTKN m^{-2} d^{-1}]	3.5	8.3
	TKN removal efficiency in VSSF (%)	71%	66%
	TKN removal efficiency in VSSF +HSSF (%)	96%	86%
Total N	Applied total N load in VSSF [gN m^{-2} d^{-1}]	5.3	13.0
	Removed total N load in VSSF [gN m^{-2} d^{-1}]	1.5	5.9
	Total N removal efficiency in VSSF (%)	28%	45%
	Total N removal efficiency in VSSF +HSSF (%)	78%	74%

10.3.2 Intensive monitoring campaigns in the C-line

The VSSF in the C-line was operated with a typical down flow configuration, where 43 liters of wastewater are applied and drained through the bed and moved to the HSSF during the next six hours. In this case, track studies involved the collection of all the water that was drained during the time intervals (0-5min, 5-10 min, 10-30min, 30min- 1h, 1-2 hours, 2-4 hours and 4-6 hours), and a sample representing each time step was taken out of this water. In the first 10 minutes a peak of drained water can be observed that decreases gradually until, after 3 hours, flow is almost constant (Figure 59). The VSSF drainage system developed during this study and in the first year almost 75% of the water was drained in the first hour, whereas in the second year these values decreased to 65%. This decrease could be correlated with the biomass development, even if clogging conditions were not observed.

The removal efficiency is higher starting from the first minutes after the application of the wastewater in the bed: the COD concentration of the inlet averaged 454 mg/L and the COD concentration at the time-step 0-5 min was 135mg/L (which means a removal of 70% of total COD in the first 5 minutes). The removal rate increased from around 75% after 30 minutes to 85% after 4 hours to 88% at the end of the cycle. Table 2 shows the fractions of COD at each time step. It is possible to observe the physical retention of COD particulate that occurs in the first minutes due to filtration.

Table 30 Average values of COD fractions during the entire cycle of C-line.

	Inlet Wastewater	0-5 min	5-10 min	10-20 min	20-30 min	30-60 min	1-2 h	2-4 h	4-6 h
Total COD (mg/L)	454	135	163	129	110	116	76	68	50
Soluble COD (mg/L)	227	69	67	37	48	45	41	45	44
Soluble COD/Total COD (%)	49	56	40	29	44	40	58	70	90
Biodegradable COD (mg/L)	277	-	57	49	30	13	11	-	9
Biodegradable COD/Total COD (%)	65	-	39	52	25	18	19	-	18

Table 31 shows the evolution of NH_4-N removal and the formation of NOx compounds during the down flow cycle. The NH_4 removal is around 50% in the first 5 minutes. It is maintained around 75% during the first hour and a slight increase is observed until the end of the cycle (85% of NH_4 removal). The nitrate formation reaches its peak after around 2 hours. Total Nitrogen removal is around 37% at the end of the cycle. The maximum specific nitrification rate (v_N) of 3.7 mgNH_4-N L^{-1} h^{-1}or 1.7 mgNH_4-N $m^{-2}d^{-1}$was obtained based on average values.

Table 31 Average values of nitrogen fractions during the entire cycle of the C-line.

	Inlet Wastwewater	0-5 min	5-10 min	10-20min	20-30min	30-60min	1-2 h	2-4 h	4-6 h
NH_4-N (mg/L)	65.6	25.9	17.1	18.5	17.0	16.1	14.5	12.8	10.5
NO^2-N (mg/L)	0.05	1.62	0.55	0.58	0.54	0.60	0.82	1.13	1.55
NO^3-N (mg/L)	0.1	31.0	32.0	43.0	42.2	41.9	45.0	35.0	39.7
Organic N (mg/L)	22.4	15.4	12.2	8.0	7.7	12.8	5.4	12.2	3.4
TKN(mg/L)	88.0	41.4	29.3	26.5	24.7	28.8	20.0	25.0	14.0
Total N (mg/L)	88.2	74.0	61.8	70.1	67.4	71.4	65.8	61.1	55.2

10.3.3 Intensive monitoring campaigns in the Recirculated E-line (Recirculated VSSF)

Recirculated VSSF and subsequently HSSF profiles for COD and nitrogen compounds are shown in Figure 72 and Figure 73 respectively. Recirculated VSSF profile is derived from analysis of the sample collected during each recirculation step, in order to evaluate the removal efficiency at each step. COD removal during each recirculation, can be seen in its fraction: Total COD, Soluble COD and Biodegradable COD (by respirometry) on Figure 72. After the first recirculation (R1) the COD removal already reaches 42% for Total COD, 56% for Soluble COD and 58% for Biodegradable COD. The removal of the COD fraction from the wastewater in its first passage through the bed is not just to the physical retention of particulate COD, but due to biological retention of soluble compounds takes place. In the case of the C-line (free drainage VSSF), around 50% of the wastewater volume passed through the VSSF bed in the first 30 minutes (Figure 59) and goes to the HSSF, where the amount of biodegradable COD remaining from the VSSF could be used in denitrification.

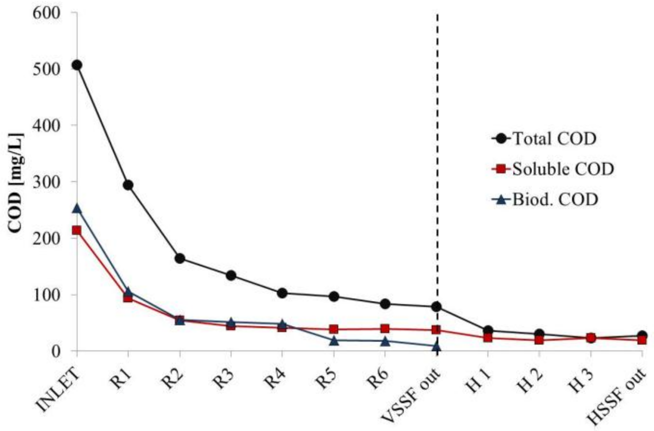

Figure 72 Time Profile for COD removal in the VSSF of Recirculated E-line and Longitudinal Profile in the subsequent HSSF.

In the system operating as a recirculated VSSF, the wastewater is maintained in the bed and is recirculated six times more, thus improving the removal of COD in the same bed: around 80% of the whole COD removal is obtained at the fourth recirculation step (R4 that corresponds to 4 hours of cycle). After 6 hours, the COD removal reaches 85% for Total COD, 82% for Soluble COD and 93% for biodegradable COD, with values of 79 mg Total COD/L, 37 mg Soluble COD/L and 10 mg biodegradable COD/L. The evolution of COD fractions along the recirculation steps can be observed in Table 32.

As shown in Figure 72, the HSSF was divided along its longitudinal profile according to the position of the taps where the samples were taken: H1, H2, H3 and Hout are a 1.5, 3.0, 4.5 and 6 meters from the HSSF inlet respectively. In the case of the Recirculated E-line, four different portions of the HSSF system (H1, H2, H3 and Hout) can be evaluated, whose average specific surfaces were 1.4, 2.8, 4.3, 5.8 m^2/PE, respectively. The HSSF had a polishing role for the removal of organic matter in that the final concentration of COD was already reached at the first tap (H1) and remained constant until the outlet (Hout), with average values around 26 mg/L of Total COD (Figure 72). This means that the first part of the HSSF (i.e. H1) would already be enough to achieve the removal performance achieved by the entire HSSF. This suggests that a significant reduction in area can be obtained when using a hybrid system with recirculated VSSF + HSSF of 2.9 m^2/PE (1.5 m^2/PE in the VSSF and 1.4 m^2/PE in the HSSF).

Table 32 Average values of COD fractions during the entire cycle of Recirculated E-line.

	Inlet Wastewater	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**VSSF OUT**
Total COD (mg/L)	517	283	180	148	115	108	93	92
Soluble COD (mg/L)	211	97	65	55	51	47	46	44
Soluble COD/Total COD (%)	41	34	36	37	44	43	49	48
Biodegradable COD (mg/L)	191	119	51	33	31	26	12	13
Biodegradable COD/Total COD (%)	37	42	28	22	27	24	13	14

In the case of the HSSF in the C-line, four different portions of the HSSF system (H1, H2, H3 and Hout) can be evaluated, whose average specific surfaces were 3.7, 7.4, 11.0, 14.7 m^2/PE, respectively. If the HSSF in the C-line was reduced at the first tap (H1) as it was suggested to do in the Recirculated E-line, a hybrid system with VSSF + HSSF of 7.4 m^2/PE (3.7 m^2/PE in the VSSF and 3.7 m^2/PE in the HSSF).

Figure 73 shows the evolution of the nitrogen compound and the redox potential (ORP) inside the bed during the recirculation. It is possible to see the decreasing profile of the NH_4 concentration in the recirculated VSSF of the Recirculated E-line. 42% of NH_4 removal in the first recirculation step, which means that this removal happen during the first passage of the wastewater through the filter material, even when the system is operated at the normal Down-flow configuration. In the C-line, this removal corresponds to 50% of the volume that is drained in the first 30 minutes and flows to the HSSF (in the Figure 59). In the case of the Recirculated E-line, the removal is occurring across the entire volume treated.

During the recirculation steps the NH_4 removal increased until 73% in the last recirculation step. The NH_4 removal is followed by the formation of NOx compounds and the increase of ORP values. The removal of NH_4 was 40 mgN/L on average and the NO_2-N + NO_3-N formation was just 20 mg/L, confirming the occurrence of nitrification and denitrification inside the bed. The amount of NO_2-N + NO_3-N formed is partially removed in the HSSF CW, and the final effluent has values of NO_2-N + NO_3-N around 12 mg/L. The average removal of Norg was 92%, which means that the sytem achieved values of 2.5 mgNorg/L in the effluent of the VSSF (Recirculated E-line), when the initial concentration was about 30 mgNorg/L. The final concentration of Norg was < 1 mgNorg/L. The removal rate of TKN reaches 82%, which means values of 16 mgTKN/L at the outlet of the VSSF CW, and around 3 mgTKN/L at the final effluent.

Table 33 Average values of nitrogen fractions during the entire cycle of Recirculated E-line.

	Inlet Wastewater	R1	R2	R3	R4	R5	R6	VSSF OUT
NH_4-N (mg/L)	58.1	35.8	31.9	29.1	25.5	23.2	21.1	20.1
NO_2-N (mg/L)	0.04	1.91	0.45	0.33	0.28	0.23	0.24	0.21
NO_3-N (mg/L)	1.5	10.5	12.7	14.6	18.8	20.3	20.4	20.1
N Org (mg/L)	22.6	22.5	16.4	9.0	4.5	3.5	4.1	1.7
TKN(mg/L)	66.2	61.8	50.6	39.9	29.1	30.4	28.8	18.5
N totale (mg/L)	67.2	72.4	60.7	50.9	43.0	45.8	43.2	43.2

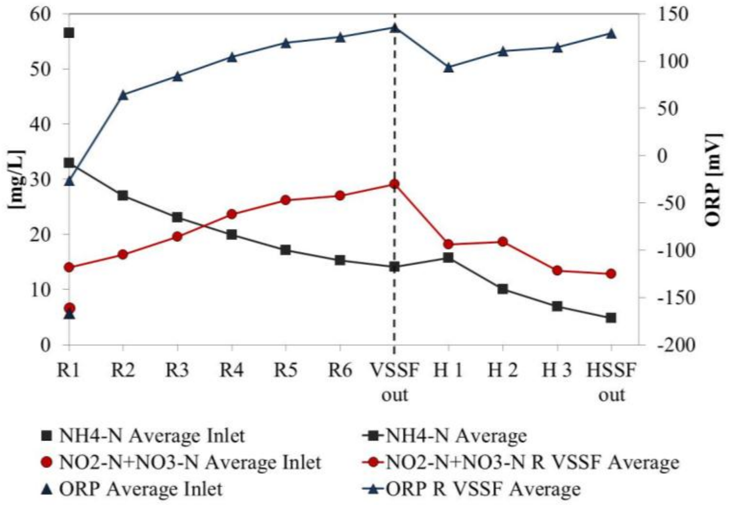

Figure 73 Time Profile for Nitrogen compounds and ORP in the recirculated VSSF and Longitudinal Profile in the subsequent HSSF in the Recirculated E-line.

Even if the COD removal did not improve after the 4th recirculation step, the same did not happen with the NH_4 removal where the removal was increasing until the last recirculation step. Regarding the HSSF, average NH_4 values were lower than 20 mg/L at the first tap (H1), reaching 10 mg/L at the second sampling point (H2). When using the entire HSSF, the specific surface of the system reached5.8 m^2/PE on average. Considering the removal of nitrogen compounds and reducing the area of the HSSF to the sampling point H2, the specific surface area would befurther reduced, leading to a hybrid system with 1.5 m^2/PE in the recirculated VSSF and 2.8 m^2/PE in the HSSF.

The influence of temperature variation from 3 to 22ºC on the maximum specific nitrification rate (v_N, expressed in gNH_4-N m^{-2} d^{-1}) was investigated during track studies. The value of v_N was estimated using the slope of NH_4-N consumption instead of the slope of NO_3+NO_2 production, because of the denitrification that was occurring inside the filter. Values of v_N as a function of temperature are indicated in Figure 74.

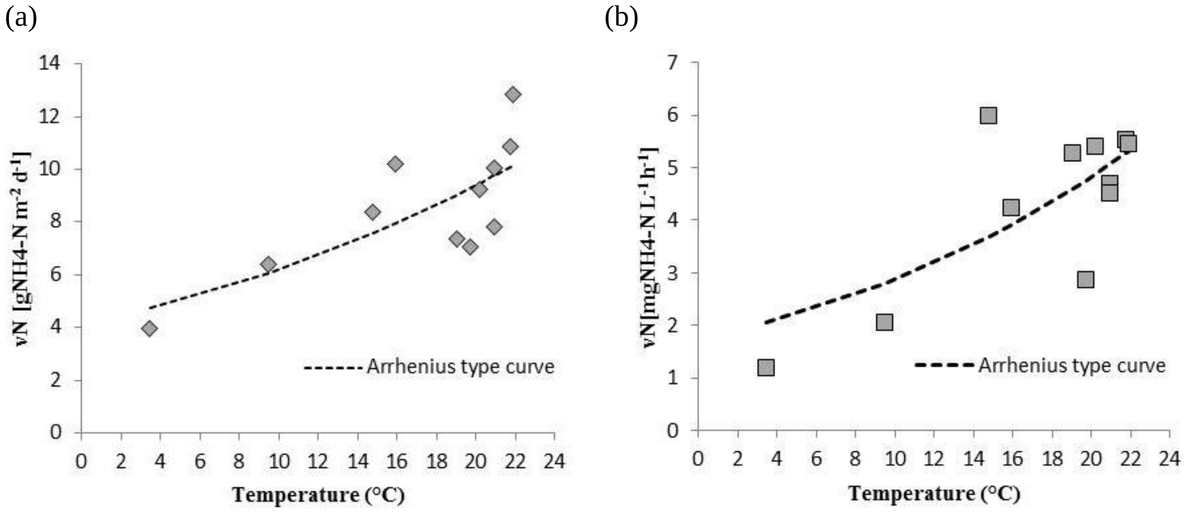

Figure 74 Maximum specific nitrification rate (v_N) expressed in (a) gNH_4-N m^{-2} d^{-1} and (b) gNH_4-N L^{-1} h^{-1}.

As temperature varied between 3.5°C and 22°C during track studies done during the experiments run for this chapter, though the values of ammonia removal rate also had a variation from 3.9 to 13 gNH_4-N $m^{-2}d^{-1}$ at 3°C and 22°C, respectively. These values are in accordance with the values obtained with the AUR test in lab cores where the maximum specific nitrification rate was of 3.2 gNH_4-N $m^{-2}d^{-1}$at a temperature of 3.5°C and 14.5-16.2 gNH_4-N $m^{-2}d^{-1}$at a temperature of 18°C (see Chapter 9).

Considering the removal efficiency expressed as a percentage, the two VSSFs (C-line and Recirculated E-line) showed similar performances. However, the main difference lies in the actual amount of pollutant removed (Kg) per m^2: the average TKN removed load was 3.5 gTKN m^{-2} d^{-1} in the VSSF C-line and 8.7 gTKN m^{-2} d^{-1} in the VSSF E-line, that isthe amount of TKN removed was more than double in the latter case. Regarding the organic load removed, the Recirculated E-line removed three times as much as the C-line.

Chapter 11

Use of aeration in VSSF CWs as a tool for area reduction[5]

11.1 Introduction

In subsurface flow constructed wetlands, plants were usually known to be the dominant oxygen-transfer mechanism. However some studieshave shown that oxygen-transfer rates convened by plants are smaller than the oxygen demand exerted bythe wastewater, even under common loading conditions (Wu et al., 2001). This has led to the development of treatment system that could provide higher rates of oxygen transfer as tidal flow CW, the use of passive air pumps on VSSF CW and artificial aeration at the bottom of the HSSF CW.

Tidal flow CWs are also known as flood and drain wetland (FaD) system and they can provide effective treatment for nitrification and nitrogen removal (Austin, 2006). The nitrification is enhanced due to the cation exchange capacity that happens during the flooding and drainage phases in these systems. When the CW is flooded, ammonium cations (NH_4^+) adsorb to negatively charge surfaces and when the wetland drains the pore volumes are filled with air. The air diffusion favours the oxygen transfer and enhances nitrification of adsorbed ammonium ions, which are nitrified under these conditions (McBride and Tanner, 2000). Nitrate (NO_3^-) and nitrite (NO_2^-) anions would be desorbed in the wastewater in the next flood cycle as terminal electron acceptors for bacterial respiration.

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[5] This chapter is based on: Foladori P., Ruaben J., Ortigara A. R. C., Andreottola G. (2012). Comparison of innovative Constructed wetland configurations aimed to area reduction. Proceedings of 9th SIDISA – Sustainable Technology for Environmental Protection. Milan, Italy, 2012. ISBN: 978-88-9035572-1

Green et al. (1998) tested the use of passive air pumps in an enhanced VSSF CW. Their system comprises of an unsaturated zone of gravel media overlaid by coarse sand (which results in surface ponding to uniform distribution wastewater across the media), vertical aeration pipes connect the outer atmosphere with the sub layers of the bed and a siphon which regulates the drain and flood phases. In this case, oxygen transfer is enhanced by a cyclical sequence of slow and fast draw of the lower layers which induces the exchange of air in the bed via the vertical pipes. The volume of effluent drained by the siphon is displaced by an equal volume of fresh airpassively sucked from the atmosphere into themedia. While the air exchange between the system and the atmosphere is mainly governed by the convection mechanism, oxygen distribution in the system is governed mainly by the diffusion mechanism (Green et al, 1998).

Artificial aeration systems have been used to improve oxygen transfer in HSSF CW. These systems usually operate under anaerobic conditions due to the limited oxygen diffusion from air to water. This is a consequence of the constant level of saturation that is maintained in these beds. In this case compressed air is diffused at the bottom of the HSSF CW by means of perforated pipes. The distribution system can be placed at the bottom of the whole bed (Nivala et al., 2007) or just in the initial section of the bed where the load is applied (Ouellet-Plamondon et al., 2006; Maltais-Landry et al., 2009). The artificial aeration can be supplied continuously or discontinuously depending on the dissolved oxygen concentration in the bed (Zhang et al., 2010).

In this chapter, an enhanced configuration of VSSF will be tested. This configuration is characterized by the saturation of the bottom of the VSSF and cyclical artificial aeration. It was tested in an attempt to increase the applied loads in the pilot plant (conventional VSSF+HSSF configuration) and investigate the efficiency of these systems under high hydraulic and organic loads, assessing the area reduction that can be achieved with this configuration.

11.2 Materials and Methods

C-line (as described in the Chapter 3) works as a traditional system of free drainage (as in Chapter 7 and 10): the supply of effluent from the Imhoff tank is every 6.56 h. The VSSF effluent flows by gravity in the sector HSSF. The specific surface area was 4.2 m^2/PE on average. The hydraulic load was 65.2 $Lm^{-2}d^{-1}$. The specific surface area in the subsequent HSSF was around 19.5 m^2/PE.

E-line operated with artificial aeration in the VSSF (Aerated E-line): the VSSF system was operated with an aerated configuration (10.8 h/cycle, 2.2 cycles/day on average). The applied organic load was twice as much as the loads applied in the C-line configuration resulting in a VSSF specific surface area of 1.9 m^2/PE on average. The hydraulic load applied in the Aerated E-line was 135 L $m^{-2}d^{-1}$. The wastewater was applied on top of the VSSF and the automatic valve at the bottom of the bed was closed in order to maintain saturated conditions in the bottom layers. During this phase, the aeration system worked for 5 minutes every 30 minutes through holed pipes installed at the bottom of the VSSF bed. This phase lasted approximately 6 h/cycle (6h-phase). Table 34 summarizes the main operational parameters of the VSSF and HSSF used in this chapter.

Table 34 Main operational parameters of the VSSF and HSSF systems in the C-line and Aerated E-line.

	Parameter	Units	C-line	Aerated E-line
VSSF	Influent flow rate (hydraulic load)	L/d	149	304
	Specific hydraulic load	L m^{-2} d^{-1}	65	135
	Surface organic load	gCOD$m^{-2}d^{-1}$	25	58
	Specific area	m^2/PE	4.2	1.9
	Cycles per day (feeds per day)	#/d	3.6	2.2
	Resting period (between feeds)	H	6.6	10.8
HSSF	Specific hydraulic load	L m^{-2} d^{-1}	16	34
	Specific area (all the bed=9m^2)	m^2/PE	19.5	8.7

11.2.1 Chemical analyses

Samples of influent and effluents from VSSF and HSSF systems were collected once a week. Intensive monitoring campaigns were conducted during the VSSF Aerated E-line operation to obtain the concentration of wastewater effluent after each aeration. Samples of wastewater were taken in correspondence of the aeration every 30 minutes using a piezometer connected with the bottom of the system. The samples were called A1 is the one just after the feeding, the A2 corresponds to 30 minutes after feeding, and so on until the A12 that corresponds to 5h 30 min from the beginning of the cycle and the outlet sample that is a composite sample of the first 20 minutes of the draining phase. At the end of the cycle the valve was opened and wastewater was discharged to the HSSF for 4 h. Concentrations of COD, TKN, NH_4-N, NO_2-N, NO_3-N, PO_4-P and total P were analysed according to Standard Methods (APHA, 2005). Soluble COD was measured after filtration of the sample on 0.45-μm-membrane and Biodegradable COD was estimated as described in Chapter 3.

11.3 Results and Discussion

11.3.1 Performance of the overall systems (C-line and Aerated E-line -VSSF+HSSF)

The average temperature of the inlet wastewater ranged from 17.1 to 20.2°C, while the temperature of the effluent of the Aerated E-line's VSSF ranged from 13.8 to 21.2 °C and the temperature of the effluent of the HSSF ranged from 12.1 to 21.8°C. For the C-line the range of temperature was from 12.3 to 20.2 °C for the VSSF and 12.1 to 21.2 °C for the HSSF. The average results obtained in the weekly analysis for the inlet and outlet VSSF+HSSF for the C-line and the Aerated E- line are shown in Table 35.

When considering the VSSF only, the COD removal efficiency of the C-line and the Aerated E-line was 78.4% and 88.6%, respectively. Considering the whole system (VSSF+HSSF), a higher efficiency was observed in the C-line, but the values obtained were similar (95.6% and 94.9% for the C-line and the Aerated E-line, respectively).

Table 35 Characterization of influent and effluent wastewater (mean ± standard deviation).

Parameter	Units	Influent wastewater	VSSF C-line	Aerated E-line VSSF	HSSF C-line	Aerated E-line HSSF
Temperature	°C	18.6±1.1	17.0±2.9	18.0±2.6	16.9±3.1	17.0±3.1
COD	mg/L	406±129	100±38	51±17	17±2	20±2
Soluble COD	mg/L	175±29	80±9	31±3	16±2	18±2
TSS	mg/L	121±20	56±6	22±16	1.2±0.4	4.9±9.3
TKN	mg/L	82.0±9.1	17.3±1.7	23.4±5.2	2.4±1.8	6.0±0.14
NH_4-N	mg/L	64.7±7.8	13.2±1.6	19.4±4.6	1.2±1.2	3.6±2.0
NO_2-N	mg/L	0.01±0.005	0.53±0.18	0.18±0.07	0.01±0.01	0.04±0.08
NO_3-N	mg/L	1.54±0.7	34.8±7.6	20.4±8.9	17.7±5.4	12.7±5.2
Total N	mg/L	83.2±8.7	51.4±12	37.2±6.3	19.2±7.7	18.5±7.0
Total P	mg/L	8.6±0.9	6.2±0.5	6.5±0.2	2.9±0.2	3.7±0.6
pH	-	7.1±0.2	7.7±0.2	7.7±0.1	7.5±0.2	7.3±0.1
ORP	mV	-315±6	66.3±11.7	76±27	100±39	92±24

The maximum organic load applied in the VSSF of the Aerated E-line was around 70 gCOD m^{-2} d^{-1}. The average value of organic load applied and removed in the VSSF of the Aerated E-line was 57.6 gCO $m^{-2}d^{-1}$ and 50.7 gCOD$m^{-2}d^{-1}$, respectively (88.6% of removal efficiency). The values applied in the VSSF of the C-line were lower, around 24.8 gCOD $m^{-2}d^{-1}$ and the removed organic load was 19.7 gCOD$m^{-2}d^{-1}$ (79% of removal efficiency).

The wastewater treated by the VSSF flows by gravity to the HSSF where further removal occurs. The applied and removed organic loads were 1.3 and 1.0 gCOD m^{-2} d^{-1} respectively for the HSSF of the C-line and 1.7 and 1.0 gCOD $m^{-2}d^{-1}$ respectively for the HSSF of the Aerated E-line. When considering the overall system (VSSF+HSSF), the applied and removed organic loads were 2.66 and 2.46 gCOD m^{-2} d^{-1} for the C-line and 11.5 and 11.0 gCODm^{-2} d^{-1} for the Aerated E-line, respectively. Figure 75 shows the applied and removed organic loads in the VSSF of C-line and Aerated E-line (a) and VSSF+HSSF of C-line and Aerated E-line (b).

The aeration provided in the VSSF Aerated E-line for 5 minutes every 30 minutes allows a shift from aerated to non-aerated periods in the saturated bottom of the VSSF bed. The NH_4 present in the influent wastewater (65 mgNH_4/L) was partially removed in both VSSF lines (average outlet concentrations were 13 mgNH_4/L in the C-line and 19 mgNH_4/L in the E-line, respectively 79% and 69% of removal efficiency).

The amount of NO_2-N + NO_3-N produced in the C-line was 34 mgN/L and the amount of NH_4 removed was 49 mgN/L. Even though the E-line was aerated, the occurrence of nitrification/denitrification could be observed when analysing the effluent of the VSSF of the Aerated E-line. The values of NO_2-N + NO_3-N produced were lower (18mgN/L) than the amount of NH_4 removed (42 mgN/L), confirming the occurrence of denitrification. The NO_2-N + NO_3-N present in the effluent of both VSSF were further denitrified in the HSSF, with higher removal in the Aerated E-line where the final effluent concentration was around 13 mgN/L on average, while in the C-line the average value was 18 mgN/L (Table 35).

(a) (b)

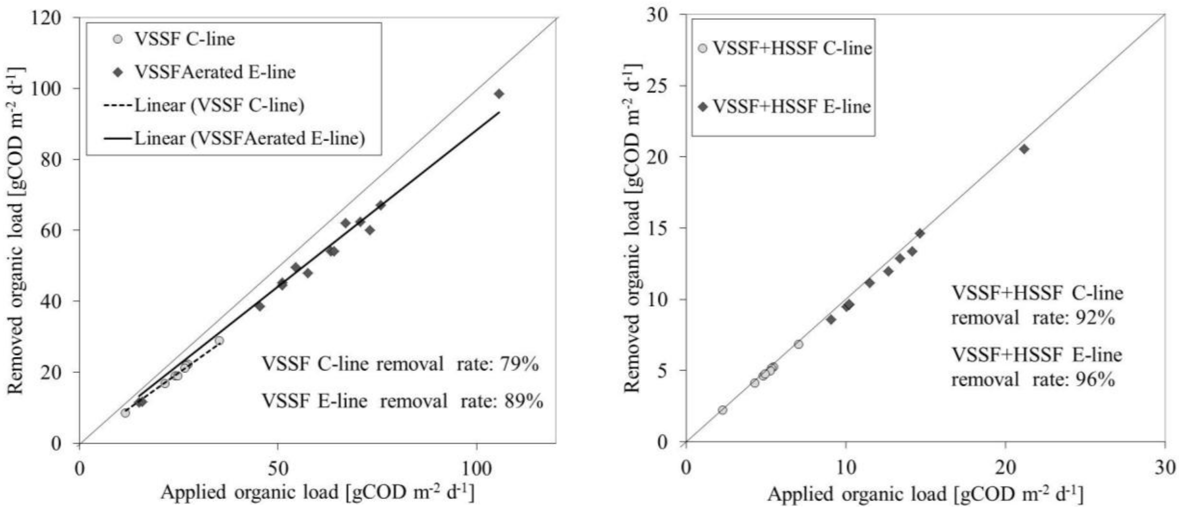

Figure 75 Applied and removed organic loads in the (a) VSSF of C-line and Aerated E-line and (b) VSSF+HSSF of C-line and Aerated E-line.

Figure 76 shows the TKN removal obtained during this phase. TKN concentrations in the inlet were around 82 mgN/L. TKN removal efficiency in the VSSF were 78% and 74% for the C-line and Aerated E-line, respectively. The removal efficiency in the VSSF in the Aerated E-line was lower than observed in the VSSF C-line due to the higher volumes of wastewater treated. The saturation of the bottom of the bed during the filling phase, and the subsequent aerationphases (5 minutes every 30 minutes) were not enough to reach the same efficiency obtained with the free drainage system. However, the amount of wastewater system was almost twice as much.

Considering the overall system (VSSF+HSSF), the average TKN removal efficiency was very similar: around 96% for the C-line and around 93% for the Aerated E-line. The average TKN applied and removed loads were 5.4 and 4.3 gTKN m^{-2} d^{-1} for the VSSF C-line and 12.4 and 9.2 gTKN m^{-2} d^{-1} for the VSSF Aerated E-line (Figure 76a). The TKN loads applied and removed considering the overall system (VSSF+HSSF) were 1.09 and 1.05 gTKN m^{-2} $d^{-1}$for the C-line and 2.48 and 2.29 gTKN m^{-2} $d^{-1}$for the Aerated E-line (Figure 2b). Total nitrogen removal is higher in the Aerated E-line VSSF (53%) than in the C-line (35%). This occurs because denitrification in the Aerated E-line already starts in the saturated bottom of the VSSF, and it continues in the HSSF. In the case of the C-line, denitrification occurs mainly in the HSSF. The final concentration in the system is 16.9 mg/L (C-line) and 21.5 mg/L (Aerated E-line).

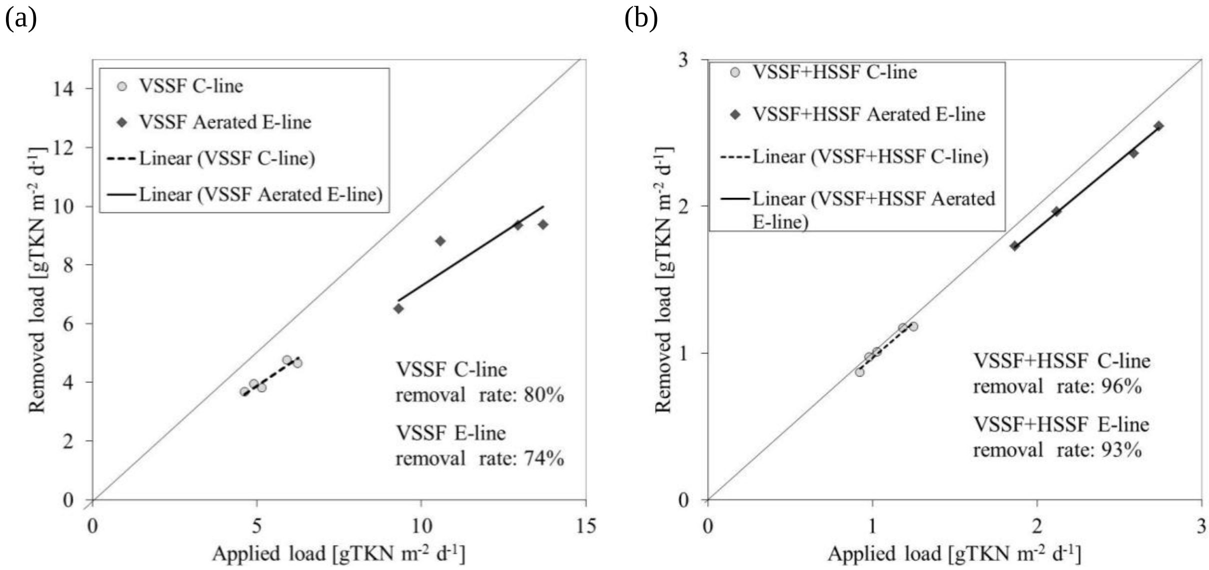

Figure 76 TKN applied and removed loads in the (a) VSSF of C-line and Aerated E-line and (b) VSSF+HSSF of C-line and Aerated E-line.

Total P concentrations in the VSSF C-line and the VSSF Aerated E-line effluents were similar (6.2 and 6.5 mg/l, respectively). Regarding the HSSF, the output was slightly lower at the C-line (2.9 mg/L) when compared to the Aerated E-line (3.7 mg/L). Table 36 shows the applied and removed loads in the C-line and Recirculated E-line and removal Efficiency.

Table 36 Applied and removed loads in the C-line and Recirculated E-line and removal efficiency.

	Parameter	C-line	Aerated E-line
COD	Applied COD load in VSSF [gCOD m^{-2} d^{-1}]	24.8	57.6
	Removed COD load in VSSF [gCOD m^{-2} d^{-1}]	19.7	50.7
	COD removal efficiency in VSSF (%)	79%	89%
	COD removal efficiency in VSSF +HSSF (%)	92%	96%
TKN	Applied TKN load in VSSF [gTKN m^{-2} d^{-1}]	5.4	12.4
	Removed TKN load in VSSF [gTKN m^{-2} d^{-1}]	4.3	9.2
	TKN removal efficiency in VSSF (%)	80%	74%
	TKN removal efficiency in VSSF +HSSF (%)	96%	93%
Total N	Applied total N load in VSSF [gN m^{-2} d^{-1}]	5.5	13.6
	Removed total N load in VSSF [gN m^{-2} d^{-1}]	1.9	7.2
	Total N removal efficiency in VSSF (%)	35%	53%
	Total N removal efficiency in VSSF +HSSF (%)	75%	76%

11.3.2 Intensive monitoring campaigns in the C-line

During the period when aeration tests were performed in the Aerated E-line, only one intensive monitoring campaign was conducted in the C-line. In general, the results obtained during this period in the C-line were similar to those shown in Chapter 10, which were obtained under the same operation conditions. During that specific campaign, the total COD concentration in the inlet was 375 mg/L, and the composite sample of the first 5 minutes the concentration in the outlet was already 170 mg/L (80% of removal efficiency) and it slightly decreased until the end of the cycle (final concentration was 17 mg/L). Soluble COD concentration in the inlet was 116 mg/L (31% of the total COD) and it decreased to 55 mg/L in the first 5 minutes of composite sample and reached 22 mg/L at the composite sample correspondent to 1-2 hours from the feeding (81% of the Total COD), showing that the bed retained the particulate COD present in the inlet wastewater.

Nitrogen fractions on the C-line are shown in Table 37. It is possible to observe a decrease in the NH_4-N concentration of 65% after the first 5 minutes of the cycle, increasing to 90% after 6 hours. NO_3-Nproduction (from almost zero in the influent to 40.2 mg/L at the effluent) confirms that nitrification occured inside the bed.

Table 37 Average values of nitrogen fractions during the entire cycle of C-line.

	Inlet Wastewater	0-5 min	5-10 min	10-20min	20-30min	30-60min	1-2 h	2-4 h	4-6 h
NH_4-N (mg/L)	76.8	26.9	27.9	22.8	19.9	17.2	13.6	10.0	7.3
NO_2-N (mg/L)	0.01	0.25	0.25	0.31	0.44	0.52	0.98	1.41	2.05
NO_3-N (mg/L)	0.1	27.9	27.8	31.4	32.7	32.9	36.7	43.1	40.2
N Org (mg/L)	11.6	7.1	4.7	4.3	3.2	0.1	0.8	0.5	1.4
TKN(mg/L)	88.4	34.0	32.6	27.1	23.1	17.3	14.4	10.5	8.7
Total N (mg/L)	88.5	62.1	60.7	58.8	56.2	50.7	52.1	55.0	50.9

11.3.3 Intensive monitoring campaigns in the Aerated E-line (VSSF)

The profile of COD fractions in the Aerated E-line is shown in Figure 77. The average COD concentration of the inlet wastewater of the E-line was around 413 mg/L, while the Soluble COD was 130 mg/L (32% of the Total COD).

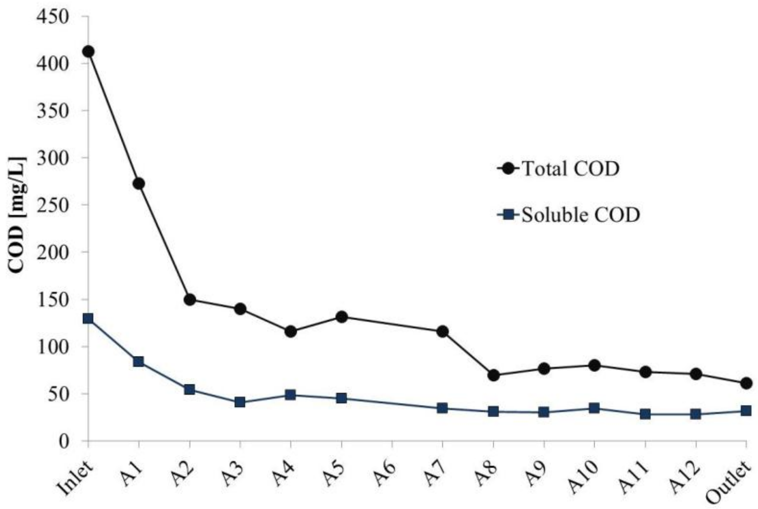

Figure 77 Average values of COD removal in the VSSF in the Aerated E-line over time.

Table 38 shows the results obtained in one intensive monitoring campaign as an example of the profile obtained in the tests. In the inlet wastewater, the concentration of biodegradable COD was 311 mg/L, corresponding to 68% of the Total COD. After the first aeration (A1) the COD removal already reached 46% for Total COD, 65% for Soluble COD and 48% for Biodegradable COD. After the ninth aeration step (A9), Biodegradable COD/Total COD ratios between 29 and 31% were observed from the A9 to the VSSF outlet. The effluent of the Aerated E-line was 62 mg/L for Total COD, 28 mg/L and Soluble COD (45% of the Total COD) and 10 mg/L of Biodegradable COD (29% of the Total COD). This remaining COD was depleted in the subsequent HSSF until it reached values of 18 mg/L of Total COD (Figure 79).

Table 38 COD fractions during the entire cycle of Aerated E-line resulting from one monitoring campaign.

	Inlet	A 1	A 2	A 3	A 7	A 8	A 9	A 10	A 11	A 12	Outlet
Total COD (mg/L)	457	211	150	116	126	44	65	55	44	55	34
Soluble COD (mg/L)	116	75	53	32	32	22	37	27	27	22	22
Soluble COD/Total COD (%)	25	36	35	28	25	50	57	49	61	40	65
Biodegradable COD (mg/L)	311	136	90	67	44	-	21	-	14	-	10
Biodegradable COD /Total COD (%)	68	64	60	57	35	-	30	-	31	-	29

Figure 78 and Table 39 show the average results obtained for nitrogen fractions and ORP during the cycle of the VSSF Aerated E-line. With regards to nitrification (Figure 78), the concentration of NH_4-N in the influent wastewater was reduced from 55 mg/L to 20.2 mg/L (values of 22.5 mg/L of NH_4-N were reached at A8), confirming the average reduction of 63% over the entire monitoring period (2 months, from August to October). In the aerated configuration, wastewater drains to the saturated bottom layers where oxygen is supplied, but wastewater is not put in contact with the nitrifying biomass present in the top layers of the bed as occurred in the Recirculated configuration shown in Chapter 10.

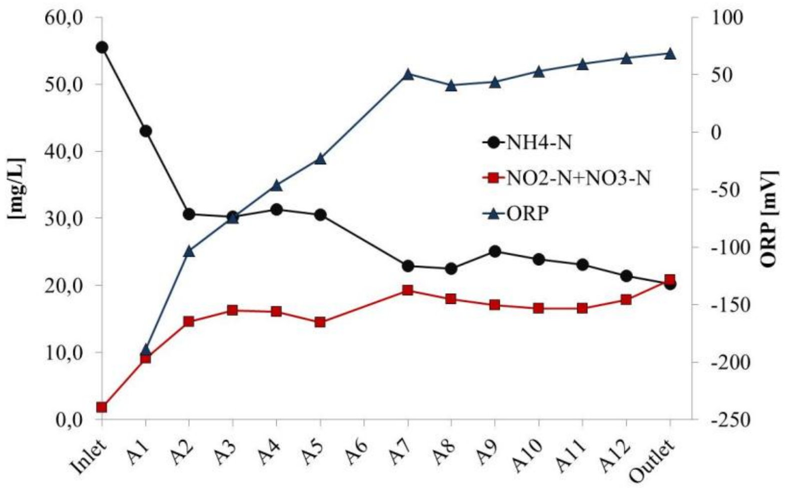

Figure 78 Time profile for nitrogen compounds and ORP in the VSSF and HSSF of the Aerated E-line.

Time profiles shown in Figure 77 and Figure 78 suggest that the number of aeration steps could be reduced. Stopping the aeration phase at the A8 aeration step, for example, would reduce the depuration cycle and, subsequently the area of the system. However, studies should be conducted to evaluate the effects of cutting the aeration cycle and discharging the effluent in the HSSF.

Table 39 Average values of nitrogen fractions during the entire cycle of Aerated E-line.

	Inlet	**A 1**	**A 2**	**A 3**	**A 7**	**A 8**	**A 9**	**A 10**	**A 11**	**A 12**	**Outlet**
NH_4-N (mg/L)	55.5	43.0	30.6	30.3	23.0	22.5	25.1	23.9	23.1	21.4	20.2
NO_2-N (mg/L)	0.01	0.35	0.32	0.18	0.13	0.11	0.14	0.13	0.13	0.12	0.01
NO_3-N (mg/L)	1.8	8.8	14.3	16.1	19.1	17.9	16.9	16.5	16.5	17.7	20.7

In the aerated configuration, the occurrence of saturated conditions in bottom layers (aerated only few minutes per hour) enabled denitrification. The NO_3-N concentration in the effluent reached 19.9 mg/L as confirmed by the ORP profile that highlights the occurrence of denitrification. The removal of Total N was 49% in the VSSF. The effects of aeration applied in the VSSF bed are different from expected. In CW systems, even when air is supplied at the bottom of the bed, it is not possible to reach all the pores and subsequently the liquid volume is not entirely aerated. This causes the formation of anoxic sites in the granular medium. This heterogeneity allows the simultaneous nitrification and denitrification in saturated layers at the bottom of the bed.

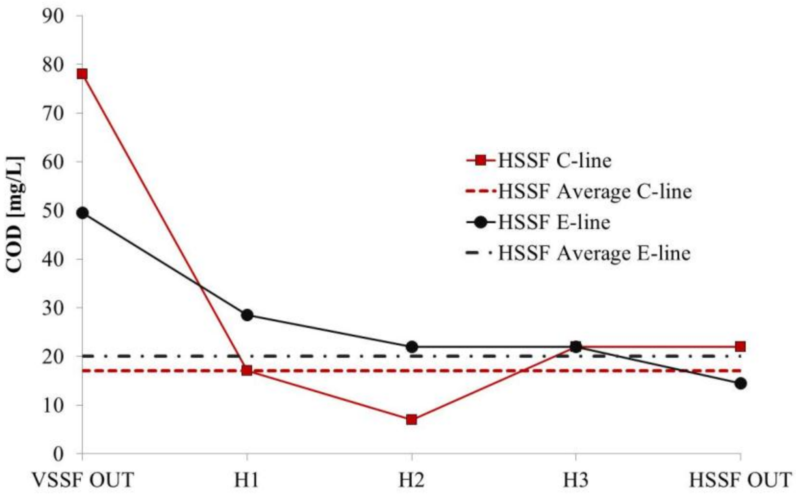

Figure 79 Longitudinal profile for Total COD in the HSSF (C-line and Aerated E-line)

After passing the VSSF, the wastewater flowed by gravity to the HSSF, where further removal of pollutants was observed. Even though few intensive monitoring campaigns were conducted in the HSSF during the aeration phase, the fact that wastewaterwas sampled at different distances from the inlet of the HSSF allowed the estimation of the land area reduction possible in this kind of system (Aerated VSSF + HSSF).

The COD concentration after the passage through the HSSF was reduced from 79 mg/L in the C-line to 20 mg/L (Figure 80). In the case of the C-line, the 9 m^2 HSSF system corresponds to a specific surface of 19.5 m^2/PE, given the organic load applied. If the surface of the HSSF in the C-line were reduced to the H1 sampling point, the specific surface of the HSSF would be around 9.7 m^2/PE, resulting in a hybrid system (VSSF+HSSF) with a total specific surface of 14.3 m^2/PE.

In the Aerated E-line, when considering that this removal (from 50 mgCOD/L to 20 mgCOD/L) was already achieved at the first sampling point (H1 in Figure 79); the total area of the HSSF could be reduced to this point. The area of the HSSF system is 9 m^2, which corresponds to a specific surface area of 8.7 m^2/PE, given the organic load applied. When considering a hybrid system composed of an Aerated VSSF and a HSSF,whose areais given by the original HSSF that has been limited at the first sampling point H1 (specific surface area = 2.1 m^2/PE), the specific surface area of the whole system would be 4.0 m^2/PE.

The fate of nitrogen inside the HSSF (Aerated E-line) can be observed in Figure 79, where the increase of NO^3-N present in the effluent and further removal of NH_4 are shown. The average results obtained were around 1.3 and 3.6 mgNH_4/L and 17.8 and 13.1 mgNO_3-N for the C-line and the Aerated E-line, respectively. Given the removal obtained at the first sampling point H1 (Figure 78), the area of the HSSF system could be reduced significantly, while still obtaining adequate removal performances.

Chapter 12

Conclusions

12.1 Introduction

The main goal of this research was to improve the applicability of CWs to the treatment of mountain communities' wastewater. The work was driven by three specific objectives:

- The provision of reliable tools for the estimation of kinetic and stoichiometric parameters in CWs, that might be used in the design phase.
- The assessment of CWs performance under conditions commonly found in mountain communities.
- The proposal of alternative configurations that can reduce the area of a CW without reducing its efficiency.

The use of CWs in small communities located in mountain areas is limited by some intrinsic characteristics of such systems, namely the large land area requirement and the reduced performance under cold climates. A literature review on CW and respirometric techniques highlighted approaches and configurations that could tackle some of the limitations of these systems when applied in mountain areas.

A comprehensive investigation of the biological processes occurring in CWs, through the use of respirometric techniques and AUR tests, was conducted in order to analyse how CWs are affected by the specific conditions characterizing mountain regions (i.e. low temperature and flow variation). Finally, some innovative CW configurations were tested in a pilot plant to analyse their ability to reduce the CW area requirements. Such configurations can also be used as a temporary solution to increase the treatment capacity during tourist peak seasons, while a traditional configuration is kept over the rest of the year. While this research focused on mountain environments, the configurations and results contained therein could be applied to a wide variety of settings, where shortage of land or difficult climate conditions would exclude CWs from the list of wastewater treatment options available.

In this chapter the main findings of the research, grouped by the three specific objectives, are discussed by reviewing their strengths and weaknesses and proposing some recommendations for future research.

12.1 Kinetic and stoichiometric parameters in CWs

12.1.1 Main findings

Investigations were performed at the lab scale to identify a viable test to measure the kinetic and stoichiometric parameters that are commonly used in mathematical models for the design of CWs. Design is an important phase for the application of CW, mainly in situations where the land area available might be a constraint, as is the case of mountain regions. In order to estimate kinetic and stoichiometric parameters of the autotrophic and heterotrophic biomass, two different tests were applied: liquid respirometric test and off gas technique.

Respirometric tests were used to investigate the mechanisms involved in the oxidation of carbonaceous substrates in CW systems by heterotrophic biomass in acclimatized cores. From respirograms of CW cores, some kinetic and stoichiometric parameters (Y_H and Y_{STO}) of heterotrophic biomass and wastewater biodegradability were evaluated. The values obtained for Y_H (0.56-0.59 mgCOD/mgCOD) are lower than the values obtained for activated sludge (0.67 mgCOD/mgCOD).

An important mechanism occurring in CW cores during the oxidation of carbonaceous substrates was the substrate storage mechanism by the heterotrophic biomass. The values of Y_{STO} for CW cores using acetate were 0.75-0.77 mgCOD/mgCOD, lower than the value of 0.85 suggested in ASM No. 3 for activated sludge, but only slightly lower than the value of 0.78 obtained by Karahan-Gület al. (2003) for activated sludge fed with acetate.

The storage mechanism is probably a response to the intermittent/low feeding in CW systems, which creates transient concentrations of readily biodegradable substrate. Kinetics varied significantly between the two different CW cores, probably due to the different amounts of heterotrophic bacterial biomass which can be present in such cores, while stoichiometric parameters Y_H and Y_{STO} were similar in both cores, because stoichiometric parameters are independent from the amount of biomass.

Respirometry was also applied during the CW core acclimatization in order to identify if this technique is able to capture the changes in biomass during the first weeks of operation and eventually establish when the biomass has reached its steady state. While the removal efficiency during the first weeks of operation was usually considered as an effect of the filter material, respirometric tests allowed new insights into the formation of the biomass inside the filter material.

A shift in the biomass towards the steady state was observed in the respirograms from the first to the tenth respirometric test conducted in the cores during the acclimatization phase. In the same period, respirometric tests were performed to study the NH_4 consumption. The respirograms showed that the Ammonia oxydizing bacteria (AOB) is already present in the filter material after the third week. Despite an increase in the NH_4 removal efficiency (from 40 to 60%), kinetic parameters such as the NH_4-N maximum removal rate does not increase significantly during the acclimatization phase.

Even though the kind of biomass present in the filter material and the amount of biomass inside the core can be better studied via microbiological tests as the qPCR and Flow cytometry, respirometric tests can provide some insights into the evolution of the biomass in CWs. Respirometric tests constitute a valuable, reliable and cheap tool when the objective is not to analyse biomass composition and structure, but to know and measure kinetic and stoichiometric parameters. These tests are also suitable if the objective is to identify when the biomass reaches its steady state in order to verify if two systems are ready to start the experimentation phase when they will be compared under different conditions.

The second kind of test that was performed in CW was the off-gas technique. This approach is usually used in activated sludge to measure the oxygen transfer efficiency of these systems. The use of this technique in CWs is rather new and the preliminary results showed the potential of the technique for the estimation of oxygen demand in CWs, as well as the possibility to estimate kinetic and stoichiometric parameters with the measurement in the gas phase.

Consistency was found in the estimation of kinetic and stoichiometric parameters between respirometric tests and off-gas analysis. Besides that, the possibility to estimate oxygen consumption in the air phase provides insights into the amount of oxygen transferred in aerated CWs, which might eventually be used to improve the aeration equipments usually used in these systems, therefore increasing CWs' efficiency per unit area.

12.1.2 Strengths and Weaknesses

Strengths

The estimation of the maximum oxygen requirements in CWs by liquid respirometry proved to be a reliable tool providing consistent results on the estimation of kinetic and stoichiometric parameters. The method requires limited and cheap equipment, the OD probes being the most expensive tool needed. The method is easy to apply in lab cores and it can be used with filter material sampled from real CWs. The sampled material would be inserted inside the cores and tested, providing reliable measurements of CW filter material parameters. The new information obtained by this research may optimize design procedures, by providing real measurements for kinetics and stoichiometric parameters, which are usually taken from literature.

Regarding the off-gas technique, its main advantage is the possibility to measure the amount of oxygen consumed in CW in the air phase, as opposed to respirometric tests, which rely on measurements performed in the liquid phase. Once the technique will be improved for application to CWs, analyses will be directly carried out in the field eliminating the transport of material to the lab.

The use of aerated VSSF CW, as it was done in this research, is still not as common as traditional CW in the field. However, some authors have been using aerated HSSF with good performance under cold climates (inter alia Oullet-Plamondon et al., 2006). It suggests that the use of off-gas technique might be an advantage also in designing and operating Aerated HSSF, due to the possibility to increase the oxygen transfer efficiency in these systems. In this case, the possibility of measuring the amount of oxygen needed and the oxygen transfer efficiencymay reduce the power consumption in this kind of system.

Weaknesses

Kinetics measured with liquid respirometry can be overestimated, due to the fully aerobic conditions that are present in the respirometer during the test. In fact, such conditions are not present in real CWs, where oxygen transfer from the atmosphere is limited and insufficent to ensure a fully aerobic environment, eventually leading to a possible overestimation of kinetics. Moreover, the fact that in liquid respirometry an unsaturated filter material (as the VSSF) is tested under saturated conditions (liquid respirometry) may sound controversial and further tests should be conducted to estimate the oxygen consumption in the gas phase.

Regarding the off gas technique, it seems to be a promising technique for the measurement of kinetic parameters and the oxygen demand in CWs, though various limitations emerged from the analyses that were conducted in this thesis. The most limiting issue of this test is the need of extremely precise and sensitive oxygen probes that can detect minimal changes in the content of oxygen in the air phase, between the inlet and the outlet. Probes used for analyses in CWs must guarantee higher precision standards than those used in activated sludge, where in fact oxygen consumption is much higher due to the higher amount of biomass in the system.

A second problem regards the aeration systems commonly used in CWs, which consist in holed pipes placed on the bottom of these systems. This kind of aeration system might be in part responsible for the low values of SOTE found during the experiments: most of the air blown by the air pump passed through the filter material without being consumed. Even though part of the air bubbles is retained inside the filter material, this effect of "holding bubbles" is not enough to guarantee higher oxygen transfer efficiency. This confirms the need of using more efficient aeration systems on CW experiments. At the lab scale, for example, the use of an air stone at the bottom of the core, under the gravel, might be an option to be tested. In real systems that are aerated, as is the case of the already operating HSSF, the use of aeration systems better than those usually applied in CWs would be advisable (e.g. membranes or perforated plates instead of holed pipes). This would be more expensive in the construction and operation phases, but the increased oxygen transfer efficiency and the energy consumption might compensate it.

12.2.3 Recommendations for future research

At lab scale, liquid respirometric tests proved to be a reliable and repeatable option for the measurement of kinetic and stoichiometric parameters. The validity of such parameters might be assessed by using them as an input of mathematical models for the design of CWs (e.g. Hydrus – CWM1). However, the calibration of the biological component of these models remains a complicated task due to model complexity and the high number of parameters to fit (Morvannou et al., 2011). Langergraber (2007) suggested the use of indirect parameter determination methods through inverse modelling of the inflow/ outflow pollutants fluxes of a VSSF CW to support the calibration of this model. The parameters determined by respirometric experiments can be used to generate reliable preliminary estimates of biological parameters, which are subsequently further refined during model inversion, eventually allowing the overall parameter uncertainty to be reduced (Morvannou et al., 2011).

Further research could be carried out in order to compare the efficiency of saturated (as the one used in this research) and unsaturated approaches for respirometric analyses. An unsaturated approach that has been already testedon CWs is that applied to household waste characterization (Dynamic Respiration Index measurement). Morvannou et al. (2010), who used this method to measure oxygen demand over time in the gas passing through a reactor, containing a mixture of an organic matrix and a bulking agent (wood), obtained different results from those obtained in this research. Comparative studies between these two approaches could be performed in order to verify in which conditions one test would be preferable to the other.

Among methods available to estimate kinetic and stoichiometric parameters as well as oxygen demand, the off-gas technique was applied here as a proposal for the measurement of oxygen consumption on aerated VSSF. However, the same technique would be a viable solution for the evaluation of oxygen consumption in the aerated HSSF too. In this case, compressed air is diffused in the HSSF CW by means of pipes installed at the bottom of the whole bed (inter alia Nivala et al., 2007; Ouellet-Plamondon et al., 2006; Zhang et al., 2010) and off-gas measurements could be done at the surface of the CW in order to estimate the oxygen transfer efficiency, and eventually assess the need to improve the aeration system.

12.2 VSSF CWs performance under conditions commonly found in mountain communities

12.2.1 Main findings

The performance of the hybrid CW system was assessed in the presence of anoscillating population and low temperatures, these being common conditions of many mountain communities that are tourist destinations. To this purpose, a CW system designed for treatingthe wastewater of the resident population was tested over a two month high-load period in summer, in order to simulate the increase in population due to the tourist presence, and it was also tested during the winter under low temperatures and load variation.

During the two-months summer period, the removal efficiency in the hybrid CW system decreased slightly from 94 to 88% for COD removal and from 78 to 75% for total N removal, even after doubling the applied hydraulic (from 60 to 123 L m^{-2} d^{-1}) and organic load (from 37 to 87 gCOD m^{-2} d^{-1}). The nitrogen load was also increased from 4.4 to 10.3 gN m^{-2} d^{-1}. During the high-load period, the nitrification in the VSSF system was stable and the specific nitrification rate was 4.7 gNH_4-N m^{-2} d^{-1} compared to 2.2 gNH_4-Nm^{-2} d^{-1} in the

Low Load period. However, a higher NH_4-N concentration in the VSSF effluent was observed (26 mgNH_4-N/L compared to 17 mgNH_4-N/L on average in the Low Load period). In the case of higher hydraulic and organic loads in the VSSF, the HSSF increased the removal of COD, nitrogen and phosphorus. In the high-load period the denitrification rate increased in the HSSF system due to the higher availability of biodegradable COD in the effluent from the VSSF unit. Clogging and problems on plant growth were not observed during the high-load period.

During operation at low temperatures, under both continuous and discontinuous feeding, COD and nitrogen removal in the two VSSF CWs were monitored. Continuous feeding period is the normal operation that was run in the winter months of 2011/12 and discontinuous feeding period was managed with feeding and sampling campaigns at intervals of 13 to 30 days, to simulate the tourist presence during the winter holidays.AUR tests were used to evaluate the temperature influence on the nitrification capacity of CW cores and granular material.

The results showed that VSSF CWs can maintain their COD removal performances: during the continuous feeding period the COD removal efficiency remained around 70% even at 2-4°C; under discontinuous feeding conditions the efficiency was around 60% in both VSSF. TKN removal is affected by temperature and the organic load applied. During the continuous feeding period, TKN removal efficiency was 54% on average in the High-Load VSSF, in comparison to an average of 71% in the Low-Load VSSF. Long periods with discontinuous feeding make the nitrification rate decline significantly mainly in the High-Load VSSF, where the TKN removal decreased from 55% in December to 0-18% in January-February. On the contrary, in the Low-Load VSSF under discontinuous feeding, the TKN removal remained always over 42% even after 3 months of discontinuous loads at low temperatures.

The AUR method was used to detect the maximum removal capacity of a VSSF at various temperatures. The AUR tests on the CW lab cores showed a strong temperature dependency, with the maximum specific nitrification rate (v_N) ranging from 14.5-16.2 gNH_4-N m^{-2} d^{-1} at 18°C to 2.8-2.9 gNH_4-N m^{-2} d^{-1} at 2°C. The value range obtained for the temperature coefficient in this research (θ =1.12-1.13), which is narrower than that found in literature (1.06-1.37), allows CWs to be designed with greater precision, thus limiting land consumption. The filter material of the pilot plant that was exposed to low temperatures and discontinuous feeding showed, during the AUR test carried out at 15°C, a $v_{N,15}$ of 7.2 gNH_4-N m^{-2} d^{-1}. The observed nitrification rate is slightly lower than that expected using the Arrhenius-type law, confirming a quick recover in the nitrifying biomass after the winter period. This recovery makes VSSF CW plants suitable for those mountain communities, whose population varies significantly throughout the year.

12.2.2 Strengths and Weaknesses

Strengths

The results showed that in the summer period (when temperatures are favourable, removal kinetics and solids mineralization is higher) the application of high loads does not significantly affect the efficiency of the hybrid CW plant. This suggests that a CW designed on the basis of the resident population can only handle the additional load due to the presence of tourists in the summer season. Such a design would allow significant land savings and subsequently a reduction of total investment. CWs proved to be a reliable system for small mountain communities also during the winter. When properly designed and constructed, these system do not have freezing problems and maintain quite a good removal of organic compounds even at low temperatures.

The AUR method that was developed for its application to VSSF, material is useful to quantify the nitrification rate of real systems at different temperatures and therefore to predict the removal efficiency throughout the year. The value range obtained in this research for the temperature coefficient is narrower than that found in literature and allows CWs to be designed with greater precision, thus limiting land consumption. The reduction of land consumption is an asset in mountain areas, where flat and moderately steep land has often a limited extension and can be allocated to other uses as well (e.g. agriculture, infrastructures, etc.)

CWs have a good performance and deal properly with the load variation observed in the wastewater produced by a community with fluctuating population throughout the year, with peaks during winter and summer. During winter CWs maintain their efficiency under normal operating conditions (continuous operation) as well as after idle periods (discontinuous operation). The ability to recover after idle periods is a major asset of CWs, that was demonstrated by the quick recover of biomass activity in the filter material after the winter months (such observed in the AUR tests).

Weaknesses

Even though neither clogging problems nor a decline in the performance were observed during the summer, and the use of CWs is a good compromise for small mountain communities due to its easy operation and maintenance, these systems are not recommended for winter tourism destinations (e.g. ski areas). In such contexts the maximum load comes in a time when biological activity is at its minimum level as a consequence of low temperatures. Under these conditions, in order to guarantee high treatment standards, the CW surface should be so large that it would be mostly underutilized during the rest of the year, when biological activity is higher.

This research analyzed above ground pilot-scale beds, CWs should be built underground to keep the temperature above the freezing point. It is important to consider that the application of CWs under winter conditions requires a particular care in equipment maintenance. In particular, pipes should be buried and kept empty to avoid freezing, pumps should be kept free of water after operation, while a insulating mulch layer should be used to cover the bed for preventing freezing.

12.2.3 Recommendations for future research

The use of CWs in mountain areas has several advantages that are related to CWs' intrinsic characteristics of easy operation and maintenance as well as adaptability to natural environment and landscapes. However, providing every mountain community with a proper wastewater treatment is still a challenge, mainly for economic reasons.

Further investigation is needed for the design of wastewater treatment strategies in territories characterized by complex morphology (e.g. steep terrain, slopes) and the presence of several scattered small communities. This situation is commonly found in mountain areas that have been inhabited for long time, as is the case study of this research. This kind of situation calls for a network of wastewater treatment systems that comprehensively provide adequate sanitation for the entire region. Designing CWs based on just the need of the single communities may result in an enormous waste of resources (e.g. an excessive number of plants to treat an amount of wastewater that could be perfectly handled by fewer plants located in the right positions). This would only be assured if the environmental and socio-economic characteristics (i.e. the distance from the nearest wastewater treatment plant and the costs for piping, the land area availability and the social, environmental and agricultural issues, etc.) of the territory are taken into account and the technology is selected taking into accountin the planning phase the already existing network in that territory and the need of the surrounding population.

12.3 Alternative configurations that can reduce the area of a CW without reducing its efficiency

12.3.1 Main findings

The pilot plant used in this research consisted of two parallel lines composed by a Hybrid CW: a VSSF followed by a HSSF. The Hybrid CW included two lines: C-line (control line) and E-line (experimental line). The main difference between these two lines is the composition of the filter material of the VSSF: the C-line was designed following the indications provided by the Province Law n. 992/2002 (0.5m) and the E-line used a thicker layer of filter material (0.3m).

The change in the sand size of the filter material in the VSSF CW (1/3 mm and 1/6 mm) with the same hydraulic conductibility, and the reduction of the main filter thickness with respect to the values recommended by guidelines (from 0.5 m to 0.3 m) had only slight effects on the performance. The two VSSF systems showed the same removed total COD loads and a stable nitrification occurred in both VSSF CWs; the average removed TKN loads and total N concentration in the effluents were similar in both VSSF CWs. A higher efficiency in TSS entrapment and in the total P removal was observed in the 0.5m-VSSF.

In general, the reduction of filter material thickness and the slight change in the sand size influenced effluent quality in a negligible way for total COD concentration and nitrification and, considering the equivalent performance of the VSSF CWs, the use of the 0.3m-VSSF seems preferable for its lower expected costs. However, this economic difference is not enough to justify by itself the choice of one of the two configurations.

E-line VSSF CWs were tested in four different configurations and compared with the C-line. The C-line was run under typical down flow conditions all the time, while the E-line was run under varying conditions. The first configuration was the simple comparison between E-line and C-line under the same load conditions, which was called Low Load VSSF. The second configuration tested in the E-line (High Load VSSF) was the simulation of tourism influence that increases organic and hydraulic loads in the summer period (Chapter 8). The third and fourth configurations tested on the E-line were the Recirculated VSSF and Aerated VSSF (Chapter 10 and 11, respectively).

Mean removal efficiency of total COD in the E-line VSSF was always high, equal to 82%, 74%, 86% and 89%, in the case of Low Load VSSF, High Load VSSF, Recirculated VSSF and Aerated VSSF configurations, respectively. The higher efficiency presented by the Recirculated and Aerated configurations, even though the organic and hydraulic loads applied were higher, can be due to the longer HRT of wastewater inside the recirculated and aerated VSSF and the enhanced oxygen availability, favoured by recirculation to the top layers or the artificial aeration.

The organic load applied in the VSSF E-line was 37, 87, 82, 58 gCOD m^2 d^{-1} for Low Load, High Load, Recirculated E-line and Aerated E-line configurations, respectively. The organic load removed increased from 29 gCOD m^2 d^{-1} in the Low Load VSSF up to 64 gCOD m^2 d^{-1} in the High Load VSSF. Almost 51 and 70 gCOD m^2 d^{-1}were removed in the VSSF of the Recirculated E-line and Aerated E-line configurations. Considering the entire E-line VSSF+HSSF system, the COD removal efficiency was very similar among the four configurations: values of 96%, 94%, 95% and 96% were found for Low Load, High Load, Recirculated and Aerated configurations, respectively. The subsequent HSSF stage offered a finishing/polishing function, allowing a reduction in the organic peak loads discharged from the VSSF system. The area of the HSSF could be further reduced taking in account the results observed in the 4 different sections of this systems and the whole system of a hybrid Recirculated VSSF+HSSF would have an specific surface area of 2.9 m^2/PE (1.5 m^2/PE in the VSSF and 1.4 m^2/PE in the HSSF), while a hybrid Aerated VSSF+HSSF would have an specific surface area of 4.0 m^2/PE (1.9 m^2/PE in the VSSF and 2.1 m^2/PE in the HSSF).

TKN removal efficiency was around 66-74%, except in the High Load VSSF (58% on average), where the higher hydraulic load applied caused a shorter HRT in the system. In the Recirculated and Aerated configurations the retention time was longer due to the accumulation of the wastewater in the lower part of the system. The Total Nitrogen (TN) removal was also benefited from the existence of a saturated lower part of the VSSF CW, which allowed the occurrence of nitrification and denitrification inside of the same system: the availability of organic matter, the longer residence time and the creation of aerobic and anoxic zones in the bed.

Regarding the COD removal efficiency, the innovative configurations tested in the E-line (Aerated and Recirculated VSSF) seems to give results similar to those observed in the High Load VSSF. However, when analyzing the nitrogen removal, such innovative configurations proved to give better results, by removing higher nitrogen loads than the typical down flow configuration and without showing any clogging formation. The effluent of the innovative configurations was more nitrified than that of the High Load VSSF, even if nitrification occurred in all four configurations.

Nitrification and denitritication occurred differently in Recirculated and Aerated configurations. In the Recirculated configuration, the recirculation of wastewater from the bottom to the top of the VSSF bed contributed to enhance nitrification efficiency in any passage through the bed, due to the continuous contact with oxygen and the biofilm attached in the sand layer. Denitrification occurred in anoxic microsites in saturated layers.

In the Aerated configuration, the wastewater drains to the saturated bottom layers where oxygen is supplied by a compressor. However, the effect of aeration in the VSSF bed is different from that observed in activated sludge tanks because in CW systems the air supply cannot reach all the porosity of the bed and thus the liquid volume results not entirely aerated. This fact contributes to the formation of heterogeneity and anoxic sites in the granular medium which allows the formation of a possible simultaneous nitrification and denitrification in the saturated layers at the bottom of the bed.

Even if both innovative configurations performed nitrification, the Recirculated VSSF presented better performance, because during the recirculation, the wastewater returns on top of the bed and flows through the entire depth of the bed, entering in contact with oxygen and the nitrifying biofilm attached to the sand in the unsaturated layers; it does not happen in the case of the Aerated VSSF.

These conditions resulted in a higher TN removal in these innovative configurations (44% and 53% in the case of Recirculated and Aerated configurations, respectively) when compared to the typical down flow configurations (24% and 40% for Low Load and High Load, respectively). Denitrification occurred also in the Aerated configuration due to discontinuous aeration (i.e. few minutes per hour), and during the cycle DO concentration dropped to levels suitable for denitrification. In all the configurations, the subsequent HSSF system had a role of finishing in the TN removal, contributing significantly to the denitrification: 85%, 86%, 80%, 72% TN removal efficiency were observed in the VSSF+HSSF systems Low Load, High Load, Recirculated E-line and Aerated E-line, respectively. The lower TN removal in the High Load Aerated configuration could be due to the passage of the DO present in the VSSF effluent into the subsequent HSSF system, limiting the denitrification activity.

12.3.2 Strengths and Weaknesses

Strengths

The use of innovative configurations in CWs provided good results, in terms of treatment performance and the reduction of land area requirements. The occurrence of nitrification and denitrification inside the same bed was also observed in the Recirculated and Aerated VSSF.

The use of innovative configurations (Recirculated and Aerated VSSF) givens to an increase in the complexity of these systems, both during the construction and operation phases. However, this complexity is a consequence of smaller and more efficient systems that require less space than a traditional CW. A potential reduction of land requirement was observed in all the configurations tested in the VSSF E-line: in it resulted in 1.3, 1.5 and 1.9 m^2/PE for High Load, Recirculated and Aerated configurations, respectively. The reduction from the standard value (4m^2/PE) proposed by the Province of Trento for VSSF design can have a significant impact and make VSSF a suitable option when not enough land is available for a conventional CW.

Innovative configurations, such as the Aeration and Recirculation of VSSF proposed in this research, have also the possibility to be used as a temporary solution for increasing the treatment capacity during tourist peak seasons, while a traditional configuration is kept over the rest of the year. In the specific case of Aerated CW, aeration pipes could also be applied on the bottom of HSSF during winter to increase temperature and mixing, as demonstrated by Muñoz et al. (2006). The use of aeration pipes on the bottom of the first meters of a HSSF or also along the whole bed of a VSSF, would allow the use of artificial aeration to prevent clogging formation in the filter, thus avoiding the need to replace the filter material.

Weaknesses

Even if clogging effects were not observed during the use of aeration in VSSF, these may be observed when operating Recirculated VSSF with organic loads higher than 100 g COD m^{-2} d^{-1}, mainly when the high organic load is coupled with a decrease in the temperature. In our study when the system reaches these values, ponding formations are observed in the VSSF surface, discouragingthe use of this system under the above mentioned conditions.

While the configurations tested allow a considerable improvement in removal efficiency and land area reduction, their actual application calls for extra investments with respect to more traditional configurations. Such investments are needed for pipe networks (for recirculation or aeration), pumps, and extra efforts during the construction and maintenance of these systems. This would not be a problem in regions like Western Europe where good sanitation infrastructures are generally found and the extra investment needed to equip VSSF CWs with aeration/recirculation systems could be compensated by the lower area requirement.

According to Paing and Voisin (2005), when sufficiently sloping sites are available, no power is needed for the operation of a traditional CW. However, the configurations presented in this research require energy for their operation. This, along with the intrinsic complexity of these configurations, is a considerable limitation if these systems are to be applied in remote communities. In such contexts, the need for energy and complex maintenance would probably prevent the use of these systems, or at least limit their adequate operation and the provision of satisfactory results in terms of effluent quality. Such limitations suggest that in many cases traditional CWs with no power requirement are preferable to ensure that all communities have their wastewater adequately treated.

12.3.3 Recommendations for future research

While this research has focused on the adaptation of CWs to mountain communities, the innovative configurations tested provide benefits for a wide variety of contexts. In particular, they can be applied everywhere the availability of land is limited and high standards of effluent quality are required. Even though problems in the recirculation and aeration systems were not observed in the pilot plant, further research is needed to test these configurations at the field scale, in order to identify whether their maintenance and the long term operation could pose particular challenges.

Further research could also be carried out on the Aerated VSSF in order to understand how the aeration can be used to prevent clogging problems in CWs. The use of aeration could help in the degradation of the organic solids accumulated inside the filter material that worsen the hydraulic conductivity and subsequently the efficiency of the biological treatment. This would be an alternative to the replacement of the filter material, increasingthe operation life of these systems.

Research is needed to understand how and where photovoltaic panels should be installed to provide the greatest benefits to the application of CWs. The energy consumption in the simplest configuration is around 0.17 KWh m^{-2} d^{-1}, while the high load configuration had a consumption of 0.22 KWh m^{-2} d^{-1}, which is not much different from the Recirculated VSSF one (0.28 KWh m^{-2} d^{-1}). The energy consumption of the aerated VSSF was around 0.57 KWh m^{-2} d^{-1}. The use of pumps is already necessary in some CW systems and in those cases the application of innovative configurations would not cause an evident increase in energy consumption (excluding the case of aeration where the energy consumption was twice the amount used, even in the recirculated configuration). While the need for energy might limit the applicability of these systems in remote areas. The use of renewable energy could help to partly tackle this problem. Considering that a photovoltaic panel installed in the region where this study was conducted can generate in December an average of 1.18

$KWhm^{-2}d^{-1}$(data from the simulation presented on http://www.solaritaly.enea.it/ CalcRggmmOrizz/Calcola.php). This means that the energy produced in the coldest month would be sufficient for the operation of a CW plant.

An attractive option, for example, is represented by the possibility to install panels above CWs. While this could be criticized for its potential negative effects on plants, it is already known that plants in VSSF play a minor role in the removal of pollutants (inter alia Sklarz et al., 2009). This option presents at least a couple of key advantages. First, the use of solar energy allows the CW to become self-sufficient and possibly to produce extra energy that can be used for additional purposes (e.g. public lights). Secondly, the integration in the same area of a CW and photovoltaic panels guarantees that the land requirement for the whole system is minimized.

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Appendix

1. In the appendix, the experimental activity developed UNESCO-IHE in Delft (The Netherlands) under the supervision of Prof. Diederik Rousseau, Jan Willen Foppen and Piet Lens will be described**.**

Appendix 1

Bacterial and Ammonia oxydizing quantification in HSSF CW by qPCR.

INTRODUCTION

The use of constructed wetlands for decentralized wastewater treatment plants, single home projects and rural communities has increased because of low maintenance requirements and operational costs, and efficiency in terms of BOD, nitrogen and suspended solids removal. The large area requirement is probably the most important drawback of this technique. VSSF CWs are considered aerobic systems that favor microbial processes as BOD removal and nitrification. In HFSS CWs, wastewater flows horizontally through the substrate. This system, which is generally considered an anoxic one, performs BOD removal by anaerobic process, denitrification and suspended solids removal. Clogging and oxygen limitation are the problems that affect the organic matter removal in particular in the case of HSSF. In order to overlap this problem,some authors (inter alia Nivala et al., 2007) have used different rates of aeration in the HSSF and they have achieved better results in BOD removal and total nitrogen (TN) when compared with systems without aeration. Another advantage of the use of aeration is that it allows a reduction of the land area requirement for building the HSSF. As far as we know the microbial biomass quantification and composition in the aerated HSSF has not been explored in much detail.

The quantification of its microbial biomass could provide information to estimate certain kinetic parameters. The amount of microbial biomass can be expressed through the concentration of volatile suspended solids (VSS), but the presence of biodegradable and inert particulate substrate in the VSS makes their use not adequate. For the quantification of active cellular biomass other, more innovative, methods may be proposed. qPCR is widely used to quantify microbial cells in various environmental fields. Ammonia oxidizing bacteria (AOB) play an important role in the TN removal in CW, mainly in VSSF, due to its aerobic conditions, and the same role is expected in the aerated HSSF. The aim of this study is to evaluate the biomass, especially AOB, in two HSSF, one of them receiving artificial aeration. The quantification of the total bacteria wasdonevia qPCR to estimate the amount of total bacteria and AOB present in the aerated HSSF.

EXPERIMENTAL SET-UP

The outdoor experiment has been conducted in the laboratory of the UNESCO-IHE Institute forWater Education in Delft, the Netherlands. Two microcosms of HF CWs were made using plastic containers packed with gravels with 40% porosity (8-16 mm diameter) and planted with common reed (Phragmites australis). The first system (S1_cont) is a normal HSSF, the second wetland ($S2\_{aer}$) includes bottom aeration. The dimensions LxWxd of the set up for S1_cont and S2_aer are60x40x38 cm with a pore water volume of 33 L for each system.The aeration was provided during the night hours through two porous PVC tubes.

Bacterial Detachment Protocol

Biomass detachment is an important step towards analyzing bacterial community in a constructed wetland, particularly when the technique to be used is qPCR. PCR is a technique developed for application on matrices of water, sludge and soil. SSF CW are systems in which the bacteria grow attached to the filter material creating a biofilm. The biofilm consists of extracellular polymeric substances (EPS) composed by polyssacaraides, lipids, protein and nucleic acids (Böckelmann et al., 2003). In order to detach the bacteria from the biofilm, before the microbiological analysis, some methods have been developed including scraping, swabbing, shaking, sonication, blending, and digestion approaches (Weber and Legge, 2010).

Foladori et al. (2010) developed a methodology for bacterial detachment from gravel using pyrophosphate and mechanical shaking that aimed at quantifying the bacterial community by flow cytometry. In order to detach bacteria from sediments (coarse sand, 2–0.5mm; medium sand, 0.5–0.2mm; fine sand, 0.2mm), Amalfitano and Fazi (2008), combine chemical and physical treatments (buffer solution of sodium pyrophosphate and polysorbate, shaking and sonication, followed by Nycodenz density gradient centrifugation). In this case a higher recovery from the finer grain-size class (90%) as compared to the coarse fraction (69%) was detected, and it could be related to the higher formation and quantity of EPS in coarser materials. Also, the production of bacterial biomasscompletely ceases after detachment and purification procedures.

Weber and Legge (2010) proposed a methodology for the detachment of culturable bacteria from pea gravel (2– 4mm). The final proposed method is based on 25 g of pea gravel media to be washed out on 100 m of phosphate buffer + enzymes mixture and 3 hour mechanical shaking (100 rpm). According to their results, the use of enzymes increase the number of culturable bacteria (CFUs) detached, resulting in a higher community diversity when compared to using the buffer solution alone.

Weber and Legge (2010) tested two kinds of mechanical detachment: a 5 seconds of a manual shake and a 3 hour mechanical shaking (100 rpm 30°C). During a simple manual-shaking they assessed that a large amount of solid material was detached from the pea gravel media. However, during the 3 h mechanical shaking period the amount of solids detached seemed to be smaller than during a hand shaking. They concluded that the bacterial population may have utilized some of the detached organic material in its growth, increasing the number of culturable bacteria in the sample.

In order to quantify the bacterial population in the initial CW sample, a 3-hour shaking is not interesting, because it could overestimate the real number of bacteria. On the other hand, the 5 seconds hand shaking could generate an underestimated number and also compromise the enzyme mixture reaction with the EPS destabilization. The protocol used consists in testing over different times with the addition of enzymes to destabilize the EPS:

- For each 25 g sample of gravel 50mL of shaking solution (buffer solution + enzyme mixture) were added to a 250mL Erlenmeyer flask and shaken at 100 rpm for 5 min– 15 min – 30 min – 1h at 30°C;

Gravel was separated from the water (that should be kept in an Erlenmeyer) and the procedure repeated with the gravel. Final suspension was made up of two 50 mL volumes used as shaking solution (100mL);2mL of the liquid were centrifuged for 3 min at 14000 x g. Supernatant was discarded and the sediment used for DNA extraction.

The phosphate buffer solution was made with a pH of 7,2 in autoclaved deionised water and the enzyme mixture used was: lipase (50U/g pea gravel), β-galactosidase (10U/g pea gravel), and α-glucosidase (1U/g pea gravel) [Sigma-Aldrich®], as proposed by Weber and Legge (2010). A buffer solution with a pH of 6 was also tested to evaluate how the enzymes action changed when the pH decreases. The differences between different times and pH used were assessed by qPCR analysis (with SYBR Green assay for total bacteria and amoA as described in this report).The material used in this test was gravel (1-3mm) from a VSSF CW. The qPCR test was done for total bacteria and AOB (amoA gene) were done in duplicate. The results showed in Table A1 and Figure A1, represent the average values obtained in qPCR for total bacteria.

Table A1 Average Ct values obtained in qPCR quantication for Total Bacteria.

Time of shaking / Ct values(amoA)	**pH 6**	**pH 7,2**
5+5	21.65	21.55
15+15	20.16	20.79
30+30	20.70	21.29
60+60	19.71	19.26

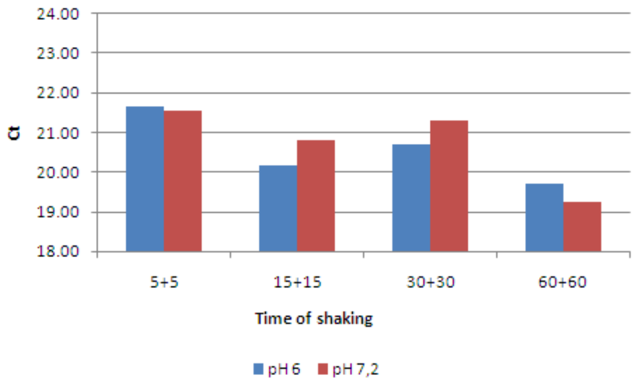

Figure A1 Average Ct values for Total bacteria quantification in the detachment protocol test over different time of shaking and different pH.

During the last hour of test an increase of the copy number was observed for both pHs (the lower the Ct the higher the amount of DNA in the samples). This could be related with bacterial growth in the sample, mainly with pH 7.2 which offers optimal conditions for bacterial growth.The results showed in Table A2 and Figure A2 represent the average values obtained in qPCR for the quantification of amoA gene.

Table A2 Average Ct values obtained in qPCR quantication of amoA gene.

Time of shaking / Ct values(amoA)	**pH 6**	**pH 7,2**
5+5	26.12	22.87
15+15	26.81	24.85
30+30	26.315	24.875
60+60	27.13	24.81

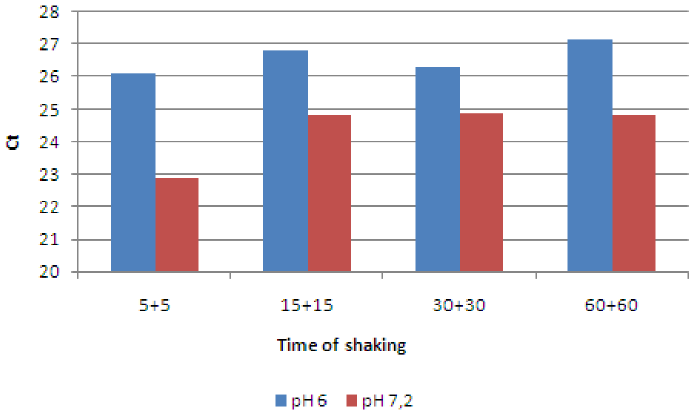

Figure A2 Average Ct values for amoA gene of the detachment protocol test over different time of shaking and different pH.

It is possible to see that on average the mixing with pH 7 gives better results than with pH 6 (the higher the Ct the lower the amount of DNA in the samples). The shaking procedure could be stopped at 30 min (15+15 minutes), because after this time the amount of AOB bacteria detached does not change significantly. Figure A3 shows the gel eletrophoresys of the products generated by the qPCR by the amplification of amoA gene (590 base pairs).

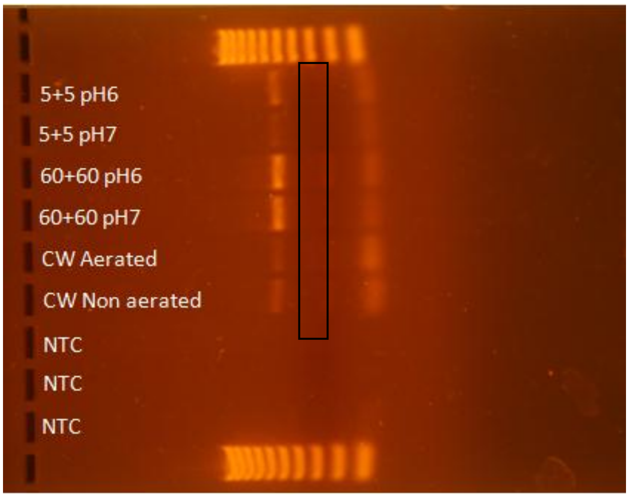

Figure A3 Gel eletrophoresys of product generated after the qPCR by the amplification of amoA gene (590bp).

Bacterial Growth during the detachment protocol

The bacterial growth in the sample over different times was assessed by plate counting (Böckelmann et al., 2003). Three tests were done in triplicate, with three different treatments: a)buffer solution without enzymes at 5°C, b) buffer solution with enzymes at 30°C, c) buffer solution without enzymes at 30°C.

The tests were performed with 2 flasks with 25g of gravel each one. The detachment protocol was applied for 60 min and the first sample will be collected. In one of the flasks the gravel was separated from the water while gravel was left in the other flask. The shakinglasted 3 hours and at the end the second sample wascollected. The difference between the counting performed in the two flasks represents the bacterial growth in the sample. Serial dilutions of the supernatants (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) were plated on 10% TSA agar. Plates were incubated at 30°C in the dark and colony forming units (CFU) were counted after 1 day.

Volatilesuspended solids (VSS) were measured to evaluate the amount of organic matter detached from the gravel. A known amount of liquid was filtered in a glass fiber filter (0.45mm). TSS is the dry weight of the filter after drying in ceramic crucibles at 105°C for 24 h. After the drying, the same sample was analyzed for organic. Organic content is determined by the amount of sample weight lost after the muffle furnace treatment at 550°C for 15 min.

Figure A4 and A5 show the results obtained from the VSS and plate counting, respectively.

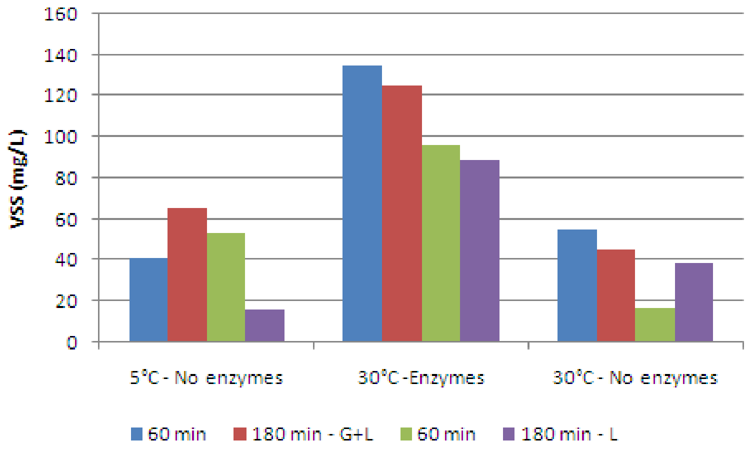

Figure A4VSS Average result from tests in triplicate

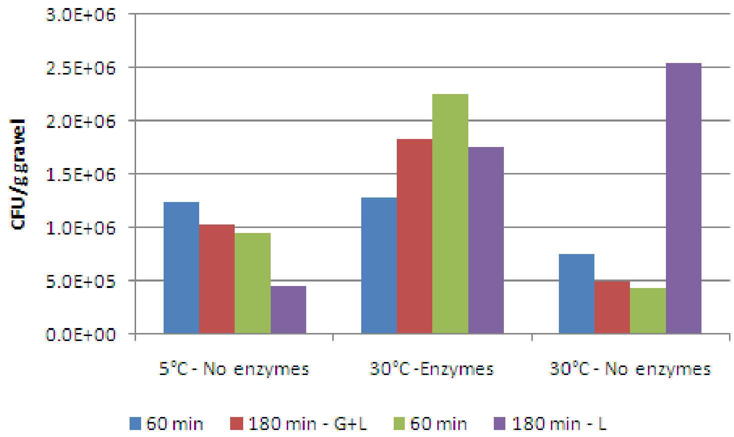

Figure A5 Average result of CFU/g of gravel from tests in triplicate

Figure A4 shows that the amount of suspended solids detached from the gravel is higher in the treatment at 30°C and with enzymes, when compared with other treatments without enzymes (at 5 and 30°C). In Figure A5, the results from plate counting show that the amount of culturable bacteria was also higher in the second treatment. However, these results do not allow us to evaluate whether there is growth in the solution with a 3 hour shaking. The use of PCR could be interesting for the quantification of total bacteria in these three treatments, and maybe it could allow the evaluation of the growth in the solution or the degree of detachment from the gravel.

qPCR APPLICATION

DNA Extraction

Molecular analyses of microbial communities in complex environmental samples such as compost require efficient unbiased DNA extraction procedures (LaMontagne et al.,2002). According to Zhou et al. (1996), every type of environmental sample, because of its own nature, requires extraction methods to be optimized. In addition, the efficiency of a soil microbial DNA extraction depends on soil quality, particularly on its clay and organic matter contents, because micro-organisms can interact with soil colloids (Lakai et al., 2007).

One sample of activated sludge was treated with different procedures in order to evaluate five different methods for DNA extraction. Three extraction procedures were based on Boom et al. (1999):

- regular procedure using 100ul of solid suspension (correspondent in the Figure 6 to samples 9a and 9b);
- regular procedure with pellet formed after centrifugation of 2 mL of solid suspension (correspondent in the Figure 6 to samples 7 and 8);
- extended procedure where 2mL of the suspension were filtred (0.2um) and the filter was used as in the normal procedure (correspondent in the Figure 6 to samples 10 and 11).

Two commercial kits were also tested using the pellet formed by centrifugation of 2 mL of suspension:

- FastDNA SPIN kit for soil (MP Biomedicals, Santa Ana, CA) (corresponding to samples 4, 5 and 6in Figure 6);
- MoBio kit (Ultraclean microbial DNA Kit extraction - provided by TU Delft)(corresponding to samples 1,2 and 3in Figure 6).

The DNA extracted from the samples was evaluated through gel eletrophoresys and also through qPCR (using the SYBR green primers). Based on the results provided by the qPCR evaluation for total bacteria, the DNA extraction method called Boom procedureusing the pellet generated by centrifugation, give a higher amount of copy number (Ct= 14), when compared to other methods, such as: FastDNA SPIN kit (Ct= 15.2), Boom extended (Ct= 16.1) and regular (Ct= 18.5), and MoBio (Ct= 17). Being the Boom Procedure a homemade procedure, it is less expensive and also allows RNA to be extracted when using the pellet in the extraction (see Figure A6 – samples 7 and 8).

However the preparation of the consumables may be a source of contamination. Anyway for the purpose of this research, Boom regular procedure with centrifuged sample was used in the DNA extraction of the samples.

Figure A6 Gel electrophoresis from DNA extract from the same sample with different methods.

Total Bacteria Quantification by qPCR

After the detachment protocol, part of the sample (sample suspension) was separated and preserved in order to avoid bacterial growth. These samples have the DNA extracted using Boom protocol (using the pellet of 2 mL centrifuged). The quantification of total bacteria was performed by qPCR. During my period at Unesco-IHE, we have tested TaqMan Probe using Harms et al (2003) and SYBR Green primers. In the report I will describe briefly our attempts with TaqMan probe (all the experimental set up and results were sent in weekly based reports), but for the final results SYBR Green primers were used.

TaqMan Primers and Probe

The total bacteria quantification was based on 16S rDNA using a TaqMan Probe and following the methodology proposed by Harms et al. (2003). This assay was designed to be a broad-based assay for the estimation of total bacteria. The primers used were 1055f and 1392r (15 pmol) and the probe: 16STaq1115 (6.25 pmol). Instead of using a Master mix (commercial mix with all the components needed to perform a PCR reaction) a homemade mix was testedthat is made up of PCR buffer (commercial mix or homemade Dynazyme buffer), dNTPs (adenosine, guanadine, cytosine, timine) and the enzyme (Taq polymerase) and water DNA free.

Negative templates controls (NTCs) are the test performed with water instead of using DNA samples. In this case you would expect negative results in the PCR reaction. However, the authors (Harms et aL., 2003) describe a Ct value of 29,7, while in our test the Ct value of the NTC was higher, around 22 and 27. A difference between the real sample and the NTC prevented a differentiation of a real sample with low DNA contents from a sub product of the PCR reaction (it could be the primer dimer formation or contamination in one of the reagents). In order to overcome the NTC problem and in order to have reliable and repeatable results of DNA samples, a 2 month period of optimization of Harms Protocol was started.

Since all reagents were replaced by “DNA free” reagents, it is suspected that the source of contamination was the Taq polymerase; which can be contaminated by bacterial DNA (Spangler et al., 2009). Based on Spangler et al. (2009), who describes Taq polymerase contamination, a series of Taq polymerase dilutions were tested (10X, 100X, 1000X). The dilutions were used to prepare PCR mixes following Harms assay. Also a control mix was included, in which the Taq polymerase concentration remained unchanged (0.5 units/ul). This experiment yielded no amplification, and therefore it was repeated two more times to reduce the probability of human errors.

A review of lab experiments during July and August revealed that,due to the use of PCR buffer instead of Dynazyme buffer, the Harm´s assay startedfailing also in the positive control amplification. A comparison between the two buffers showed a difference in $MgCl_2$ concentration, which is lower in the PCR buffer (1.5mM) than in the Dynazyme buffer (2.5 mM).$MgCl_2$ is one important component in the PCR reaction: an incorrect magnesium concentration will reduce or prevent amplification of your PCR product. In this way, the $MgCl_2$ concentration was increased until the concentration we already have in the Dynazime buffer (2.5 mM) and also higher concentrations (3.75mM, 5mM as used by Harms et al., 2003, 6nM and 6,25mm of $MgCl_2$). The experiment yielded amplification in the positive control, but the NTC values were around Ct 22, 23. After having observed the effect of magnesium concentration in the reaction, the Taq polymerase dilution was tested again to reduce the Ct value of the NTC. Unfortunately this test did not show any improvement in the Ct values.

The effect of temperature on the annealing process was also assessed when PCR buffer was used. The temperatures proposed by Harms were 3 min at 50°C, 10 min at 95°C, 45 cycles at 95°C for 30 s (denaturation), 50 °C for 60 s (primer annealing), and 72 °C for 20 s (primer extension). The annealing temperature was varied on a range between 55°C and 65°C using the FAM general protocol. In the first test the temperature of 65°C showed good Ct values for NTC, but the results were not repeatable. Other tests were also done with different Taq Polymerase (AmpliTaq Gold) with no encouraging results.

Finally we decided to use the PCR buffer increasing the $MgCl_2$ concentration instead of Dynasyme buffer, which could be a source of contamination. The temperature of annealing was fixed at 60°C with FAM protocol. Despite our efforts and even after several attempts, the assay did not provide reliable results in terms of positive and negative controls.

SYBR Green

After collaboration with TU Delft, SYBR Green primers were tested and adopted in our laboratory. The primer set for total bacteria quantification was based on 16S rDNA:forward primer BAC341F (5'CCTACGGGAGGCAGCAG3') and a combination of two reverse primers Bac 907rC (5' CCGTCAATTCCTTTGAGTTT3') and Bac 907rA (5'CCGTCAATTCATTTGAGTTT3') were used for quantification of total bacteria. The PCR protocol for total bacteria quantification wasas follow: 5min at 95˚C, and then 40 times 30sat 95˚C, 40sat 55˚C, 40sat 72˚C, 25sat 80˚C.

PCR Efficiency

PCR efficiency is 100% if the product doubles at every cycle 2-4-8-16-32 etc. In normal amplification this can vary due to high contaminants, primer-template mismatching and the lack of pipeting skills. The PCR efficiency can be determined by dilutions of the positive control. This is so because, in case of dilutions, the DNA needs more cycles to amplify to the same amount of product as an undiluted sample would. The following equation was applied to determine the PCR efficiency (%):

$$PCR^{efficiency} = \left(\left(dilution\ factor^{\frac{1}{cycles\,delay}} \right) - 1 \right) * 100 \qquad \text{A1}$$

Preliminary qPCR efficiency was determined using different dilutions (1x, 5x and 25x) of the same positive control. Figure A7 shows the results of such application.

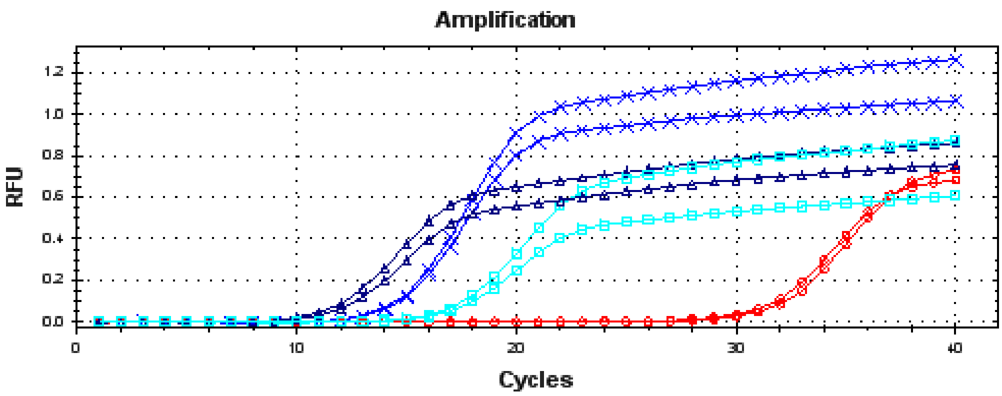

Figure A7 Preliminary results obtained in the efficiency test. Triangles represent 1fold dilution (Ct 10.57 and 10.74). Crosses represent 5fold dilution (Ct 13.55 and 13.53). Squares represent 25 fold dilution (Ct 15.96 and 15.74). Red circles represent NTC (Ct 30.55 and 31.05).

According to the information given by TU Delft, for the standard total bacterial qPCR using the primer set BAC341-907 we would expect 65-80% PCR^{efficiency}. Based on the preliminary results showed in Figure 5 and applying equation A1, an efficiency of about 85% was achieved.

Ammonia oxidizing bacteria by qPCR

Chemolithoautotrophic ammonia-oxidizing bacteria obtain energy from the oxidation of ammonia to nitrite. The oxidation proceeds in two steps. First, ammonia is oxidized to hydroxylamine. This step is catalyzed by the enzyme ammoniamonooxygenase (AMO) Afterwards, hydroxylamine is oxidized to nitrite by hydroxylamine oxidoreductase (Bjerrum et al., 2002). AMO is a membrane-bound enzyme containing multiple subunits: *amoA*, *amoB* and *amoC*. The genes are present in multiple copies in most ammonia-oxidizing bacteria. *Nitrosomonas* strains usually carry two gene copies whereas most *Nitrosospira* strains carry three (Klotz and Norton, 1998 apud Bjerrum et al., 2002).

PCR primers have been developed to target *amoA*, a functional gene coding for the active subunit of ammonia monooxygenase, a key enzyme in ammonia oxidation by AOB (Okano et al., 2004). At the beginning TaqMan was used with the primers: A189F(300 nM) and amoA-2R´(900 nM) and the probe A337 (100 nM) (Okano et al., 2004). However, after some attempts with no amplification observed (due to the higher specificity of the probe, which matches only with *Nitrossomonas europaea*), a different set of primers (SYBR green primers instead of TaqMan), as proposed by experts at TU Delft. The forward primer AMOa-1F deg6 (5' GGGGHTTYTACTGGTGGT3') andthe reverse primeramoA-2R deg36 (5' CCCCTCBGSRAAVCCTTCTTC3') developed by Hornek et al (2006) were used in the experiments. According to the authors, these primers recover full length amoA sequences from cultured AOB and could be used to screen AOB in the environment. The PCR protocol for AOB quantification was as follows: 5 min at 95˚C, and then 40 times 30s at 95˚C, 40s at 57˚C, 40s at 72˚C, 25s at 80˚C.

Preliminary qPCR efficiency was determined using different dilutions (1x, 5x, 25x and 125x) of the same positive control. Figure A8 shows the results of such application.

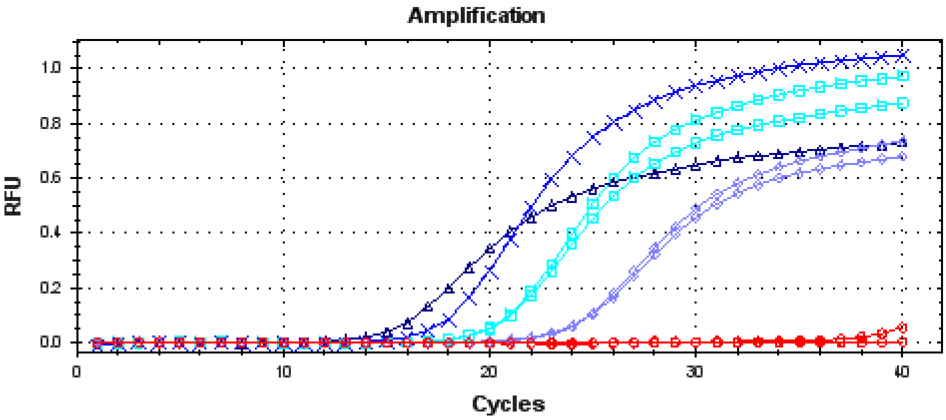

Figure A8 Preliminary results obtained in the efficiency test. Triangles represent 1fold dilution (Ct 14.63). Crosses represent 5fold dilution (Ct 16.83). Squares represent 25 fold dilution (Ct 19.51 and 19.59). Diamonds represent 125 fold dilution (Ct 22.98 and 23.03). Red circles represent NTC (Ct N/A and 39.38).

Based on the preliminary results showed in Figure A6 and applying equation A1, an efficiency of about 78% was achieved. This analysis also allows the detection of some inhibition in the undiluted sample (triangles in the Figure A6). The inhibition can be caused by products, for example humic acids, that were not completely extracted during the DNA extraction. To avoid inhibition, when using CW samples, I recommend the use of 5 fold dilution before analyzing the samples.

Relative quantification of Total Bacteria and AOB in aerated and no aerated wetland

Samples were collected from the two horizontal wetlands (aerated and no aerated). Two different methods of detachment were used: Trento (30 min shaking with pyrophosphate) and Delft (2 hours shaking with enzymes at 30°C). Table A3 show the results obtained from the qPCR analysis of Total bacteria and amoA for both detachment methods.

Table A3 Ct values obtained from the qPCR analysis of Total bacteria and amoA.

	Total Bacteria		amoA	
	Pirophosphate (30min)	Enzymes (120min)	Pirophosphate (30min)	Enzymes (120min)
Aerated	23.39	23.49	30.38	29.88
Non aerated	22.16	21.72	30.50	30.15

As we do not have a standard curve, it is not possible to determine exactly the number of bacteria. However, based on the results showed in Table A3, the amount of total bacteria is higher in the non aerated wetland than in the aerated one (relative quantification).

The amount of AOB bacteria identified in both wetlands was similar. Considering the amount of AOB found and the ammonia removal efficiency in the aerated wetland when compared to non-aerated wetland, the AOB community seems to be present in both wetlands, with similar quantity, but in the aerated wetland they are more active.