Comparative performance evaluation of constructed wetland systems for wastewater treatment.

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Master’s dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Sanitation
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Ghent University, August 18, 2016

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Abstract

High strength wastewater effluents are a frequent scenario of contamination in developing countries, driven by a combination of accelerated population growth and lack of adequate sanitation. A treatment method of interest in developing countries is constructed wetlands due to its high buffering capacity, easy implementation and operation. These qualities favor the implementation of such natural treatment system instead of conventional systems, such as activated sludge. The scarcity of researches comparing the performance of wastewater treatments to high strength wastewater scenarios led to the present study, which aimed to compare the aforementioned technologies of secondary treatment level regarding buffering capacity, treatment efficiency and resilience.

A design and set-up of laboratory-scale constructed wetlands and activated sludge were conducted. Performance of both systems was monitored along a stabilization period, which contributed to predict theoretical effluent concentrations of high strength wastewater scenario with a discretized plug flow model. Sample size determination was achieved by power analysis and the relevant formulated hypotheses for high strength wastewater scenario, were tested by a mixed model approach.

The main results comprise that constructed wetlands and activated sludge systems presented high buffering capacity and removal efficiency regarding organic matter (~90%). These removal values resembled the ones reported for both systems in field conditions, but they also complied with the discharge standard limits of wastewater treatments for BOD$_5$ and COD given by European Community and Flemish Government. Regarding the resilience capacity, both treatment systems showed fast adaptability to treat fluctuating organic loads.

This experiment demonstrated that constructed wetlands can be effective for treating wastewater with elevated organic load at fluctuating influent concentrations.

Keywords: Constructed wetlands, wastewater, natural treatment systems, pollutant removal.
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List of abbreviations

AOB: Aerobic Oxidizing Bacteria
AS: Activated Sludge
AWW: Artificial Wastewater
BOD$_5$: Biological Oxygen Demand
COD: Chemical Oxygen Demand
CSTR: Completely Stirred Tank Reactor
CW: Constructed Wetland
DO: Dissolved Oxygen
EC: Electrical conductivity
ES: Effect Size
FC: Fecal Coliforms
FSF: Free Surface Flow
HLR: Hydraulic Loading Rate
HRT: Hydraulic Retention Time
HSAWW: High Strength Artificial Wastewater
HSSF: Horizontal Subsurface Flow
MBR: Membrane Bioreactor
MLSS: Mixed Liquor Suspended Solids
MLVSS: Mixed Liquor Volatile Suspended Solids
MSAWW: Medium Strength Artificial Wastewater
N: Nitrogen
NH$_3$: Ammonia
NH$_4^+$: Ammonium
NHST: Null Hypothesis Significance Testing
NO$_2^-$: Nitrite
NO$_3^-$: Nitrate
NOB: Nitrite Oxidizing Bacteria
OECD: Organization for Economic Cooperation and Development
**OLAND:** Oxygen Limited Autotrophic Nitrification/Denitrification

**P:** Phosphorus

**PAO:** Phosphorus Accumulating Organisms

**REML:** Restricted Maximum Likelihood

**SBR:** Sequential Batch Reactor

**SRT:** Sludge Retention Time

**SS:** Suspended Solids

**SVI:** Sludge Volumetric Index

**T:** Temperature

**TC:** Total Coliforms

**TDS:** Total Dissolved Solids

**tN:** Total Nitrogen

**TOC:** Total Organic Carbon

**tP:** Total Phosphorus

**TS:** Total Solids

**TSS:** Total Suspended Solids

**VSS:** Volatile Suspended Solids

**VSSF:** Vertical Subsurface Flow

**WWTP:** Wastewater Treatment Plant
1. **Introduction**

The increase in water demand and water scarcity arises proportionally to the increase in population. This situation incites the development of a wide range of wastewater treatment technologies, which can be classified into (i) conventional (high cost in operation and maintenance) or (ii) nonconventional treatment (low cost in operation and maintenance). Nowadays, the selection of an adequate and sustainable technology that involves low energy consumption, a good sanitation and that considers the increment in global temperature is of outmost importance (Verstraete and Vlaeminck, 2011).

Constructed wetlands have gained interest in wastewater treatment due to their low cost, low energy consumption, easy operation, sustainability and their integration with the landscape (Boets, et al, 2011, Mahmood et al., 2013). On the other hand, activated sludge is considered a conventional wastewater treatment technology, with advantages such as low land requirements, reliable processes, operational flexibility, high removal efficiency of organic matter and possible high removal of nutrients as well (Von Sperling, 2007b). Thereby, a comparison between the performance of the aforementioned treatments results of interest for selecting a suitable treatment alternative. Notwithstanding, to the knowledge of the author, there is a lack of experimental comparisons of both systems at high strength wastewater scenarios, which predominates overall in developing countries where fast growth of populations without proper water sanitation leads to water quality deterioration and scarcity (Kivaisi, 2001).

The main objectives of this study were to analyze and compare the treatment efficiency, buffering capacity and resilience within and between two treatment systems, activated sludge and constructed wetland during a peak exposure to high strength wastewater. To this end, a systematic approach was conducted. During 6-month start-up period with medium strength wastewater, besides the purpose of stabilizing the systems, the removal efficiencies related to COD, BOD$_5$, N, and P were used in developing first order kinetic models and power analysis tests for the preparation of sampling campaigns during the exposure of high strength wastewater. More specifically, the first order models were built as a description of expected results.
of each system during the peak while power analysis supplied statistically sufficient sample size. Subsequently, four statistical hypotheses regarding the comparison among the replicas of each system and between systems were developed and tested via likelihood ratio test based on restricted maximum likelihood (REML) estimation.

This thesis includes six sections, the first one provides background information related to the importance of wastewater treatment and to the two treatment systems that will be compared. Furthermore, some operational and controlling factors of relevance to the experiments performed are explained as well. The second section refers to the methodology and research questions. The third part includes the obtained results while the fourth one includes the respective result discussion that finalizes with a perspective of applicability and implementation. Finally, some general conclusions and further research suggestions are presented in the last two sections.
2. Literature review

The first part of this literature review explains the characteristics of wastewater, the importance of its treatment and the general classification of sewage treatments (subsection 2.1). As the main objective of this research involves the comparison of two different biological treatments, subsection 2.2 describes the removal processes of two selected treatment technologies: activated sludge and constructed wetlands. Moreover, as the optimal performance of any treatment technology requires monitoring and controlling certain parameters, subsection 2.3 provides information about relevant physicochemical conditions for activated sludge and constructed wetlands systems. Finally, subsection 2.4 presents a general comparison of some environmental and economic features commonly considered for the selection between both treatments.

2.1 Wastewater

2.1.1 Composition

Water is a resource for a wide variety of anthropogenic activities, ranging from agriculture to industry one. In general, after accomplishing its initial purposes, the water is discharged in sewers/surface water bodies as wastewater due to the presence of contaminants in it (Tchobanoglous et al., 2003).

The composition of wastewater varies from common soaps and food craps in domestic wastewater to more toxic chemicals, such as heavy metals, pharmaceuticals and volatile organic compounds in industrial wastewaters (Akuzuo Ofoefule, 2011).

Table 1 presents main components and properties of wastewater that are normally examined for water quality assessment. In some cases, for one component several forms can be monitored and each one of them can be related to a specific repercussion on the environment or in human health.

<table>
<thead>
<tr>
<th>Component or properties</th>
<th>Monitored form</th>
<th>Effect on environment / human health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS)</td>
<td>Total dissolved solids (TDS)</td>
<td>-Turbidity that interferes with the penetration of light through the water column</td>
</tr>
<tr>
<td></td>
<td>Total suspended solids (TSS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volatile suspended solids (VSS)</td>
<td>-Protection of pathogens</td>
</tr>
<tr>
<td>Component or properties</td>
<td>Monitored form</td>
<td>Effect on environment / human health</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>Nitrite (NO₂⁻), Nitrate (NO₃⁻), Ammonium (NH₄⁺), Ammonia (NH₃), total Nitrogen (tN), Kjeldahl nitrogen (organic N)</td>
<td>- Depletion of dissolved oxygen by ammonia conversion to nitrites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Free ammonia toxicity mainly to fishes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Nitrates are associated with development of hypoxia in tissues</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Water alkalinity consumption during nitrification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Excessive algae growth</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>Total phosphorus (tP), phosphate (PO₄³⁻ or P₂O₅)</td>
<td>Excessive algae growth</td>
</tr>
<tr>
<td></td>
<td>Biological oxygen demand (BOD₅)</td>
<td>Dissolved oxygen consumption by microorganisms for degrading organic matter</td>
</tr>
<tr>
<td>Organic matter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(proteins, carbohydrates, lipids)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total coliforms (TC)</td>
<td>Fecal coliforms (FC), Helminths’ eggs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical conductivity (EC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>Dissolved oxygen (DO)</td>
<td></td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>Liquid temperature</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>Acidity/alkalinity</td>
<td></td>
</tr>
</tbody>
</table>

An emerging concern related to wastewater composition involves the micropollutants; these compounds are present in wastewater typically in low concentrations, from micrograms to nanograms per liter; nevertheless, they pose a negative potential effect to environment and public health (Jiang et al., 2013). Generally, these kind of contaminants comprise pharmaceuticals, personal care products, endocrine disrupting chemicals, microplastics and pesticides (Besseling et al., 2012, Bolong et al., 2009). Though, as this is a research topic on its own, it will not be covered in this thesis further on.
2.1.2 Wastewater treatment

Sewage treatment became a concern since the beginning of the twentieth century as consequence of two main reasons: first, sewage represented an environmental hazard by overpassing the natural buffer capacity of the water bodies and, second, it increased health problems (Bani, 2011, Molle et al., 2005). The natural buffer capacity, or stream self-purification refers to the capability of a water body to recover the equilibrium state by natural mechanisms after a discharge (Whitehead and Lack, 1982). Moreover, when inhabitants started to dispose of their sewage to the water bodies, potential pathogens were carried along and arrived to downstream populations, causing waterborne diseases (Bani, 2011, Akuzuo Ofoefule, 2011).

Generally, wastewater is collected after its discharge and transferred to wastewater treatment plants (WWTP). In order to decide the adequate treatment, wastewater must be characterized by measuring the main components and properties that determine water quality (Akuzuo Ofoefule, 2011). In accordance to the detected pollutants’ concentration, three strengths of wastewater are commonly established (Table 2).

**Table 2. Wastewater strengths according to the contaminants load (Tchobanoglous et al., 2003).**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Low strength (mg. L⁻¹)</th>
<th>Medium strength (mg. L⁻¹)</th>
<th>High strength (mg. L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>390</td>
<td>720</td>
<td>1230</td>
</tr>
<tr>
<td>TSS</td>
<td>120</td>
<td>210</td>
<td>400</td>
</tr>
<tr>
<td>VSS</td>
<td>95</td>
<td>160</td>
<td>315</td>
</tr>
<tr>
<td>BOD₅</td>
<td>110</td>
<td>190</td>
<td>350</td>
</tr>
<tr>
<td>COD</td>
<td>250</td>
<td>430</td>
<td>800</td>
</tr>
<tr>
<td>tN</td>
<td>20</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>tP</td>
<td>4</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>TOC</td>
<td>80</td>
<td>140</td>
<td>260</td>
</tr>
</tbody>
</table>

TS= Total Solids; TSS= Total Suspended Solids; VSS= Volatile Suspended Solids; BOD₅= Biological Oxygen Demand; COD= Chemical Oxygen Demand; tN= total Nitrogen; tP= total Phosphorus; TOC= Total Organic Carbon

The most suitable sewage treatment is determined based on the wastewater type and socioeconomic factors. With regard to the wastewater type, high strength wastewater is usually treated by anaerobic reactors while low strength wastewater with aerobic
treatments (Chan et al., 2009). Related to socioeconomic factors, the following are taken into account for treatment selection: infrastructure, available technologies, land requirements, operation and maintenance costs, and experienced staff (Von Sperling, 2007b).

Bani (2011) mentioned that there are two main treatment methods: conventional and non-conventional. The former typically applies automated technologies and requires pumping and power. Some conventional technologies comprise activated sludge, trickling filters and rotating biological contactors. Non-conventional techniques are usually low-priced, less sophisticated in operation and maintenance, e.g., waste stabilization ponds, constructed wetlands and oxidation ditches.

**2.2 Wastewater treatment systems**

**2.2.1 Mechanical treatment: activated sludge**

Activated sludge (AS) is considered as a biological process for wastewater treatment which consists of microorganisms growing in a reactor of a WWTP. (Tchobanoglous et al., 2003). This technology is known as artificial treatment and is part of the conventional methods mentioned in the previous section. Furthermore, AS has been applied preferentially in the last 40 years for biological removal of nutrients (Ekama, 2015).

**Principle of technology**

In general, the influent of a WWTP passes through a physical treatment (e.g. grids and pre-settling tanks) before being mixed in a reactor with a microbial community in suspension, known as mixed liquor suspended solids (MLSS) (Jeanningros et al., 2010). Principally, heterotrophic microorganisms are responsible for decomposing biodegradable organic matter from wastewater for their growth. In order to separate treated water from the microorganisms, flocs from 50-200 µm are desired, so they can precipitate in a settling tank (Tchobanoglous et al., 2003). After settling, the clear effluent can be discharged to surface water bodies and/or reused according to its quality, whereas separated sludge is returned to the reactor or disposed.

**Organic matter removal**

During AS process, aeration allows a proper mixing of MLSS to achieve the formation of flocs in appropriate sizes, an adequate suspension of sludge and the aerobic
removal of organic carbon (see Equation 1) (Tchobanoglous et al., 2003). As a consequence, a pumping system is generally needed (Pittoors et al., 2014), which increases the investment and operational costs (Verstraete and Vlaeminck, 2011).

\[
\text{COHNS} + O_2 + \text{nutrients} \rightarrow CO_2 (g) + NH_3 + C_5H_7NO_2 + \text{other end products} \quad (1)
\]

**Nutrient removal**

According to the objectives of a WWTP, different configurations of AS can be selected. The simplest configuration would only consider an aeration tank and a clarifier (Bani, 2011). However, for achieving removal of phosphorus compounds, the configuration needs to include an anoxic section before the aerobic tank or after the aerobic tank (Coma et al., 2012). The first case is known as the Ludzack-ettinger process and the second case as Wuhmann process (Figure 1) (Baba et al., 2009, Chen et al., 2015).

**Ludzack-ettinger configuration**

![Ludzack-ettinger configuration diagram](image)

**Wuhmann configuration**

![Wuhmann configuration diagram](image)

*Figure 1. AS configurations for removal of nutrients.*

Nitrogen (N) removal is mainly accomplished by autotrophic bacteria called aerobic oxidizing bacteria (AOB) and the nitrite oxidizing bacteria (NOB) which are responsible for the conversion of ammonium to nitrites followed by nitrites to nitrates through the nitrification process (see Equation 2) (De Clippeleir et al., 2013, Tchobanoglous et al., 2003). After that, nitrates are converted to nitrogen gas by a
wide range of anaerobic oxidizing bacteria under anoxic conditions (see Equation 3), known as denitrification (Von Sperling, 2007a, Rousseau et al., 2008, Tchobanoglous et al., 2003).

\[ NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \]  
\[ (2) \]

\[ C_{10}H_{19}O_3N + 10NO_3 \rightarrow 5N_2(g) + 10CO_2(g) + 3H_2O + NH_3 + 10OH^- \]  
\[ (3) \]

Phosphorus (P) removal involves as well autotrophic bacteria known as phosphate accumulating organisms (PAO), which absorb the available phosphates under aerobic or anoxic conditions to produce poly-phosphates reserves and generate biomass. Phosphorus uptake is enhanced by a previous anaerobic exposure (Yin et al., 2015).

2.2.2 Natural treatment: constructed wetlands

Constructed wetlands (CW) are considered as non-conventional or natural method for the treatment of primary and secondary domestic wastewater as well as industrial and agricultural sewage (Randerson, 2006). CW systems involve mainly biological processes performed by microorganisms and macrophytes. In general, their easy operation, efficient maintenance and cheap cost are some factors that ease its application in developing countries (Kivaisi, 2001).

The classification of CW technology according to the direction of the water flow results in three categories: free water surface (FWS), vertical subsurface flow (VSSF) and horizontal subsurface flow (HSSF) (Figure 2). The former includes macrophytes growing in substrate which are surrounded by the water of column to be treated. The second and third category include the macrophytes which are planted in a substrate through which the wastewater flows horizontally or vertically in order to be treated (Mahmood et al., 2013).
Principle of technology

Frequently CW systems includes an artificial substrate bed for the plant, a biofilm and emerging macrophytes over the water surface. The substrate regularly consists of a mixture of sand and gravel where the helophytes’ root settle and form rhizomes. The biofilm includes algae, fungi and bacteria over substrate and plant stem surface. Finally, the commonly selected helophytes plants comprise *Phragmites australis*, *Typha* spp. and *Scirpus* spp. which are adapted to grow in anaerobic or low oxygen conditions (Rousseau et al., 2004).
Macrophytes in wetlands contribute to the structure of the system and are a source of carbon and organic nitrogen for the microbial community (Taylor et al., 2011). Moreover, they also contribute with the formation of an aerated rhizosphere that supports oxidative reactions (Van de Moortel et al., 2010).

During the operation of a VSSF CW, the influent enters the system from the top and drains vertically down to the bottom, where the effluent is collected. The hydraulic regime can be unsaturated, permanently saturated, intermittently unsaturated or flood and drain wetlands (Garcia et al., 2010). Whenever saturated conditions exist, the negative electric potential increase; thus, the reducing ecosystem counts with a great potential for processing nutrients and other materials. On the other hand, the unsaturated conditions allow to obtain oxidized conditions which are able to treat higher contaminants load (Kadlec et al., 2000).

**Organic matter removal**

Organic matter removal in CW occurs by physicochemical and biological processes. Sedimentation, filtration and sorption are the main physicochemical processes while microbial metabolism corresponds to the biological one.

Sedimentation of suspended solids (SS) from the influent takes place over the substrate whereas small particles may be filtered by or adsorbed onto the substrate due to ion exchange (Kadlec et al., 2000). These processes could cause an effective SS removal, which after a long time may clog the substrate bed and decrease the accomplishment of effective treatment (Liu et al., 2015).

Related to the microbial metabolisms removal pathway, Dordio and Carvalho (2013), mentioned that microbial communities present in the soil and roots of the wetland are responsible for the major transformations and decomposition of organic compounds such as nitrogen, iron, sulphur and carbon. This degradation is executed in aerobic and anaerobic conditions principally by heterotrophs and autotrophs present in bacterial biofilms. In this way, organic carbon is degraded to carbon dioxide by aerobic respiration (Equation 4) or by fermentation (Kadlec et al., 2000, Randerson, 2006). During the most prevalent anaerobic conditions, the fermentative bacteria generate as main product fatty acids, such as acetic acid (Garcia et al., 2010).

\[
(CH_2O) + O_2 \rightarrow CO_2 + H_2O
\] (4)
**Nutrient removal**

Similar to organic matter, the nutrients in CW are removed by a combination of biological and physicochemical processes, such as microbial decomposition, volatilization, adsorption, chemical precipitation and plant uptake.

Nitrogen transformations occur through ammonification, nitrification and denitrification processes (Randerson, 2006). The former means the mineralization of organic nitrogen to ammonium. Concerning the last two processes, similarly to the AS system, they require aerobic and anoxic conditions, respectively. Ammonia volatilization and adsorption are less relevant for nitrogen removal (Kadlec et al., 2000).

Phosphorus removal occurs by its storage in soil, plants and microorganisms. In addition, phosphorus is removed by chemical precipitation due to linkage to iron, aluminum, calcium and magnesium within the substrate matrix. Under certain pH circumstances, these are released to the bulk solution, forming precipitates with the phosphate of the wastewater (Garcia et al., 2010). Therefore, phosphates can be bound to the derived calcium carbonate in case that the substrate includes a gravel medium (Macci et al., 2015). For increasing P removal in SSF CW, the substrate bed can be chosen with high sorption capability (Dordio and Carvalho, 2013), for example with high porosity or high iron oxide content.

Regarding plant uptake, Zurita et al. (2006) mentioned that pollutants and nutrients are directly immobilized in a significant extent during the initial and rapid growth period of plants. Once growth rate decreases, elevated concentrations of nutrients could become phytotoxic. To solve the restricted plant growth period, it is possible to use plants that can be harvested frequently, such as ornamental plants.

**2.3 Controlling factors for treatment performance**

To ensure a good performance of the treatment system it is important to be aware of the different organisms present in the microbial community and parameters that affect their selection, survival and growth (Tchobanoglous et al., 2003). Special emphasis should be given to maintain the most beneficial physicochemical conditions for heterotrophic and autotrophic bacteria community responsible for nitrogen and phosphorus removal as they grow at smaller rate than heterotrophic bacteria (De Clippeleir et al., 2013).
According to Von Sperling (2007a) some controlling parameters for treatment performance with AOB, NOB and PAO include pH, temperature, dissolved oxygen, nutrients concentrations, loading rate, sludge volumetric index and retention time. Table 3 shows the range of some physicochemical conditions commonly applied in AS treatment and the optimal range suggested in a WWTP for carbon and nutrients’ removal.

Table 3. Ranges of physicochemical conditions for carbon, nitrogen and phosphorus removal (Von Sperling, 2007a).

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>COMPONENT REMOVAL</th>
<th>Carbon</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6 - 9</td>
<td>7</td>
<td>6.5 - 8</td>
<td>7 - 8</td>
</tr>
<tr>
<td>DO (mg O₂. L⁻¹)</td>
<td>&gt;0.5</td>
<td>2</td>
<td>&gt;2*</td>
<td>&lt;0.2**</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20</td>
<td>20</td>
<td>5 - 50</td>
<td>35 - 50*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 – 36**</td>
</tr>
</tbody>
</table>

* Dissolved oxygen and temperature value correspondent to nitrification.
** Dissolved oxygen and temperature for denitrification.
*** Oxygen value corresponding for absorption of phosphates by PAO.

2.3.1 pH

Tchobanoglous et al. (2003) remarked that the optimal pH for the growth of heterotrophic bacteria is between 6.5 and 7.5 whereas Von Sperling (2007a) indicated that the most suitable pH range would be between 7 and 8 for all bacteria involved in the decomposition and removal of organic carbon, nitrogen and phosphorus. Above pH 8 nutrient removal is still possible, but the removal rate can be very different from the optimal one.

The importance of maintaining the above mentioned pH range lies in the optimal pH range required for ammonification by heterotrophs (6.5 - 8.5), nitrification by AOB and NOB (7.6-8.6), denitrification by anaerobic oxidizing bacteria (7-8) and phosphorus removal by PAO (Table 3) (Kadlec et al., 2000).
2.3.2 Dissolved oxygen (DO)
Oxygen is important for accomplishing nitrification; in case that the aerobic conditions are scarce, incomplete nitrogen removal will be noticed in the effluent as nitrites. In contrast, the anoxic conditions are determinant for denitrification. Additionally, for phosphorus removal anoxic or aerobic conditions are necessary.

With the objective of promoting the floc forming bacteria, it is necessary to maintain DO in higher levels than 1 mg O₂ L⁻¹. Besides, heterotrophic bacteria, AOB and NOB, and PAO also require a control of DO for removing carbon, nitrogen and phosphorus (Table 3). Hence, the best value for supporting all the processes would be between 2-3 mg O₂ L⁻¹.

2.3.3 Temperature
The adequate temperature that allows bacteria growth and that facilitates the decomposition reactions during wastewater treatment depends on each member of the bacteria community (Tchobanoglous et al., 2003). In Table 3 can be seen that the optimal value for carbon removal by heterotrophic bacteria equals room temperature. On the other hand, removal of organic matter in CW is possible in cold temperatures due to oxygen release by the roots of some plants, which stimulates the bacteria (Taylor et al., 2011).

For nitrification processes the optimal temperature should be maintained between 25-40 degrees. If the value is lower than 15 degrees, the nitrification rate is highly diminished.

2.3.4 Nutrients
Organic matter, nitrogen and phosphorus content in sewage influence the production of new biomass. The requirements of each of these nutrients depends on the composition of the microbial community. For AS system, a ratio of 100:5:1 is reported for BOD₅:N:P, respectively, with a hydraulic retention time of 4-6 days (Von Sperling, 2007a).

If nutrients are not present in appropriate amounts, the bacteria can be subjected to a physiological shock, for instance, a lack of a substrate leads to an uncoupled metabolism that impedes biomass production (Coma Bech et al., 2015). In contrast, nutrient abundance can be counterproductive in some cases; for example, free
ammonia at pH >8 and free nitrous acid at pH <7.5 inhibit nitrifiers by decreasing their growth rate (Van Hulle et al., 2010).

### 2.3.5 Loading rate

Von Sperling (2007a) describes the importance of the loading rate, which refers to the organic matter load (kg BOD·d⁻¹) in the influent related with the amount of microorganisms in the reactor (kg MLVSS). The recommended load range goes from 0.25 to 0.40 kg BOD·kg MLVSS⁻¹ day⁻¹. The AOB and NOB are sensitive to substrate concentrations in order to do the nitrification and denitrification; a reduction in the loading rate also decreases the process.

### 2.3.6 SVI

This parameter resembles the settling ability of the sludge: a high SVI value reflects less settling and vice versa. As sludge flocs should be separated from the effluent, the recommended SVI value ranges from 50-100 mL·g⁻¹ (Von Sperling, 2007a).

### 2.3.7 Retention time

With regard to AS, the hydraulic (HRT) and sludge retention (SRT) time should be sufficient, inasmuch as the sludge age increases; in this way, the community of autotrophic bacteria can develop and enhance nitrogen and phosphorus removal. Gupta and Sharma (1996) recommended a combination of two days HRT and 30 days SRT for good removal of pollutants.

### 2.4 Comparison of wetlands versus activated sludge

#### 2.4.1 Environmental aspects

**Water quality**

AS and CW effluent quality determine the future reuse of the treated water, from discharge on water surface bodies to potable water. Because of this, the contaminant removal performance of each technology dictates the range of possible reuses in some extent. The other main factor that delimits the water reuse is the regional standard limits (Von Sperling, 2007b). Table 4 shows a comparison between the effluent quality of AS and CW.
Table 4. Effluent quality after treatment of domestic wastewater with AS and CW (Von Sperling, 2007b)

<table>
<thead>
<tr>
<th>Technology</th>
<th>AS with N removal</th>
<th>CW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Removal efficiency (%)</td>
<td>Effluent concentration (mg. L⁻¹)</td>
</tr>
<tr>
<td>BOD₅</td>
<td>85-93</td>
<td>15-40</td>
</tr>
<tr>
<td>COD</td>
<td>80-90</td>
<td>45-120</td>
</tr>
<tr>
<td>SS</td>
<td>87-93</td>
<td>20-40</td>
</tr>
<tr>
<td>Ammonia</td>
<td>&gt;80</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>&gt;75</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>&lt;35</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Fecal Coliforms (FC)</td>
<td>1-2 (log units)</td>
<td>10⁶-10⁷ FC/100mL</td>
</tr>
</tbody>
</table>

Based on the typical effluent quality of each technology, in both cases the effluent cannot be discharged in sensitive water bodies according to the less stringent standards of the European Community Directive of urban wastewater (Von Sperling, 2007b). However, when AS and CW are combined with advanced technologies, the effluent quality improves enabling a different reuse. For instance, AS combined with membranes and ozonation allows the reuse of wastewater as potable water (Verstraete and Vlaeminck, 2011). Furthermore, CW integrated with membrane bioreactors (MBR) can treat high strength wastewaters, which can be reused for agriculture, horticulture and energy forestry after treatment (Randerson, 2006, Liu et al., 2015).

**Treatment of different wastewaters**

When a treatment system faces a large variation in wastewater composition, the pollutant removal efficiency depends on its robustness. Particularly, CW are considered as robust systems with a high buffering capacity, which allows to get a stable effluent quality (Rousseau et al., 2008). On the other hand, AS is more sensitive to different contaminant loads due to its total dependence on the microbial community (Liu et al., 2015) and the control parameters to sustain it. Despite of this, both systems currently treat a variety of wastewater strengths.

In general, both technologies can cope with high strength wastewater when coupled with another technology or when selecting an enhanced variant of the same
technology. Some AS variants for treating high strength wastewater include: granular sludge in sequencing batch reactor (SBR) (Coma et al., 2012), membrane bioreactors (MBR) and oxygen limited autotrophic nitrification/denitrification (OLAND) in a rotating biological contator (De Clippeleir et al., 2013). For CW, the placement of sand filters previous to the wetland boosts the treatment of high strength sewage (Shepherd et al., 2001). In addition, the arrangement in series of different configurations of CW, such as HSSF, VSSF and FSF improves pollutant removal as well; this alternative is known as hybrid wetlands (Ávila et al., 2014).

**Residue generation**

The AS process frequently deals with excess sludge production due to the increase of microorganism biomass. Therefore, surplus sludge is removed from the system and disposed of continuously. Disposal is preceded by thickening, digestion, conditioning, dewatering and disinfection of the sludge (Von Sperling, 2007b) which can be reused in agriculture in some cases. Nevertheless, Milieu mentioned that AS residue generation can reach $10^{10}$ kg of sludge dry matter per year (as cited by (Verstraete and Vlaeminck, 2011) which represents an inconvenience for WWTP economy and sustainability.

Residues generated by CW comprise substrate and plant cover, of which the production frequency depends on substrate clogging. In general, residue disposal occurs after 10-15 years of functioning (Von Sperling, 2007b). The biomass harvested can be used for biogas production, composted for horticulture or used as animal feed (Kivaisi, 2001), whereas the substrate typically is replaced for new substrate (Nivala and Rousseau, 2009).

**2.4.2 Economic aspects**

**Treatment facilities**

AS is considered as a centralized sewage treatment method, hence, WWTP dimensions are determined by the type of AS technology selected (e.g. conventional or MBR) and by the inhabitant equivalents capacity (Von Sperling, 2007b). On the other hand, CW facilities have high land requirements due to their limitation to low hydraulic loading rates (HLR). As a result, CW is considered as decentralized sewage treatment preferentially applied for sanitation of small populations with land availability in surrounding areas (Ávila et al., 2014, Kivaisi, 2001). Table 5 shows
construction cost and the corresponding land requirements in order to compare the treatment facilities costs between AS and CW. It is clearly shown that the construction cost for AS is twice as high when compared to CW, though the latter has a much higher land requirement.

**Table 5. Construction costs and land requirements for AS and CW treatment facilities. Modified from (von Sperling, 1996, Von Sperling, 2007b)**

<table>
<thead>
<tr>
<th>System</th>
<th>Construction cost (euro. inhabitant⁻¹)</th>
<th>Land requirements (m². inhabitant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>40-62</td>
<td>0.12-0.25</td>
</tr>
<tr>
<td>CW</td>
<td>18-27</td>
<td>3-5</td>
</tr>
</tbody>
</table>

**Operation costs**

Given that AS belongs to the category of conventional treatment technologies, it is usually associated with intensive maintenance and capital costs (Boets et al., 2011). The range of the mentioned costs differs depending on the size of the WWTP: small sized plants (<50,000 inhabitants) cost twice as much as large plants (>150,000 inhabitants) (Von Sperling, 2007b). Independently of the size, the addition of an external COD source for nutrient removal, sludge treatment and disposal represents a big investment of the operational cost (Coma Bech et al., 2015). However, some AS variants such as OLAND promote less sludge production and less aeration in order to decrease the expenses (De Clippeleir et al., 2013).

With respect to energy requirements, Verstraete and Vlaeminck (2011) stated that AS technology is not optimal regarding energy efficiency, carbon footprint and recycling. Only 20% of the overall consumed electricity is recovered through anaerobic sludge digestion.

In general all the technologies that require energy in high quantity are more expensive than CW (Kadlec et al., 2000). In a comparative study performed by Von Sperling (2007b) CW energy requirements during systems’ operation were considered as nulls. With respect to operation and maintenance costs, CW are around four to six times cheaper than AS (Table 6). Moreover, the expenses can be reduced with the economic alternative of using ornamental flowers in CW instead of the typical macrophytes;
this results successful overall in developing countries with subtropical climate (Zurita et al., 2006).

**Table 6. Operation and maintenance costs for AS and CW treatment systems. Modified from (Von Sperling, 2007b).**

<table>
<thead>
<tr>
<th>System</th>
<th>Power (Watt.inhabitant⁻¹. year⁻¹)</th>
<th>Operation and maintenance (euro. inhabitant⁻¹. year⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>15-22</td>
<td>3.5-8</td>
</tr>
<tr>
<td>CW</td>
<td>0</td>
<td>0.9-1.3</td>
</tr>
</tbody>
</table>
3. Materials and methods

3.1 System set up

The designs of the CW and AS were based on reported laboratory scale experiments (Lianfang et al., 2009, Wiessner et al., 2005, Sun and Austin, 2007, Coma et al., 2012). In the case of the former, the selected configuration was a SSVF CW; in the case of the latter, the chosen configuration corresponded to the Wuhmann process.

Each system was comprised by three replicas and one replica included one compartment for CW while for AS a replica was constituted by two compartments of the same size. The main features considered for the installation of a replica of AS and CW are shown in Table 7.

Table 7. Main features considered for CW and AS systems.

<table>
<thead>
<tr>
<th>System</th>
<th>Container</th>
<th>Volume to treat (L)</th>
<th>Flow (L.d⁻¹)</th>
<th>Hydraulic Retention Time (d)</th>
<th>Height (m)</th>
<th>Surface area (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CW</td>
<td>PVC pipe</td>
<td>7.5 ± 0.7</td>
<td>3.4 ± 0.5</td>
<td>2.2</td>
<td>0.8</td>
<td>0.031</td>
</tr>
<tr>
<td>AS*</td>
<td>Transparent plastic aquaria</td>
<td>3</td>
<td>3 ± 0.2</td>
<td>0.49</td>
<td>0.08</td>
<td>0.036</td>
</tr>
</tbody>
</table>

* Features corresponding to one of the compartments of AS replica. Similar values were considered for the second compartment.

After the design, the following step consisted in setting up the systems in an insulated room with controlled temperature in the water (22-25.7 °C) and fluorescent light cycles of 16 hours per day.

3.1.1 CW

In the CW system, PVC tube dimensions were 1 m height with a base diameter of 0.2 m; at the bottom of the pipe an impermeable plastic valve for sampling and/or draining the treated water was placed (Figure 3a). Concerning the setup, each of the replicas was arranged in the following way: a layer of coarse substrate was placed at the bottom of the pipe up to the valve level; from that point upward, a layer of substrate with porosity of 30% (courtesy of Prof. Rousseau D. research group,
Kortrijk) was distributed until reaching the 0.80 m of total height; both substrates were rinsed thoroughly before being placed in the systems to avoid intrusion of undesired particles. After arranging substrate, two individuals of *Typha latifolia*, (donated by Geert Verhoeyen, Oeverplanten company) were planted in the substrate (Figure 3b). Finally, the systems were inoculated with approximately 1L of activated sludge (from WWTP Aquafin Ossemeersen) for a faster development of the bacteria community.

*Figure 3. Upper and bottom part of CW replica after set up. a) on the bottom of the pipe the draining valve is located, b) Helophytes planted in the substrate.*
3.1.2 AS

The AS system was set up including two aquaria in sequence per replica. Both aquaria were filled with 1.5 L approximately of concentrated returned sludge from an existing WWTP (Aquafin WWTP Ossemeersen) and 1.5 L of tap water. In addition, to maintain the sludge in suspension and control the dissolved oxygen, the first compartment was aerated (2-6 mg O₂. L⁻¹) and a magnetic stirrer was introduced at the base of the aquaria; the wastewater moved to the second section through a rubber tubing. The second compartment included a magnetic stirrer over a glass base to maintain mixing but anoxic conditions (<1mg O₂. L⁻¹) (Figure 4). Afterwards, the exit sludge was collected in a common barrel for the replicas to allow its recirculation in a daily basis. The recirculation consisted in concentrating the exit sludge by draining the supernatant from the collecting barrel, which corresponded to the treated water. Then, the aeration and mixing in the aquaria were interrupted to allow the distribution of the concentrated sludge in the six different compartments in a way that each aquaria included approximately half volume sludge and half volume supernatant before reestablishing the functioning of AS system.

*Figure 4. Replica compartments. The aquaria in higher level corresponds to the aerobic section. On the anaerobic section is visible the mixing effect by the magnetic stirrer.*
3.2 Chemicals used
3.2.1 Artificial wastewater

After the setup, CW and AS systems were fed from the same barrel with synthetic wastewater which composition was based on the OECD guideline for testing of chemicals in activated sludge units (Painter and Nyholm, 1996); a tubing pump (Ismatec BVP standard) supplied continuously the influent with a flow of $3.4 \pm 0.5$ L.d$^{-1}$ to fulfill the HRT planned during the design. New artificial wastewater (AWW) was prepared (every one or two days approximately) by mixing the nutrients from a stock nutrients solution with fresh peptone and meat extract (Figure 5). Under these controlled conditions, it was possible to monitor the influent and effluent of the systems at reference conditions until their stabilization; this would allow to compare future effects caused by different exposure conditions.

Figure 5. Synthetic wastewater composition according to the OECD guidelines. The reactant’s brands used in the study are indicated inside the brackets.

AWW described above corresponds to a medium strength AWW category (MSAWW) according to classification given by Tchobanoglous et al. (2003). Moreover, in a second part of the present study, MSAWW composition was increased the triple to obtain a high strength wastewater (HSAWW) and feed CW and AS systems for comparing their performance. The composition of the two types of AWW used can be
expressed in terms of contaminants usually measured for water quality purposes; therefore, an estimation of their concentration in the influent can be obtained.

In the present study BOD$_5$, COD, tN, tP were the contaminants monitored in the influent and effluent of each system. Table 8 presents the expected concentrations in the two types of AWW applied.

**Table 8. Expected concentrations of pollutants of interest in two types of AWW.**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>MSAWW (mg. L$^{-1}$)</th>
<th>HSAWW (mg. L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD$_5$</td>
<td>125</td>
<td>375</td>
</tr>
<tr>
<td>COD</td>
<td>212</td>
<td>637</td>
</tr>
<tr>
<td>tN</td>
<td>43</td>
<td>129</td>
</tr>
<tr>
<td>tP</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

### 3.2.2 Tests kits

Conventional test kits were used to measure the concentrations of most of the above mentioned pollutants in wastewater. The specifications of the tests and the equivalent standards are shown in Table 9.

**Table 9. Reactants used for contaminant analysis in wastewater.**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Test kit used</th>
<th>Brand</th>
<th>Standard followed</th>
</tr>
</thead>
<tbody>
<tr>
<td>tN</td>
<td>Crack set 20 Nitrate test</td>
<td>Merck Spectroquant</td>
<td>DIN EN ISO 11905-1 DIN 38405-9 EPA 365.2+3</td>
</tr>
<tr>
<td></td>
<td>Crack set 10</td>
<td>Merck Spectroquant</td>
<td>APHA 4500-P E DIN EN ISO 6878 DIN ISO 15705</td>
</tr>
<tr>
<td>tP</td>
<td>Phosphate test (o-phosphate)</td>
<td>Merck Spectroquant</td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>COD test wide range (25-1500 mg COD.L$^{-1}$)</td>
<td>Merck Spectroquant</td>
<td>EPA 410.4 APHA 5220 D ASTM D1252-066 B</td>
</tr>
</tbody>
</table>

For the determination of BOD$_5$ were used as reactants the nutrients and inhibitor of nitrifiers indicated by the European standard NB EN 1899-1 equivalent to ISO 5815:1989, modified. Additionally, the seeds for inoculating seeding microorganisms were Polyseed InterLab.
3.3 Scenario Analysis

3.3.1 Stabilization period

The stabilization period refers to preliminary tests held after the setup of the systems to monitor their removal efficiency for \( \text{BOD}_5 \), \( \text{COD} \), \( \text{tN} \) and \( \text{tP} \) from the MSAWW. Nevertheless, for \( \text{BOD}_5 \) the monitoring was more frequent than the last three contaminants due to test kits availability. In addition, \( \text{pH} \), \( T \), \( \text{DO} \) and \( \text{EC} \) were monitored daily to ensure adequate conditions for the removal process. After some weeks, physicochemical values were stable, which allowed to keep monitoring three times per week.

The treatment performances during stabilization would allow to assume enough stability in the systems for testing the treatment of a different kind of synthetic wastewater; in such a case, the pollutants removal obtained during the stabilization period would be considered as reference conditions for comparing to a different exposure scenario.

Stabilization period comprised a total of six months (December 2015-May 2016). Nevertheless, the monitoring for the CW system comprised 1.5 months (February-16th March) and for the AS system one month period (15th March-17th April). The different periods for monitoring were result of the setup of the two systems in different moments.

3.3.2 Disturbance

This experimental scenario had the purpose of assessing the response of CW and AS systems to five days exposure of HSAWW. With an approach of one month duration, the scenario included an initial exposure to MSAWW during one week before the five-day exposure to HSAWW; after this, the influent was returned to MSAWW composition during the last 17 days. The described lengths of different AWW supply allowed to count with reference conditions that could be compared to the exposure and to follow the reaction of the systems along time.

In this new scenario were measured the same physicochemical parameters and contaminants removal than in the stabilization period. As the reaction of CW and AS systems to the exposure was unknown, the measurements of \( \text{EC} \), \( \text{DO} \), \( \text{pH} \) and \( T \) were
done daily. In addition, the AWW supplied to the treatment systems was prepared every day to diminish decomposition of the components inside the feeding barrel.

### 3.3.3 Research questions and hypotheses

To distinguish changes in the performance of CW and AS systems during HSAWW scenario, four different research questions were formulated for making comparisons within the system (at replica level) and between systems (comparing CW with AS). The set of four questions was applied to each of the contaminants monitored along the experimental month: BOD$_5$, COD, tN and tP. The comparisons could be approached in terms of treatment efficiency, buffering capacity and resilience. The corresponding research question and the null hypothesis to test for this scenario are presented in Table 10.

**Table 10. Research questions to test for BOD$_5$, COD, tN and tP during the HSAWW scenario.**

<table>
<thead>
<tr>
<th>Research question*</th>
<th>Concept involved</th>
<th>Hypothesis number</th>
<th>Null hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are the original background concentrations equal?</td>
<td>Treatment efficiency</td>
<td>1</td>
<td>The background concentrations are equal.</td>
</tr>
<tr>
<td>Are the responses during HSAWW exposure the same?</td>
<td>Buffering capacity</td>
<td>2</td>
<td>The responses to HSAWW exposure are the same.</td>
</tr>
<tr>
<td>Are the final concentrations equal?</td>
<td>Treatment efficiency</td>
<td>3</td>
<td>The final concentrations are equal.</td>
</tr>
<tr>
<td>Are the resulting and original background concentrations equal?</td>
<td>Resilience</td>
<td>4</td>
<td>The resulting and original background concentrations are equal.</td>
</tr>
</tbody>
</table>

*All research questions need to be posed to compare the replicas of each system and to compare between systems. An exception is the number four, which is necessary only for the comparison between systems.

### 3.4 Design of HSAWW scenario

#### 3.4.1 Modelling theoretical pollutant evolution

Theoretical systems’ effluent behavior for each of the pollutants along the month of HSAWW scenario was modelled from BOD$_5$ data collected during the preliminary test. The aim of this model was to have a clear idea of the expected concentrations in the
effluent of AS and CW during the whole scenario for designing the sampling from the systems.

The model was constructed by considering the dilution and degradation of the contaminants when entering AS and CW systems. To do this, a first order kinetic approach was used for the pollutant removal and a non-ideal plug flow with completely stirred tanks reactors (CSTR) processes in series as hydraulic regime (Equation 5). The choice of the mentioned hydraulic regime assumes that the relative low flow of 3.4 L. d⁻¹ might not cause significant mixing of incoming and existing contaminants.

\[
\frac{C_e}{C_i} = \eta \cdot t \tag{5}
\]

With Ce= effluent pollutant concentration, Ci= initial pollutant concentration, η= removal efficiency during stabilization period, t= time in days.

For AS model, the removal efficiencies for BOD₅ corresponded to the obtained in stabilization period while for COD, tN and tP were made assumptions based on efficiencies reported by (Von Sperling, 2007b) in field conditions (85%, 76% and 34% respectively).

For CW model, the removal efficiencies obtained in the preliminary tests were included in the calculations.

3.4.2 Sample size determination

Power analysis can be used to determine an accurate sample size required for an experiment in order to reject the null hypothesis (H₀) in the null hypothesis significance testing (NHST) (Beaujean, 2014). This means, the number of observations necessary to detect an effect of the treatment it this exists. As a result, in order to estimate an adequate sample size for testing the hypotheses formulated for HSAWW scenario, a power analysis was performed based on computer program with the statistical assistance of Prof. Dr. Ir. Olivier Thas.

The sample size determination was obtained considering its interrelation with effect size, the statistical type 1 error and statistical power. Beaujean (2014) mentioned that whenever three of the mentioned concepts are known, the fourth one can be estimated.
In this study the effect size (ES) referred to the unit’s difference of each contaminant that are necessary to be considered as biological relevant difference. This was calculated for each contaminant as a range of units by considering the standard deviation of pollutants’ data collected during the stabilization period. On the other hand, a value for type 1 error was given (0.05) and we assumed a range of number of samples physically viable along the HSAWW scenario (16). In this way, statistical power values were determined for multiple combinations of ES ranges and feasible samples ranges at the given $\alpha$ value.

Some additional features considered to do the power analysis were: model of interest to analyze the samples (in our case linear mixed models) and data variability.

### 3.4.3 Data collection and analysis

Considering the 16 feasible samples along HSAWW scenario, a sampling campaign for influent and effluent of CW and AS was decided bearing in mind the following:

a) the initial exposure to MSAWW required few sampling moments in a non-daily frequency due to an expected performance similar to stabilization period; b) the exposition to HSAWW included the highest number of samples collected in daily basis to follow the reaction of the systems; c) the returned exposure to MSAWW included a more distant sampling frequency than previous periods to get an idea of systems’ resilience with the few samples remaining. The exact amount of samples per period depended of the power analysis obtained. Furthermore, the influent was measured whenever a system was sampled.

In relation to sampling times, AS and CW systems were sampled in the same days before the exposure to HSAWW. In contrast, the sampling time during the exposure to HSAWW differed for each system depending on the occurrence of the high concentrations in the effluent. Additionally, both systems were sampled at the same time after the mentioned exposure.

Each sampling day was collected an approximated volume of 350 mL of fresh AWW and of replicas’ effluent from both systems. In the case of CW, the samples were obtained by the valve located at the bottom of the VSSF CW. For AS, the samples were collected in the anaerobic compartments after sludge setting previous to recirculation of exit sludge; in specific, the supernatant was collected near to exit
tube of the compartment. Before any analysis, all the samples were filtered through a 1.2 µm pore size (Whatman Cat No. 1822-047) to separate the biomass, sludge flocs and suspended solids that could interfere with any of the laboratory assays performed. Filtrates were analyzed for COD, tN and tP in attachment to test kits’ manuals and the description given for BOD$_5$ in section 3.2.2. Besides, in the case of BOD$_5$ test, oxygen consumption was determined with an oximeter probe (Oxi 3210, Wellheim).

For AS system, the physicochemical parameters were measured inside both AS compartments and inside the influent barrel with laboratory probes. In the case of CW systems, the measurements were in a small effluent volume (70 mL approximately) which was collected at that moment. Temperature was measured simultaneously with pH (GLP pH meter HI98140 Hanna). For monitoring DO, an oximeter Oxi 330 (WTW, Wellheim) was employed. Finally, the conductivity meter “Cond 315i” (WTW, Wellheim) was used in the case of EC.

SVI was determined once per week in the HSAWW according to the methodology indicated by Clescerl et al. (1999).

### 3.5 Statistical analysis

Mixed effects models are suitable when dealing with data that has repeated measurements over time, a temporal correlation, nested or hierarchical data and heterogeneity (Zuur et al., 2009). In the present study the different pollutants were measured repeatedly over time, they were correlated along time as well and they represented nested data of replica within CW and AS systems. Therefore, for testing the hypotheses related to HSAWW scenario (section 3.3.2), mixed effects models were used with the likelihood ratio test based on restricted maximum likelihood estimation method (REML) (West et al., 2014).

The general mixed model equation applied to each pollutant concentration included time as the fixed effect, the replicas as random effect and the replica within the system as a nested random effect.

To generate the models for each hypothesis per replica and per system, the data collected during the HSAWW scenario were split according to the experimental number of samples defining each period of the scenario. In this way, for CW were
considered 4 samples before HSAWW exposure, 8 during the exposure and 4 after the exposure. On the other hand, for AS the corresponding divisions were 4, 7 and 5 for each period, respectively. Statistical analysis was performed in R.

To test whether or not the random effect in mixed models was necessary, anova function in R (West et al., 2014) for REML based likelihood ratio tests was performed between the generated mixed model and its equivalent model without the random effect.
4. Results

4.1 Stabilization period

After monitoring the effluent of Constructed Wetland (CW) and Activated Sludge (AS) throughout the preliminary tests, the obtained pollutants’ concentrations allowed to calculate the respective average removal efficiencies. In Table 11 the performances of CW and AS for the four monitored contaminants under reference conditions are summarized.

Table 11. Average removal efficiencies (%) of water quality parameter (BOD₅, COD, N, P).

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>PARAMETER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOD₅ (%)</td>
</tr>
<tr>
<td>CW</td>
<td>97</td>
</tr>
<tr>
<td>AS WITH NITROGEN REMOVAL</td>
<td>96</td>
</tr>
</tbody>
</table>

* NM= Removal efficiencies not determined during stabilization period.

For the natural system, the obtained efficiencies were higher than 90% with exception of nitrogen which was only removed by 43 % in average. Contrastingly, for the conventional system, BOD₅ was the only contaminant measured along preliminary tests, which removal reached high efficiency (96 %).

Treatment efficiencies patterns of CW and AS let perceive stability at the end of preliminary tests. In case of the former, BOD₅ removal efficiency ranged from 95-99%, from 93-97% for COD, from 38-51% for tN and from 80-91% for tP. In the case of the latter, BOD₅ removal efficiency remained between 95-98%.

In relation to the physicochemical parameters, the values were comprised in the followed ranges: pH 7-8, T between 21-24°C and EC 600-850 µS.cm⁻¹ for both systems. Concerning the DO, the values were <1 mg O₂. L⁻¹ in CW effluent and anaerobic aquaria of AS, while 4-6 mg O₂. L⁻¹. in the aerobic aquaria. DO in the MSAWW varied from 8 to <1mg O₂. L⁻¹ as passed the time after its preparation. Lastly, the SVI was maintained in the recommended value of 50-100 mL MLSS. g sludge⁻¹(Von Sperling, 2007a). The maintenance of physicochemical parameters as stable as possible.
4.2 Design of HSAWW scenario

4.2.1 Modelling theoretical pollutant evolution

Figure 6 depicts the models elaborated for BOD$_5$ removal in the CW and AS system. It can be observed that the time framework of the model comprises three different periods for around one month: the first period as reference conditions previous to the exposure; the second at HSAWW exposure and, the third at reference conditions after the exposure. With respect to the models elaborated for COD, N and P removal, they behave similar to Figure 6 (Appendix 1 and Appendix 2).

Due to AS had a shorter HRT (1 day) than CW (2.22 days), it shows first the increase of pollutants’ concentration in one day approximately after the start of peak supply whereas the CW presents the pollutant’s peak in around two days after the exposure. In addition, after the peak, pollutants tend to reach similar concentrations as the ones observed prior to the peak.

The different level of effluent concentrations is a reflection of the removal efficiency of each system. In this way, Figure 6 shows that for AS exists an expected removal

![Theoretical evolution of BOD5 in different systems](image)

*Figure 6. Dynamic of BOD$_5$ concentrations in AS and CW effluent after the peak exposure of HSAWW. The peak in the influent with a duration of five days is expected to appear in the effluents with one and two days of retard in accordance to HRT of the treatment system.*
efficiency of ≈40 % along the system, while for CW it is ≈28 %. In the case of COD, the expected values are ≈36 % AS and ≈26 % CW. For tN ≈34 % AS and ≈15 % CW, and for tP ≈ % and ≈24 % respectively.

4.2.2 Sample size determination

Several power analyses were obtained with data of BOD₅ for the formulated hypothesis (Appendix 3 and Appendix 4). A combination of statistical power higher than 0.8 preferably (Ellis, 2010) and number of samples viable in laboratory (16) comprised the criteria considered to select the analysis for each hypothesis in HSAWW scenario. Unfortunately, power analyses could not be performed for COD, tN and tP due to insufficient available data collected from preliminary tests.

Table 12 shows the selected power analysis for three of the formulated hypotheses. As noticed, the biggest sample collection corresponds to the peak exposure period (eight samples). On the other hand, four samples may result sufficient to answer the hypotheses for the reference conditions and the post exposure period. Overall, 16 samples were required for the whole experimental period with a high power for hypotheses 1 and 3 in the table, but lower for the fourth one. The distribution of the 16 sampling points along the theoretical model for BOD₅ is illustrated in Figure 7.

Table 12. Ideal combination of sample size and its distribution for BOD parameter in CW and AS. The best combination for each hypothesis and the respective power of test are detailed in the different rows.

<table>
<thead>
<tr>
<th>Hypothesis*</th>
<th>Samples required per replica</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start period</td>
<td>Peak</td>
<td>End</td>
</tr>
<tr>
<td>1. Is the original background concentration equal?</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>3. Is the final concentration equal?</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>4. Are the resulting and original background concentrations equal?</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

*The hypothesis of the peak period was not considered for the power analysis.
4.2.3 Data collection

Sampling frequencies and timing for CW and AS are presented in Figure 7. Both systems share sampling days during the periods before and after the peak in the influent; nevertheless, during the exposure to HSAWW the sampling days depend on the HRT of each system. Furthermore, the schedule for influent’s samples is paired to the systems; in other words, AWW samples should be collected whenever a system is sampled.

4.3. HSAWW hypotheses within systems
4.3.1 AS

Background concentration (Hypothesis 1)

Pollutants’ concentration in AS followed a stable pattern before the HSAWW exposure in accordance to Figure 8. The graphs for BOD$_5$ and COD show lower concentrations (higher removal) and more stable behavior than the ones for tN and tP regarding the initial concentrations. The mixed model analysis applied to this period indicated that the replicas of AS behave the same for all the parameters; the p values obtained are: 0.301 for BOD$_5$, 0.9223 for COD, 0.826 for tN and 0.7677 for tP.
Pollutants’ concentration in AS present fluctuations during the HSAWW as shown in Figure 9. The graphs for BOD₅ and COD show lower concentrations than the ones for tN and tP with respect to the influent. Despite the visible fluctuations in COD and tP graphs, the mixed model analysis applied to this period indicated that the replicas of AS behave similarly for all the parameters; the p values obtained are: 0.2674 for BOD₅, 0.2926 for COD, 0.0945 for tN and 0.6411 for tP.

Exposure to HSAWW (Hypothesis 2)

Note: BODs values of this period fell within a range of 13-64% oxygen consumption in the laboratory assay.
Concentration after HSAWW exposure (Hypothesis 3)

Pollutants’ concentration in AS present more stable pattern after the HSAWW exposure than the previous period (Figure 10). Graphs for BOD$_5$ and COD illustrate concentrations more distant to the influent than the ones for tN and tP. Despite the visible fluctuations in tP graphs, the mixed model analysis applied to this period showed that the replicas of AS behave the same for all the parameters; the p values obtained are: 0.0775 for BOD$_5$, 0.5888 for COD, 0.2868 for tN and 0.9999 for tP.
AS replicas throughout the whole scenario

Figure 11 displays the changes in AS effluent concentrations across the whole HSAWW scenario. In accordance to the results described for the three independent periods, the three AS replicas perform likewise throughout the complete experiment. The p-values obtained for each of the hypotheses are summarized in Table 13.

Pollutant removal is shown clearly in Figure 11. Thus, in general, BOD$_5$ and COD effluent concentrations are smaller than the ones of tN and tP.
Figure 11. Variations of AS effluent during the complete HSAWW scenario. The influent concentrations stay over the system’s effluent, and the dissimilarities between nutrients and organic matter removal is evident.

Table 13. Statistical results for AS replicas through HSAWW scenario.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Contaminant</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The background concentrations are equal between AS replicas.</td>
<td>BOD$_5$</td>
<td>0.301</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>0.9223</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>0.826</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>0.7677</td>
</tr>
<tr>
<td>2. The responses to HSAWW exposure are the same between AS replicas.</td>
<td>BOD$_5$</td>
<td>0.2674</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>0.2926</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>0.0945</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>0.6411</td>
</tr>
<tr>
<td>3. The final concentrations are equal between AS replicas.</td>
<td>BOD$_5$</td>
<td>0.0775</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>0.5888</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>0.2868</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>0.9999</td>
</tr>
</tbody>
</table>
Additional parameters

In relation to the SVI, the values for aerobic and anaerobic compartments remained in general close to the range of 50 to 100 mL sludge/g sludge before the exposure and at the end of the scenario (Appendix 6 and Appendix 5); however, during the supply of HSAWW the increase in settling volume elevated the SVI above 200 mL sludge/g sludge (Figure 12b). Some effect of the peak can be still observed after the exposure of the systems (Figure 12c and Appendix 6).

Concerning the physicochemical parameters, the variability is observed in the Appendix 7 and Appendix 8. The electrical conductivity seems to be the steadiest parameter between the replicas. In case of temperature, it fluctuates similarly in both AS compartments. However, pH varies in both compartments and DO in the aerobic compartment; some changes produced during the exposure period contribute to this variability, such as the depletion of oxygen in aerobic tanks and the increase of pH in both tanks. Overall, the values of T comprised 22.5-26 °C; for pH 7.3-8; for EC 600-1000 µS.cm⁻¹ and 0-2, 0-5.5 mgO₂.L⁻¹.

Figure 12. Effect of the influent on AS settling volume through the HSAWW scenario. In the picture a) the settling volume corresponded to 200 mL sludge. L MLSS⁻¹ before the exposure; b) shows a contrasting settling volume of 940 mL sludge. L MLSS⁻¹ during the exposure c) corresponds to a settling volume of 130 mL sludge. L MLSS⁻¹ after the exposure and d) present a final settling volume of 210 mL sludge. L MLSS⁻¹ at the end of the scenario.
4.3.2 CW

Background concentration (Hypothesis 1)

In Figure 13 can be observed that the pollutants’ concentration in CW followed a stable pattern in the pre-exposure period. Similar to AS system, BOD$_5$ and COD graphs show more stable pattern and lower pollutants’ concentrations to the influent than in tN and tP graphs. After analyzing with the mixed model, the replicas are considered to perform equally for BOD$_5$, COD and tN; the corresponding p values are

![Graphs of BOD$_5$, COD, Total Nitrogen, and Total Phosphorus in CW](image)

*Figure 13. Compilation of CW graphs for the period preceding HSAWW exposure. BOD$_5$, COD, tN and tP removal looks to have a stable pattern between the three replicas. HSAWW influent starts at day 9, which gives the impression of counting with less data for the influent. Note: BOD$_5$ values of this period fell within a range of 23-65% oxygen consumption in the laboratory assay.*
0.9745, 0.9999 and 0.1737 respectively. On the contrary, tP replicas did not behave identically (p=0.0267).

**Exposure to HSAWW (Hypothesis 2)**

For the period of HSAWW exposure, the concentration of the effluent fluctuated for most of the parameters (Figure 14). Nevertheless, after the mixed model analysis only the COD parameter had an uneven conduct between the replicas (p=0.0064); the rest of parameters have replicas with equivalent performance (p=0.9999 for BOD$_5$, 0.408 for tN and 0.7768 for tP). Moreover, tN and tP show higher concentrations than BOD$_5$ and COD with respect to the influent.

![Figure 14. Graphs of the four water quality parameters in CW during the HSAWW exposure. COD, tN and tP removal have fluctuating patterns considered statistically similar for the nutrients. BOD$_5$ pattern seems to be steady. The apparent missing value in influent is reflection of HRT in systems. Note: BOD$_5$ values of this period fell within a range of 23-99% oxygen consumption in the laboratory assay.](image-url)
Concentration after HSAWW exposure (Hypothesis 3)

CW replicas exhibit more stable patterns in organic matter parameters than in the nutrients (Figure 15). Additionally, the graphs for BOD$_5$ and COD illustrate concentrations below the influent whereas tN and tP surpass them.

Regardless of the visible fluctuations in the nutrients, the replicas of CW behave likewise for all the parameters in agreement with the applied mixed model analysis. Hence, the p values are: 0.7539 for BOD$_5$, 0.9999 for COD, 0.9999 for tN and 1 for tP.

Figure 15. Compilation of CW graphs after HSAWW exposure. BOD$_5$ and COD removal looks to have stable pattern between the three replicas, whereas tN and tP levels fluctuate. Note: BOD$_5$ values of this period fell within a range of 27-45% oxygen consumption in the laboratory assay.
**CW replicas throughout the whole scenario**

Figure 16 shows the changes in CW effluent concentrations throughout the whole HSAWW scenario. In agreement to the three independent periods, the replicas of CW system perform likewise for tN and BOD$_5$ along the month of experiments. Contrary, tP and COD removal differ between replicas as a consequence of the discrepancies during the first and second period respectively. The p-values obtained for each of the hypotheses are summarized in Table 14.

The removal trend of each pollutant by CW replicas can be observed in Figure 16. Thereby, BOD$_5$ and COD effluent concentrations reach smaller values than the ones

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**Figure 16. Variations of CW effluent during the complete HSAWW scenario. In the case of the nutrients, the influent concentration is surpassed by the one of system's effluent. Furthermore, the dissimilarities between nutrients and organic matter removal is evident.**
of tN and tP when comparing with influent concentrations.

**Table 14. Statistical results for CW replicas through HSAWW scenario.**

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Contaminant</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The background concentrations are equal between CW replicas</td>
<td>BOD$_5$</td>
<td>0.9745</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>0.1737</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>0.0267*</td>
</tr>
<tr>
<td>2. The responses to HSAWW exposure are the same between CW replicas.</td>
<td>BOD$_5$</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>0.0064*</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>0.408</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>0.7768</td>
</tr>
<tr>
<td>3. The final concentrations are equal between CW replicas.</td>
<td>BOD$_5$</td>
<td>0.7539</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>1</td>
</tr>
</tbody>
</table>

* Value statistically significant, where the null hypothesis is rejected.

**Additional parameters**

Some effects in CW due to the exposure to HSAWW are illustrated in Figure 17. They include the increase of turbidity in the AWW and a higher release of bubbles; the die off of leaves of wetlands and the promotion of new leaves.

![Figure 17. General effects of the HSAWW exposure to CW replicas. The figure a) shows the AWW appearance during the exposure. In b) some leaves turned yellow after the exposure and finally on c) is represented the end of the scenario with new leaves.](image-url)
With respect to the physicochemical values, the T is the parameter that varies the most whereas the pH and DO remain steady during the HSAWW scenario (Appendix 7 and Appendix 8). The median values are similar between the replicas with exception of replica three in pH. Overall the values of T comprised 21-24 °C; for pH 7.5-8; for EC 850-1300 µS.cm⁻¹ and 0-2 mgO₂.L⁻¹.

4.4 Theoretical vs obtained concentrations

4.4.1 AS

Figure 18 shows that along HSAWW scenario the obtained influent had similar values to predictions. Some fluctuations are visible on the height of peak influent for BOD₅, COD and tP. Regarding AS effluent, the obtained concentrations of pollutants are in general lower than expected throughout all the scenario, with exception of tN which almost fit totally the modelled effluent.

**Figure 18.** Modeled versus obtained AS effluent dynamic. The predicted width of the peak during the exposure resembles with the obtained results for all the parameters, whereas the height coincides the best for tN.
4.4.2 CW

In Figure 19 the obtained influents had similar trends to predictions along the complete HSAWW scenario. Some fluctuations in pollutants concentrations are visible mainly in the height of peak influent for BOD$_5$, COD and tP. Regarding the effluents, the obtained concentrations of pollutants are in general lower than expected throughout all the scenario, with exception of tN and tP, which concentrations increased after the exposure period.

Figure 19. Modeled versus obtained CW effluent dynamics. The predicted width of the peak during the exposure resembles the BOD and COD results; in the case of the predicted height there is coincidence for tN.
4.5 HSAWW hypotheses between systems

4.5.1 Hypotheses 1-3
The mixed model analysis between CW and AS indicated that both systems behave
the same in each period of the HSAWW scenario. Therefore, they perform equivalently
along the complete experimental phase. This being said, the p value was 1 for all the
comparisons through the three periods (Table 15).

Table 15. Statistical results obtained for the four hypothesis between AS and CW
systems.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Contaminant</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The background concentrations are equal between CW and AS.</td>
<td>BOD₅</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>1</td>
</tr>
<tr>
<td>2. The responses to HSAWW exposure are the same between CW and AS.</td>
<td>BOD₅</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>1</td>
</tr>
<tr>
<td>3. The final concentrations are equal between CW and AS.</td>
<td>BOD₅</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>1</td>
</tr>
<tr>
<td>4. The resulting and original background concentrations are equal between CW and AS.</td>
<td>BOD₅</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>0.162</td>
</tr>
<tr>
<td></td>
<td>tN</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>tP</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

* Value statistically significant, where the null hypothesis is rejected.

4.5.2 Hypothesis 4
Regarding the comparison between background and resulting concentration, both
systems acted in identical way for BOD₅ and COD removal (p=1 and 0.162). In
contrast, in Table 15 is shown that nutrient removal is different between both
systems (p=<.0001). For AS the initial and final concentration of tP and tN are in
similar level while for CW the resulting concentration is higher than the background
one (Figure 11 and Figure 16).
The performance of CW and AS is expressed as removal efficiencies in Figure 20. The similarity between the systems described above is evident for BOD$_5$ and COD parameters, which shows high removal (>80 %). Contrastingly, the difference between nutrients’ removal efficiency can be observed in a clearer way, though the pattern results are, to some extent, similar.

![Evolution of BOD$_5$ removal](image1)

![Evolution of COD removal](image2)

![Evolution of N removal](image3)

![Evolution of P removal](image4)

*Figure 20. Removal efficiency of CW and AS system and its evolution through the complete HSAWW scenario. The highest removal is noticed for COD and BODs. For the nutrients, the removal shows a marked depletion during the exposure period that increases towards the end of the scenario.*
5. Discussion

5.1 Stabilization period

Table 16 shows that BOD$_5$ and COD removal efficiencies of the CW system during the stabilization period are in agreement with the reported results from field conditions of Von Sperling (2007b): ≈90 % for BOD$_5$ and ≈85 % for COD. In the case of tP removal, the value obtained was higher than reported (90 % vs < 35 %), which suggests a high absorptivity by the new substrate. For AS, the obtained value for BOD$_5$ removal resembles the values reported in field conditions as well (96 % vs 93 %). The high removal efficiencies for organic matter reflect that both systems had an acceptable operation and maintenance regarding biological treatment.

<table>
<thead>
<tr>
<th>System</th>
<th>Efficiencies in field conditions (%)</th>
<th>Average efficiencies during stabilization period (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOD$_5$</td>
<td>COD</td>
</tr>
<tr>
<td>CW</td>
<td>80-90</td>
<td>75-85</td>
</tr>
<tr>
<td>AS with N removal</td>
<td>85-93</td>
<td>80-90</td>
</tr>
</tbody>
</table>

5.2 Design of HSAWW scenario

5.2.1 Modelling theoretical pollutant evolution

As the removal efficiencies and HRT from stabilization period were applied for calculating the first order kinetic approach, BOD$_5$ models in Figure 6 are intended to reflect as much as possible the performance of the installed systems at HSAWW. However, the models obtained represent optimal conditions (e.g. flow and treatment performances by the systems), which in practice could change due to HSAWW exposure and the buffering capacity and resilience inherent to the systems.

Related to the expected treatment efficiencies, Figure 6 shows that the expected removal of BOD$_5$ is higher in AS system compared to CW system along all the scenario (≈40 % vs ≈28 %). These removals are in agreement with the behavior reported in field conditions of higher BOD$_5$ removal by AS than CW systems in Table 16 (93% vs 90%), but the estimated values differ in a big proportion. Besides, the expected removals of
BOD$_5$ result contradictory with the results of stabilization period (97 % CW vs 96% AS), where CW performance was slightly higher than AS. One possible source of underestimation in removal efficiencies may be the approach of tanks in series applied for modelling both systems which include a non-optimal plug flow regime; this means that there will be a background concentration of pollutants that are not degraded.

Regarding the models of COD, tN and tP in Appendix 1 and Appendix 2, the same underestimations are shown concerning the removal efficiencies along the whole HSAWW scenario, with expected values of ≈36 % AS and ≈26 % CW for COD, ≈34 % AS and ≈15 % CW for tN and ≈23 % AS and ≈24 % CW for tP.

5.2.2 Sample size determination

Total number of samples in the present study (16) was chosen based on the available resources in laboratory and the physically viable samples per day. However, the division of these samples along the different periods in Table 12, was based on the frequency criteria mentioned in section 3.4.2, which lead to higher number of samples during the exposure to HSAWW for testing all the hypotheses.

With regard to the power results obtained for BOD$_5$ in Table 12, they are considered acceptable for hypothesis 1 and 3 (0.841 and 0.904) as they are ≥0.8 (Ellis, 2010). As higher the power, higher the probability to detect a significant statistical effect in case that this exists. Therefore, this probability is lower for hypothesis 4, which power obtained (0.58) is not as suitable as the ones for hypothesis 1 and 3. One way to rise this power would be to increase the number of samples, which would also raise the precision for estimating effect size. (Nakagawa and Cuthill, 2007); in other words, statistical power is positively related to effect size and both can be improved by augmenting the sample size.

Concerning effect size value in Table 12, its calculation involved the mean differences from the data collected during the stabilization period, which is known as unstandardized effect size statistics, an alternative for calculating effect sizes (Nakagawa and Cuthill, 2007). This approach for calculating effect size allows to approach to a tailored experimental design due to the inclusion of the current data of the study.
The limitation of power results to BOD$_5$ in the present study highlight the relevance of sufficient data collection during the stabilization period to calculate the variability of each parameter in the systems. As for BOD$_5$ the number of measurements comprised from 9-12 for each system, the variability and effect size could be calculated. On the contrary, for the other three pollutants the small number of measurements just allowed to determine the effect size based on standard deviation.

5.3 HSAWW hypotheses within systems

5.3.1 AS

Hypothesis 1-3

With regard to the variations of the effluent concentrations in the period before the exposure, Figure 8 showed that AS replicas remained stable for all the parameters as expected based on the stable behavior reached in the preliminary test. On the other hand, the exposure period reflects fluctuations in the effluent concentrations that may be related to fluctuations in the influent; this can be appreciated in Figure 9 in a clearer way for tP, in which influent and effluent pattern are similar. As the wastewater was prepared in the same way during this period, one possible source of fluctuations in the influent could be variations in external sources, such as the composition of tap water used for dissolution. For the period after exposure, Figure 10 shows a smooth fluctuation in the effluent of most of the parameters that disappears probably as the system readapts to the new strength wastewater.

AS replicas throughout the whole scenario

During the three different periods of the HSAWW scenario the ratio BOD$_5$/COD was higher than 0.5, which means that the AWW content was mostly biodegradable and therefore suitable for biological treatment (Chan et al., 2009, Von Sperling, 2007b). This being said, in Figure 11 are displayed the low concentrations of organic matter parameters in AS effluent, which reflects coherently the high depletion of organic load after biological treatment. On the other side, AS was not that suitable for removing nutrients; nevertheless, it is visible that the N level was depleted more than the P in comparison with the influent concentration probably as result of the aerobic and anoxic compartments which promoted nitrification and denitrification.
The similar performance obtained in all the parameters between the replicas along the complete scenario supports the reproducibility of the experiment. Nonetheless, when comparing Table 13 with Figure 11, some p-values suggest similarity between replicas for parts of the graph that look different. For example, tP in hypothesis 3 shows the highest p-value (0.999) and the Figure 11 shows that it is the pollutant with most fluctuations at the end of the scenario. This means that the replica behave similarly despite the fluctuations; this apparent contradiction remarks that statistical significance not always equals biological or practical difference (Nakagawa and Cuthill, 2007).

**Additional parameters**

Regarding the controlling parameters monitored during the HSAWW scenario, the ranges of T and pH stayed within the suggested values for achieving good performance before and after the exposure (see section 2.3). On the other hand, DO levels were out of the optimal range, which in general did not seem to affect AS performance before and after the exposure (Appendix 7).

During the exposure to HSAWW, the excess of organic matter supplied could be responsible for the drastic decrement of DO levels in the aerobic compartment and the increase of pH in both sections of the system (Appendix 7). A possible cause for these physicochemical changes is the decrease in the production of acidifying agents (e.g. CO₂ and protons) by aerobic bacteria as the oxygen availability became restricted by the carbonaceous decomposition process (Von Sperling, 2007b). Despite of the increment to pH 8, this stayed within the suggested range, whereas the anoxic conditions probably favored the dominance of anaerobic and filamentous microorganisms that produced a low settling rate (Chan et al., 2009) and the elevated SVI (Figure 12) (Gupta and Sharma, 1996).

**5.3.2 CW**

**Hypothesis 1-3**

Figure 13 showed stability in effluent pattern between CW replicas for all the parameters at the beginning of HSAWW scenario, which coincides with the stable behavior reached in the preliminary test. COD and tP fluctuations in the CW effluent during the exposure and post-exposure periods may be related to variations in the
influent (Figure 14 and Figure 15); on the other hand, the pattern of the influent is not observed in the effluent for the variables BOD$_5$ and tN (Figure 16). The source of fluctuations in the influent could be the same than explained for AS because both systems were fed with the same AWW. In any case, the variations in effluent after exposure period became stable probably as the system adjusted to the SAWW, showing the capability of the CW to adapt to variations in the influent (Shepherd et al., 2001).

**CW replicas throughout the whole scenario**

In Figure 16, the effluent concentrations of BOD$_5$ and COD with respect to the influent are lower than those for the nutrients through the whole scenario. This could be due to CW being considered as secondary wastewater treatment which mainly removes organic matter and suspended solids by biological mechanisms; additionally, some nutrients can be treated as well (Von Sperling, 2007b). Furthermore, nutrient removal looks to be altered by HSAWW exposure as the effluent concentrations following the start of the HSAWW supply overpass or equal the influent concentration for tN and tP respectively. This suggests that CW decompose better organic matter than nutrients, which was stated by Von Sperling, 2007b, with CW systems presenting removal efficiencies of <60% for tN and <35% for tP versus 90% for organic matter.

The similar performance between replicas for tN and BOD$_5$ shows that the experiment was reproducible during the whole scenario. Besides, the similarity for COD and tP in two of the three periods contributes to the reproducibility idea. The apparent discrepancy for tP before HSAWW exposure observed in Table 14, may be influenced by the small number of samples (three per replicate) for generating and analyzing the corresponding models in this period. Nevertheless, a power analysis for tP data would result useful to accept or discard this idea. Still, the collected data suggests that the replicas acted very similarly along the three experimental periods (Figure 16).

**Additional parameters**

In accordance to the suggested ranges for physicochemical parameters (section 2.3), the temperature and pH were maintained within adequate levels during the complete HSAWW scenario (Appendix 7 and Appendix 8). Besides, *Typha latifolia* is known to tolerate ranges of pH from 4 – 10 and ranges of temperature from 10 – 30 °C (Reed
et al., 1995; Mahmood et al., 2013). With regard to the dissolved oxygen, no optimal range was suggested previously but the anoxic conditions measured in the effluent confirm that denitrification was viable in the lower parts of the VSSF CW. As for nitrification, it is probable that the input of oxygen during drainage of the systems provided sufficient unsaturated media for oxidation reactions (Von Sperling, 2007b).

Despite some fluctuations in the electrical conductivity (850-1300 µS.cm⁻¹), this did not represent any threat for the cattail selected for the experiments, which tolerates ≈ 46248 µS.cm⁻¹ (Reed et al., 1995, Mahmood et al., 2013).

About the observed effects during the exposure period in Figure 17, it can be mentioned that the observed turbidity is typically produced by concentrated sewage (Von Sperling, 2007b). In relation with the plants, cattail is known to tolerate various types of AWW (Koottatep et al., 2005), but also it has been reported that it may present wilting leaves, yellowish color and bad growth when treating high strength wastewater (Kantawanichkul et al., 2009, Shepherd et al., 2001).

5.4 Theoretical vs obtained concentrations

5.4.1 AS

With respect to the organic matter influent in Figure 18, the variation between the predicted and observed concentrations is related to wastewater preparation and monitoring. The value shown by the model is the average of the influent during the stabilization period, however, during the HSAWW scenario the degradation of carbon sources was controlled before feeding the systems.

Figure 18 depicts that effluent of AS system had lower concentrations of BOD₅ and COD than predicted in the model. The apparent improvement in organic matter removal could be the result of the following reasons: according to Chan et al. (2009), aerobic systems are suitable for treating wastewater with <1000 mg.L⁻¹ COD, which coincides with the HSAWW concentration; besides, high BOD₅ removal is achieved by having higher sludge retention time than hydraulic retention time (Von Sperling, 2007b) which was achieved by recirculating the sludge during the complete scenario. Contrastingly, the difference between predicted nutrient removal and the removal obtained is less evident; nitrogen and phosphorus may be degraded in less extent.
than carbonaceous matter due to the slower growth rate of bacteria responsible of their degradation (Von Sperling, 2007b).

Most of tN concentrations coincide with the predictions whereas for tP they are lower. The graphs in Figure 18 suggest that a source of discrepancy could be in the influent; supplied concentration of phosphorus may be smaller due to unexpected insolubility and precipitation in the feeding barrel before reaching the CW. However, even if the foreseen and obtained concentrations of phosphorus are different, the model correctly predicted P removal, which was alike both through the model (22%) and along the scenario (19%).

5.4.2 CW

Figure 19 shows that the modelled influent for the four contaminants resembles the trend of the supplied wastewater during HSAWW scenario. The minor deviations occurring for BOD$_5$, COD and tN are probably caused for the same circumstances explained in the case of AS (section 5.3.1).

In general, the obtained BOD$_5$ and COD effluents were lower than the concentrations predicted by theoretical model, which highlight the efficiency of CW replicates to treat organic matter as secondary wastewater treatment. Furthermore, the effluent concentration of BOD during the exposure has a flat appearance as the oxygen consumption overpassed the upper limit of detection in BOD$_5$ test dilutions. Therefore, the corresponding current concentrations of that period are higher than the plotted values (Figure 19).

With respect to tN concentrations, the predicted values are higher than the obtained during HSAWW supply which could indicate that N removal was enhanced. A possible cause is that higher availability of carbon source could improve denitrification while still sufficient oxygen present for nitrifiers and while the ratio C to N stayed higher than 5:1 (Baker, 1998). Figure 19 shows that the expected tN concentration decreased after the exposure; however, the apparent decrement in tN removal could be related to the re adaption of the system to the reference conditions, which was not considered in the model. Additionally, some desorption of ammonia due to equilibrium processes could have happened when changing the concentrations to MSAWW, contributing to increase nitrogen levels in the effluent (Vymazal, 2007).
As for tP, the effluent values obtained during the first two periods of the scenario can be considered lower than the ones from the model. Notwithstanding, when comparing the respective tP concentrations, the expected and obtained removals look alike (Figure 19). After the exposure, removal of phosphorus remained higher than expected, but less efficient than in the beginning of the scenario probably due to the hindered adsorption by substrate. If the concentrations of phosphorus in the water column are the same than in the substrate, the adsorption can decrease due to equilibrium processes (Vymazal, 2007). This was not considered when modelling the expected effluent concentration.

5.5 HSAWW hypotheses between systems

5.5.1 Hypotheses 1-3

Pollutants’ removal obtained throughout the complete scenario reflects that CW and AS followed similar patterns with better removal for organic matter than for nutrients, which is an expected behavior for secondary wastewater treatments (Figure 20).

5.5.2 Hypothesis 4

Organic matter

When comparing background and resulting concentration of BOD₅ and COD, CW and AS can be considered as well to perform the same (Table 15); this supports the suitability of the systems for treating fluctuating organic matter loads. AS showed a faster recovery capability than CW in terms of returning to the initial removal efficiency (Figure 20).

Nutrients

Regarding nutrient removal at the start and the end of the scenario, AS achieved lower tN concentration in the effluent than CW before the exposure, which reoccurred at the end of the test period (Figure 20). According to Oliveira and Von Sperling (2008), effluent concentrations are influenced by several factors, such as: influent variations, environmental factors and the process itself. Thus, as the first two factors are very similar for both systems, the main cause for the discrepancy obtained could be that AS configuration improved N removal process, which was not the case for CW. In addition, this difference can be related to the capability and speed of the
systems to adapt to a changing environment, which looks faster for the AS than for CW.

**Nitrogen**

In Figure 20 is shown that AS increases the removal of N removal at the end of the period, which could indicate that the removal efficiency was enhanced in some way. One possible explanation could be that during the exposure period heterotrophic bacteria and nitrifiers consumed quickly the DO (Figure 21) and provoked anoxic conditions in the first compartment, allowing denitrifiers to act in both compartments and increase nitrate treatment. After reestablishment of aerobic conditions in the first compartment with low-medium strength wastewater, nitrogen treatment resembled once more to reference conditions. However, a monitoring of nitrate concentrations and characterization of bacterial community would aid to endorse the previous idea.

With respect to CW, the increase of tN effluent concentration after the exposure proposes that nitrogen removal mechanisms (e.g. nitrification and plant uptake) were somehow hampered by HSAWW. As microorganisms are the main contributors for
pollutant removal in CW (Dordio and Carvalho, 2013), one possible explanation is that the high organic load depleted drastically the dissolved oxygen required for maintaining nitrification. The possible introduction of oxygen to the system by the plant as well as the nitrogen uptake could be hindered by the die off observed during the exposure (Figure 17).

**Phosphorus**

CW effluent shifted from having lower concentrations than AS before the exposure to the opposite situation after the exposure (Figure 20). The increase of tP concentration after exposure may be linked to phosphate adsorption and precipitation by the substrate bed (Macci et al., 2015). As biofilms develop on the substrate’s surface or this becomes saturated with the adsorbates, the sorption effect is limited (Dordio and Carvalho, 2013) which could led to the increase in tP after exposure.

**Removal efficiencies**

Considering the performance of AS and CW in terms of removal efficiency, the high values obtained for BOD (93 % and 90 %) and for COD (82 % and 80 %) were comparable to the values reported in field conditions (Table 17). However, this was not the case for the removal efficiencies obtained for tN (50 to -93 % CW and 66 to -30% AS) and for tP (57 to -107 % CW and 37 to -43 % AS) which had big fluctuations along the scenario. Hence, the removal of nutrients could be stabilized by operational control measures, for instance, allowing higher HRT, which results in more buffering capacity (Oliveira and Von Sperling, 2008).

<table>
<thead>
<tr>
<th>System</th>
<th>Efficiencies in field conditions (%)</th>
<th>Efficiencies dictated by European Community (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOD</td>
<td>COD</td>
</tr>
<tr>
<td>CW</td>
<td>80-90</td>
<td>75-85</td>
</tr>
<tr>
<td>AS N removal</td>
<td>85-93</td>
<td>80-90</td>
</tr>
</tbody>
</table>

Based on the standard limits shown in Table 17, CW and AS from the present study accomplish with the values dictated for BOD and COD; nevertheless, the removal
efficiency values for nutrients are not accomplished even by the values reported on field (with exception of tN removal for AS). This indicates that an improvement on tP removal is required to discharge AS effluent (e.g. inclusion of anaerobic compartment before the aerobic) and for CW it is necessary to enhance removal of both nutrients (e.g. applying CW in series).

5.6 Applicability and implementation of the study

Overall, the present study highlighted the importance of a pre-sampling period in any research to elaborate an adequate experimental design that will avoid unnecessary expenses nor experimental work and that will ensure significant results to answer the hypothesis. In particular, the experiments performed endorsed a parallel performance comparison between AS and CW wastewater treatment which generates data for aiding the selection of a treatment technology.

This research contributes to the existing knowledge as it combines a study among replicas and different treatment systems. In case that further trials were intended with similar systems to the ones in this study, this manuscript give some insights for the respective installation, maintenance and surveillance of the wastewater treatment performance.

In addition to CW and AS, the present work establishes the possibility of comparing different types and configurations of water treatment technologies at laboratory scale and for testing different scenarios for a variety of pollutants with controlled fluctuations in the influent. Once monitored, the response of the systems could be of great value for generating sampling campaigns of which the data could be used as input for models.

As mentioned in Benedetti et al. (2006), benchmarking process requires sufficient data input to reduce uncertainties when applying the International Water Association’s Activated Sludge Models (IWA ASM). In this way, designing and monitoring experiments like the one in the present study and complementing it with collected data from field work would enrich experimental data for ASM.
6. General conclusions

From a performance perspective, both, the natural and conventional system achieved similar removal efficiencies to the ones reported in field conditions for secondary treatment level (93 % AS and 90 % CW for BOD$_5$; 82 % and 80 % for COD). Nevertheless, according to the treatment goal, the obtained performances can be considered as non-satisfactory for direct discharge into surface water bodies in agreement with the Flemish Government and the European Community standards.

Overall, buffering capacity of constructed wetlands and activated sludge showed a high removal for biodegradable organic matter (90% and 93 % for BOD$_5$) and relatively high for nutrients (42 % and 28 % for tN, 54 % and 29 % for tP respectively) during the period of exposure to high strength wastewater. This criterion was tested through a relevant hypothesis that resulted in similar behavior for both systems related to all tested pollutants.

With regard to their treatment efficiency, the highest corresponded to organic matter removal for both systems (~ >90), whereas activated sludge had a better efficiency for the nutrients (up to 66 % for tN and 37 % for tP). The statistical test of the hypothesis for removal efficiency criterion resulted in equivalent performance between the systems when exposed to medium strength wastewater (~190 mg BOD$_5$. L$^{-1}$).

Regarding the resilient property, activated sludge was the only to recover or improve its original treatment efficiency for all the pollutants at the end of the experiments (~90 % for BOD and COD, ~50 % tN and ~20 % tP). On the other hand, constructed wetland recovered the original treatment efficiency for organic matter (~90 %), whereas for nutrients the trend showed that the original efficiency would be achieved in longer period of time. The corresponding relevant hypothesis for this criterion ended in a higher adaptability of activated sludge to fluctuations in the influent.

In relation to the physical effects, the high SVI (>200 mL sludge. g sludge$^{-1}$) in activated sludge and the die off leaves in wetlands caused by the exposure faded as time passed. Recovery was evident in the appearance and settle ability of the sludge, and in the growth of new leaves in the case of wetlands.
7. Recommendations for further research

For the stabilization period, it is desirable to obtain the experimental removal efficiencies of all the parameters of interest with the aim to model a closer representation of the expected removal at changing scenarios. With the same insight, the collected data of the parameters must suffice to calculate their variability for the power analysis. For continuation of this study, as the measurements obtained during the first period of the HSAWW scenario equal the reference conditions, they could complement the missing values for calculating the needed variability of COD, tN and tP in the power analysis.

In order to model a closer behavior to actual systems’ performance, it would result useful to determine the reaction rate of the systems. In order to do so, one option could be to test AS and CW laboratory scale systems at different scenarios of AWW and with different HRT. In this way, the first order kinetic model could be applied with an ideal plug flow as hydraulic rate. As the determination of reaction rate could take some time, the approach applied in this study could be improvements in the meantime. Hence, an increase of the number of tanks in non-ideal plug flow with completely mixed tanks in series could increase the accuracy of the model by reducing the background concentration of contaminants.

Concerning the buffering capacity and resilience, the results obtained in the present study could be used in future research to enrich the data base used for modelling the expected removal efficiencies of activated sludge and constructed wetland systems. In addition, some variations in retention time could be assessed in the modelling and experiments to try to improve the treatment efficiency of the pollutants in both wastewater treatment systems.

Lastly, the type of investigation in this study can promote new lines of research, such as designing longer scenarios with several intermittent fluctuations representing concentrations due to drought events or industrial discharges and representing dilutions due to rain fall events. In addition, the fluctuations in the influent could be related not only to organic load and nutrients, but to specific type of contaminants considered a current concern, such as micropollutants.
8. List of references


Appendices

Appendix 1. Dynamic of COD and tN concentration in AS and CW effluent after a peak exposure of HSAWW. The peak in the influent with a duration of five days is expected to appear in the effluents with one and two days of retard in accordance to the treatment system. After the peak supply, the COD and tN concentrations diminish to ideally reach the original background concentration.
Appendix 2. Dynamic of tP concentration in AS and CW effluent after a peak exposure of HSAWW. The peak in the influent with a duration of five days is expected to appear in the effluents with one and two days of retard in accordance to the treatment system. After the peak supply, tP concentration diminish ideally to the original background concentration.
Appendix 3. Extract of multiple power analysis calculated for testing Hypothesis 1 for BODs contaminant. nstart= samples before exposure, nend= samples after exposure, ntime= total number of samples in HSAWW scenario, nrep= number of replicas, effectsize2= residual of tests, pwr= statistical power obtained per row.

<table>
<thead>
<tr>
<th>nstart</th>
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<th>ntime</th>
<th>nrep</th>
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Appendix 4. Extract of multiple power analysis calculated for testing Hypothesis 3 for BOD$_5$ contaminant. nstart= samples before exposure, nend= samples after exposure, ntime= total number of samples in HSAWW scenario, nrep= number of replicas, effectsize2= residual of tests, pwr= statistical power obtained per row.

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Appendix 6. SVI values for replicas’ compartments with respect to the different HSAWW scenario. The values for each period correspond to one weekly measurement.

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Appendix 5. Sludge appearance at the end of the HSAWW scenario. The AS system recovered the characteristic light brown color for the aerobic sludge (right) and the dark brown for the anoxic one (left).
Appendix 7. pH and DO variability along the HSAWW scenario. The box plots of the AS show a higher variation than the replicas of CW or the influent. Nonetheless, the media values remain similar between the replicas of the same system/compartment.
Appendix 8. T and EC variability along the HSAWW scenario. In general, the T of the replicas has a higher variability than the EC. Furthermore, the influent presents the lowest values of both parameters. The median values between replicas are similar, with