Combined system for the treatment and reuse of urban wastewater: the efficiency of anaerobic reactors + hybrid constructed wetlands + ozonation

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ABSTRACT

The research developed a combined system in batch flow and in pilot scale for the treatment and reuse of urban effluents. The system was fed raw effluent from a university campus in Brazil and composed of four anaerobic reactors, three constructed wetlands (CWs) and an ozonation unit. The three sequential hybrid constructed wetlands were composed of a floating treatment wetland, an aerobic-anoxic baffled constructed wetland (CW) and a saturated vertical flow CW. Later, during the last trimester, weekly samples of the treated effluent were ozonated by bubbling with an application rate of 240 mg.h⁻¹ O₃. The system presented high removal rates efficiencies in terms of carbonaceous organic matter (78.9%), nitrogen (91.0%), color (96.7%) and turbidity (99.1%). In addition, it worked well for disinfection and acute ecotoxicity, but P was only efficiently (75%) removed in the first 8 months, with removing efficiency declining after this period. Ozonation provided significant color removal and an increased pH. The combination of floating, alternated upflow and downflow and saturated vertical flows improved the treatment of wastewater. This was due to the presence of both aerobic and anaerobic zones, as well as the filter substrate, through an integrated system with simple construction and operation and increased lifespan.

Key words | combined system, domestic wastewater, hybrid constructed wetlands, ozonation, wastewater treatment

INTRODUCTION

The sustainable management of water integrates an important topic of clean technologies when approaching the concept of a circular economy. This is notable due to the current necessity related to the recovery of energy, nutrients, landscape integrations, recovering degraded areas and effective reuse of wastewater.

Another important context for better management of water treatment and reuse is associated with the development of decentralized treatment systems. Since centralized systems may not be reliable in countries with large territories, this decentralization in treatment is presented as a solution for the lack of basic sanitation in many countries. This is the case of Brazil, which has a considerable fraction of the population living in communities and small towns, which makes it difficult to collect and route sanitary effluents to centralized stations. In Brazil, approximately half of the households are connected to the sewage collection network. Currently, about 40% of the total volume of domestic wastewater generated in the country is adequately treated (Von Sperling 2019).

The technology most applied for decentralized sewage treatment is the septic tank, due to its simplicity and ability to remove total solids and organic matter. However, it lacks some type of post-treatment that would reduce nutrients and pathogenic organisms and improve the water quality (Withers et al. 2014). This is especially true when the main objective is reuse of the water, in which case Brazilian standards like NBR 13969/97 and international standards like EPA/R-12/618 need to be met.

In this context, both developed and developing countries are building, and studying constructed wetland
(CW) systems, since all the aspects related to environmental sustainability and decentralization are present in these treatment technologies (Dotro et al. 2017). Constructed wetlands (CWs) are proven to be efficient for treating urban and domestic wastewater (Álvarez et al. 2017). CWs demand research continuity to improve their operational aspects. Maintenance, as well as the combination of CWs with other sustainable processes can be used to increase the amount and nature of pollutants treatable through the application of macrophytes (Horn et al. 2014).

Over the last decades, several studies have been developed that combine CWs with other consolidated treatment processes and emerging technologies, such as membrane bioreactors, electrochemical oxidation, and microbial fuel cells (Liu et al. 2015).

One of the most important aspects that limit water reuse after the treatment by CWs is the lack of a guarantee of effective disinfection. Treated water may still contain pathogenic microorganisms, requiring a post-treatment for removal. The most common technologies for disinfecting wastewater are: chlorination, chloramination, ozonation, ultraviolet radiation, chlorine dioxide, potassium permanganate and nanofiltration. When combined with an appropriate primary treatment and a polishing method, the CWs are able to produce water of acceptable quality to permit its reuse for, among other uses, irrigation, landscaping and even domestic use (e.g. toilet flushing), therefore reducing the demand of fresh water resources (Mishra et al. 2018).

In this scenario, several researchers have studied combinations of different CWs with advanced oxidative processes, aiming to promote not only the disinfection of the wastewater but also improving effluent quality, mainly by removing color, a fundamental aesthetic aspect for local water reuse. Examples of authors that developed research projects in this context are Tripathi & Tripathi (2011), Miranda et al. (2014) and Horn et al. (2014), who evaluated systems combining CWs + ozonation (O3). Systems combined with other biological treatments besides CWs have also been researched, such as aerated biofilter + O3 (Zhang et al. 2014) and biofilter + O3 (Wang et al. 2008).

Therefore, the present study developed and monitored an integrated system combining Anaerobic Reactors (ARs) +3 staged hybrid constructed wetlands (CWs) + O3 for the treatment and reuse of urban effluents from a university campus.

**METHODS**

**System’s setup**

The research was developed at the wastewater treatment plant (WWTP) of the University of Santa Cruz do Sul (UNISC), in southern Brazil over a period of 11 months (from February 2017 to December 2017). The collected wastewater, mainly drained from urinals and toilets (Silveira et al. 2016) was pumped directly from the WWTP equalization tank, after passing through a preliminary system (screening and grit removal). The feeding of the combined system, as well as sample collections and analysis, were...
performed weekly. Figure 1 presents a simplified flowchart of the integrated system.

At first, the raw wastewater was pumped to the ARs, a system formed by a sedimentation tank +3 bioreactors. The reactors were composed of four high-density polyethylene (HDPE) drums with a working volume of 100 L each. During the first 6 months of operation, the wastewater from the anaerobic bioreactors was recirculated weekly between the last three reactors on the third day of anaerobic digestion. Recirculation was maintained for 1 h using a centrifugal pump (1 HP), at a rate of 1 m³ h⁻¹. The total hydraulic retention time (HRT) of the ARs was 7 days.

After 7 days in the bioreactors, the effluent was repressed to the three-stage hybrid flow constructed wetland (HYFCW) system. The first and last CWs (CW1 and CW3) were formed by high density polyethylene (HDPE) boxes that measured 0.92 m in length × 0.55 m in width × 0.4 m in height (200 L working volume). The CW2 was constructed from a fiber box with the dimensions of 0.9 m in length × 0.3 m in width × 0.45 m in height (approximately 100 L working volume). Each CW had a HRT of 7 days, i.e. 21 days total. The CWs were arranged in a metal type ladder support, so that draining from one CW to the other could occur by gravity due to the level difference between the boxes. Figure 2 presents a general overview of the hybrid system.

CW1, designed as a floating treatment wetland, acted as a settler for suspended solids after the ARs. A combination of naturally emergent macrophytes (*Hymenachne grumosa*) and floating macrophytes (*Pistia Stratiotes* and *Lemna gibba*) were planted in order to cover the surface of the water and consequently minimize the proliferation of mosquitoes and algae as well as potentiate the removal of pollutants. For *H. grumosa*, a floating macrophyte filter (FMF) was constructed from ethylene vinyl acetate to support and maintain its aerial part, while the root system was permanently immersed in the effluent.

**Figure 2** | Schematic representation of the hybrid constructed wetlands. A – CW1 (floating treatment wetlands); B – CW2 (aerobic-anoxic baffle CW); C – CW3 (vertical flow constructed wetland).
CW2 was built with an aerobic-anoxic baffled surface flow wetland, with the box compartmentalized by vertically arranged baffles, so that the flow direction of the effluent therein could alternately vary upward and downward. The objective was to create aerobic and anoxic regions through the alternating flow. This box was initially only planted with floating macrophytes (P. stratiotes and Lemna gibba).

The last box (CW3), can be classified as a vertical flow constructed wetland (VF) with a saturated bottom, and was the only one with the presence of substrate. The bottom layer (15 cm) was filled with pebbles to facilitate drainage, whereas the upper layer (25 cm) was filled with gravel number 2 to support macrophyte development and effluent filtration. CW3 was only planted with H. grumosa, with a density of 24 seedlings m\(^{-2}\). Effluent feed was performed with a perforated polivinyl chloride (PVC) fork to promote even distribution of the liquid on the surface of the substrate. The height of the effluent was controlled to avoid the presence of water blade above the substrate. Therefore the saturated zone had a height of 40 cm., on average.

An ozone generator (RADAST 2C) was used to produce ozone by electrical discharge, with the generation rate adjusted to 240 mg O\(_3\) h\(^{-1}\) (when oxygen is used as gas feed). This feeds atmospheric air through a fogger pump (60 L h\(^{-1}\)) and uses porous stone to promote bubbling and facilitate diffusion of the O\(_3\) in the effluent. Ozone determinations were performed with the iodometric method from Flamm (1977). The ozonation assays were conducted in a reactor with 1.4 L of working volume for 1 hour and aliquots were collected and analyzed every 10 minutes to check the effects of O\(_3\) over time.

**Analytical characterization**

Five sampling points were defined at the treatment system: (P1) raw wastewater, (P2) output of the ARs unity, (P3) after CW1, (P4) after CW2 and (P5) output of CW3. Both sampling and analytical analysis of the collection points were performed systematically on the same days every week.

The characterization parameters of the raw and treated effluents though each unit included analysis of biochemical oxygen demand (BOD\(_5\)), chemical oxygen demand (COD), total dissolved solids (ppm), conductivity, pH, turbidity (NTU), total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), soluble phosphorus (P), ammonia nitrogen (N-NH\(_3\)), total nitrogen (TN), absorbometric color (420 nm), root length, thermotolerant coliforms and *Escherichia coli*. All these analyses were conducted according to APHA/AWWA/WEF (2012). Samples were taken weekly and analyzed upon collection.

**Acute ecotoxicity assays**

Acute ecotoxicity assays using the microcrustacean *Daphnia magna* Straus, 1820, were carried out at the Analytical Center of UNISC. The methodological procedures followed the Brazilian Association of Technical Standards (ABNT NBR 12713:2016). Samples collected after the treatment by the integrated system were tested and prepared with volumetric precision at a geometric progression ratio of \(\frac{1}{2}\), with concentrations ranging from 100% to 6.25%. A relative toxicity scale with classification criteria of 25th, 50th (median), and 75th percentiles were used to classify the results of the 48-h EC\(_{50}\) values (Düpont & Lobo 2012).

**Phenological monitoring and application rates**

Phenological aspects of macrophytes, such as root length and generated biomass, were also monitored. Only one pruning occurred during the execution period of the research (in October 2017), since the fodder (ripening) of the macrophytes was expected. Throughout the monitoring of the combined system, the control of the pollutant loads applied in the CWs was carried out by comparing the values used with those recommended in the literature (Table 1).

The monitoring was divided into two phases: the first with a lighter effluent load, and the second with double loading at CW1, so that all the effluent from the box could be changed weekly. In general, the values adopted were in accordance with the recommendations of the specialized literature (Wallace & Knight 2006; Dotro et al. 2017).

**RESULTS AND DISCUSSION**

**Characterization of the raw effluent and application rates**

The studied wastewater can be classified as urban wastewater since it was generated in urinals and toilets of a university campus and may be, therefore, regarded as black and yellow waters. Table 2 shows that the wastewater presents a strong eutrophying load, especially due to high levels of N-NH\(_3\) when compared to previous studies (Düpont & Lobo 2012; Horn et al. 2014). This value is four times higher than the limit allowed by Brazilian Resolution 355/2017. This resolution was established by the Rio Grande do Sul State Council on the Environment
(CONSEMA). In another comparison, it can be considered eight times higher than the maximum value allowed for emissions from domestic wastewater. This is according to the European guidelines for urban wastewater treatment (Directive 1991). However, the P values were in accordance with CONSEMA Resolution, they were four times higher than what the European guidelines allow. Furthermore, the high BOD values, mainly when considering the European and EPA guidelines, are also an important indicator of the pollutant potential of the untreated effluents.

**Main operational adjustments of the combined system**

After the system became operational, some important changes were made in its operation. The first was the replacement of the floating system at CW1 with an expanded polyethylene system (swimming pool floats). This was done because the original system suffered several deformations due to the increased weight of the growing macrophyte. In addition, there was damage caused by solar radiation. Some advantages of this new system are the smaller surface area (which allows a greater gas exchange of the effluent with the atmosphere) and the greater mobility at the effluent surface.

Another important modification in the system was the replacement of the floating macrophytes *P. stratiotes* with *H. grumosa* (also supported by a floating system) and the floating macrophytes *Salvinia*. The compartments of the box were alternately planted with these two macrophytes, to create intercalated oxic and anoxic

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**Table 1** | Hydraulic and pollutants application rates in each of the CWs

<table>
<thead>
<tr>
<th>System</th>
<th>CW1</th>
<th>CW2</th>
<th>CW3</th>
<th>CW1</th>
<th>CW2</th>
<th>CW3</th>
<th>WCSL</th>
<th>WCFV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydraulic load (m.d⁻¹)</td>
<td>0.021</td>
<td>0.043</td>
<td>0.021</td>
<td>0.043</td>
<td>0.057</td>
<td>0.029</td>
<td>–</td>
<td>0.08</td>
</tr>
<tr>
<td>TN (g.m⁻²d⁻¹)</td>
<td>2.22</td>
<td>1.39</td>
<td>0.44</td>
<td>5.5</td>
<td>5.64</td>
<td>1.61</td>
<td>1.5 (NTK)</td>
<td>1.0 (NTK)</td>
</tr>
<tr>
<td>N-NH₃ (g.m⁻²d⁻¹)</td>
<td>1.85</td>
<td>1.35</td>
<td>0.36</td>
<td>4.2</td>
<td>4.43</td>
<td>1.19</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P soluble (g.m⁻²d⁻¹)</td>
<td>0.09</td>
<td>0.16</td>
<td>0.07</td>
<td>0.22</td>
<td>0.27</td>
<td>0.12</td>
<td>0.1 (Total P)</td>
<td>–</td>
</tr>
<tr>
<td>TOC (g.m⁻²d⁻¹)</td>
<td>0.43</td>
<td>0.34</td>
<td>0.18</td>
<td>1.5</td>
<td>1.14</td>
<td>0.36</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BOD (g.m⁻²d⁻¹)</td>
<td>2.40</td>
<td>2.75</td>
<td>2.99</td>
<td>4.8</td>
<td>3.69</td>
<td>4.00</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

**Table 2** | Mean concentration and standard deviation (±Sd) of raw wastewater and quality parameters from the Brazilian and the international guidelines

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw wastewater</th>
<th>CONSEMA 355/17 Resolution (Q &lt; 200 m³d⁻¹)</th>
<th>UWTD 91/271/EEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg L⁻¹)</td>
<td>481 ± 266.58</td>
<td>330.0</td>
<td>–</td>
</tr>
<tr>
<td>BOD₃ (mg L⁻¹)</td>
<td>192 ± 66</td>
<td>120.0</td>
<td>25</td>
</tr>
<tr>
<td>N-NH₃ (mg L⁻¹)</td>
<td>81 ± 11</td>
<td>20.0</td>
<td>10</td>
</tr>
<tr>
<td>TN (mg L⁻¹)</td>
<td>92 ± 27</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TOC (mg L⁻¹)</td>
<td>140 ± 50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TC (mg L⁻¹)</td>
<td>224 ± 24</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>3.98 ± 0.57</td>
<td>4.0 (total P)</td>
<td>1.0 (total P)</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>99.3%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total coliforms (colony forming units (CFU)/100 mL)</td>
<td>1.0 × 10⁹</td>
<td>1.0 × 10⁶</td>
<td>–</td>
</tr>
<tr>
<td><em>E. coli</em> (CFU/100 mL)</td>
<td>43 ± 8.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EC₅₀ (l) 48 h</td>
<td>61.56%</td>
<td>TF = 1 for acute toxicity EC₅₀ = 100%</td>
<td>–</td>
</tr>
<tr>
<td>TDS (ppm)</td>
<td>509 ± 49</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH</td>
<td>7.31 ± 0.25</td>
<td>6 to 9</td>
<td>6 to 9</td>
</tr>
<tr>
<td>Color (λ = 420 nm)</td>
<td>0.92 ± 0.39</td>
<td>No change to the receiving body color</td>
<td>–</td>
</tr>
</tbody>
</table>


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**CONSEMA**: To indicate the regulatory body in Brazil for environmental guidelines and regulations.
environments. This is made possible by the difference in lengths of the roots of the two species.

**Characterization parameters of the treated wastewater**

The combined system presented good removal efficiencies of organic carbonaceous matter, with average reductions of 84.4% for BOD$_5$, 81.6% for COD, 78.9% for TOC and 86.9% for TC (Table 3).

The units that demonstrated the best performance regarding these parameters were the ARs, due to anaerobic digestion of organic matter, and CW1, through the digestion of organic matter and the sedimentation of suspended solids (Figure 3). According to Merino-Solis et al. (2015), who evaluated a system combining upflow anaerobic filter + horizontal flow CW, the first stage of treatment, under anaerobic conditions, also presented higher BOD$_5$ reduction, since the most biodegradable organic matter is first removed in wastewater treatments.

Zhang et al. (2009), when using hybrid CWs in China, obtained similar results. For BOD$_5$, the authors found mean reductions of 80.1%, 46.8% for TN and 79.7% for total P. Two factors that may explain the high P removal in this system are the application of specific substrates to adsorb this material and HRTs, ranging from 19.5 to 72 days for hybrid systems. However, it is important to highlight that, starting in August, there was a reduction in the removal efficiency of these parameters by ARs and CW1. This period coincided with the end of the application of the recirculation in the ARs and can also be explained by the dead macrophyte biomass that was decomposing in these systems and the accumulation of sludge.

In general, the combined system was not very efficient in removing soluble P. This removal did not occur uniformly throughout the monitoring; after the eighth month of operation the system’s efficiency drastically reduced. In the first quarter of monitoring, the system presented an average reduction of 93.6%, while in the last 3 months this value had fallen to a mere 29.6% (Figure 3). This dramatic drop may be related to the fact that the main mechanisms of removal of P in CWs are adsorption and precipitation, especially in systems that are initiating operations. This explains the better performance of CW3 in terms of P removal since it was the only one containing filter material. However, the substrate tends to saturate and lose its ability to retain phosphorus over time (Yu et al. 2015).

Merino-Solis et al. (2015) have also noted that the main mechanism for P removal is substrate retention, sediment accumulation and biofilm adsorption. The authors verified P removal from 24.2% to 43.8%. As an alternative,

### Table 3: Mean concentration and standard deviation (±SD) of treated raw wastewater and quality parameters from the Brazilian and the international guidelines

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw wastewater</th>
<th>Treated wastewater</th>
<th>Mean efficiency</th>
<th>NBR 13969/97 (Reuse)</th>
<th>Guidelines for water reuse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Class 1</td>
<td>Class 2</td>
</tr>
<tr>
<td>COD (mg L$^{-1}$)</td>
<td>481 ± 266.58</td>
<td>86.83 ± 84.33</td>
<td>81.60%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BOD$_5$ (mg L$^{-1}$)</td>
<td>192 ± 66</td>
<td>30 ± 24</td>
<td>84.4%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N-NH$_3$ (mg L$^{-1}$)</td>
<td>82 ± 9.8</td>
<td>4.5 ± 1.2</td>
<td>94.5%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TN (mg L$^{-1}$)</td>
<td>92 ± 25</td>
<td>5.9 ± 1.3</td>
<td>91.0%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TOC (mg L$^{-1}$)</td>
<td>138 ± 50</td>
<td>29.1 ± 2.6</td>
<td>78.9%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TC (mg L$^{-1}$)</td>
<td>222 ± 21</td>
<td>29.1 ± 3.2</td>
<td>86.9%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P (mg L$^{-1}$)</td>
<td>3.98 ± 0.57</td>
<td>3.2 ± 0.6</td>
<td>19.6%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>464 ± 239</td>
<td>4.3 ± 5.1</td>
<td>99.1%</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Total coliforms (colony forming unit (CFU)/100 mL)</td>
<td>1.0 × 10$^9$</td>
<td>24.5 ± 24.8</td>
<td>99.9%</td>
<td>&lt;200</td>
<td>–</td>
</tr>
<tr>
<td>E. coli (CFU/100 mL)</td>
<td>43 ± 8.5</td>
<td>0</td>
<td>100%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EC$_{50}$ (l) 48 h</td>
<td>61.56%</td>
<td>100%</td>
<td>100%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TDS (ppm)</td>
<td>509 ± 49</td>
<td>416 ± 119</td>
<td>18.3%</td>
<td>&lt;200</td>
<td>&lt;500</td>
</tr>
<tr>
<td>pH</td>
<td>7.31 ± 0.25</td>
<td>8.7 ± 0.1</td>
<td>–</td>
<td>6 to 8</td>
<td>–</td>
</tr>
<tr>
<td>Color ($\lambda = 420$ nm)</td>
<td>0.92 ± 0.39</td>
<td>0.03 ± 0.01</td>
<td>96.7%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Res. Cl (mg.L$^{-1}$)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.5 a 1.5 &gt; 0.5</td>
<td>–</td>
</tr>
</tbody>
</table>

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maintenance of the treatment system is suggested when the sedimented solids reach a determined volume.

The removal efficiencies of the first two CW units are in agreement with the literature, since, on average, only 5% to 10% of the P is removed by the incorporation of the macrophyte tissues (Vymazal 2007). Similar results were obtained by a group of researchers from Nepal who evaluated a system for the treatment of urban effluents of a small community (approximately 80 residences) using a preliminary treatment (grating), compartmented ARs and hybrid CWs (HFCW + VFCW), but with HRTs of around 30 h (Singh et al. 2013). Likewise, the authors verified that ARs promoted an increase in the concentration of total P. In contrast, when considering the combined system, Singh et al. 2009 obtained similar performances from BOD₃ (90%), COD (70%) and total P (26%) to those obtained in the present research.

In relation to N-NH₃ (which constituted the bulk of the total N), the HFCW system showed good removal rates, especially in CW3 (Figure 3). In general, VFCWs, because they have continuous flow, do not present good TN removal capacity due to the absence of anoxic regimes. Nevertheless, systems with a saturated bottom tend to have two distinct zones: one closer to the surface and to the roots of the plants, and one more anoxic in the deeper region, thus promoting both nitrification and denitrification (Kim et al. 2014).

In spite of presenting relatively lower efficiencies, the first two CWs (with FMF systems) were also able to promote the removal of both N-NH₃ and TN, on average 31% and 33% for CW1 and 43% and 38% for CW2, respectively. An important aspect that must be highlighted was the increase in TN, N-NH₃ and TOC removal efficiencies after maintenance and pruning in the CW1 and CW3 systems. The removal of decaying materials as well as the accelerated growth of the macrophytes after pruning were due to the imbalance between the aerial parts and the roots that contribute to improved system performance.

A system composed of an Imhoff decanter + hybrid CWs (HFCW + VFCW) used for the treatment of effluents produced at a hotel in Italy was studied by Masi & Martinuzzi (2007). The authors found removal rates higher than those obtained in the present work in terms of COD (94%) and P (94%), even though the system had already been in operation for 3 years. Regarding N-NH₃ and TN,
the reductions were lower than those of the present study, with mean values of 86% and 60%, respectively.

Ávila et al. (2015) verified a similar performance by the evaluation of a combined system for the treatment and reuse of sanitary effluents and drainage water. The system included an Imhoff Tank followed by hybrid flux CWs (VF CW + HF CW + free water surface constructed wetlands (FWSCW). Removal rates higher than 90% for turbidity, N-NH3 and NT and 89% for COD were obtained. Regarding total P, the average reduction was 47%. This low P removal can be explained by the operation of the combine system over the years.

On average, the ARs + hybrid wetlands + O3 system presented color removal rates (λ = 420 nm) of 97.6%. Considering only the ozonation stage, reductions of 58.7% of the apparent color were observed in relation to the effluent from the CWs, considering an O3 rate of 240 mg.h\(^{-1}\). The ability of O3 to discolor effluent is related to the degradation of organic matter (mainly C = C, C = O bonds) present in the effluent, such as phenols, polyaromatic hydrocarbons, ketones and aromatic aldehydes (Wang et al. 2008).

Although it is a very high dose of ozone, it was applied aiming more for the removal of color from the wastewater than the oxidation of organic matter, since de BOD5 and λ(420 nm) only though ozonation. When considering the apparent color were observed in relation to the effluent from the CWs, considering an O3 rate of 240 mg.h\(^{-1}\). The ability of O3 to discolor effluent is related to the degradation of organic matter (mainly C = C, C = O bonds) present in the effluent, such as phenols, polyaromatic hydrocarbons, ketones and aromatic aldehydes (Wang et al. 2008).

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These discoloration values were higher than those obtained by other researchers (Horn et al. 2014; Celente et al. 2019), and in these cases the wavelength evaluated was also at 420 nm. In other studies combining biological treatment and ozonation systems, regions of the spectrum with higher resistance to degradation were controlled, such as Tripathi & Tripathi (2011), which found a mean color reduction (λ = 254 nm) of 72.6% with the FWSCW + O3 treatment. Zhang et al. (2014) observed a mean reduction of 82.3% for color (λ = 254 nm) with a biofilter + O3 system. Wang et al. (2008), when applying O3 at a rate of 10 mg.L\(^{-1}\) (for 4 min), reached discoloration values of 75% (λ = 254 nm) only though ozonation. When considering the biofilter system combined with ozonation, the authors found discoloration rates of around 90%.

Concerning turbidity, the present work produced removal rates higher than 99%, Tripathi & Tripathi (2011) verified a slightly lower value for the FWSCW system of effluent from the activated sludge unit, which presented an average reduction of 85.7%.

Regarding thermotolerant coliforms and E. coli, the combined system reached inactivation of the microorganisms in the CWs stage. Samples were analyzed at four treatment points: after the ARs and after each of the CW stages. The results obtained for the thermotolerant coliforms were: 1.0 \times 10^9 (ARs); 232 ± 143 (CW1); 29 ± 2.8 (CW2) and 24 ± 25 (CW3), with average efficiency higher than 99.99%. However, when considering E. coli., the obtained results were: 43 ± 8.5 (ARs); 1 ± 0.5 (CW1) and 0 for both CW2 and CW3.

The results are in agreement with the values verified for hybrid CW systems operating in the Mediterranean region found by Masi & Martinuzzi (2007), which indicated average pathogen removal efficiencies of 98–99.9995%. In addition, it is important to highlight that ozone acts as an efficient agent for the removal of pathogens, and thus ozonation serves as a safety process for the disinfection of the effluent in the combined system. This ensures the inactivation of pathogens after the CWs, especially when water reuse is the aim.

Zurita & White (2014), when studying a system combining a sedimentation tank + hybrids CW for the treatment of wastewater from a university campus, verified efficient removal of pathogenic organisms (E. coli and total coliforms). They obtained higher rates of disinfection with the VFCW, which was explained by the more aerobic conditions in this stage, since these conditions are not great for growing microorganisms and promote an abundance of predators. According to the authors, one single unit of CW will hardly remove high rates of disinfection, at least two different CWs would be necessary, especially when water reuse is the main objective.

Small reductions in IC (8.5%), CT (6.3%) and soluble P (4.6%) were verified after ozonation. Regarding COD, TOC, TSD and conductivity, the results remained practically constant. According to Wu et al. (2018), increasing the ozone dosage in O3/biological aerated filter treatments generally increases the COD removal as well. However, when the increase of the ozone dose reaches a determined content, the COD removal tends to become slow depending on the quality of the wastewater being treated. For that reason, adding more ozone in order to remove COD would have little or no effect.

On the other hand, it was possible to observe a significant increase in pH during ozonation, resulting from the reaction of O3 with sodium bicarbonate ions (Silva & Daniel 2015). However, Miranda et al. (2014) did not vary significant differences in the pH and COD after the ozonation of the effluents from the CW.

**Ecotoxicity assays**

Analyses were carried out with raw wastewater and with samples collected after the ozonation step. Table 1 shows
The raw effluent presented a value of 61.56% for acute ecotoxicity. Therefore, it is classified as moderately toxic, according to Diüppont & Lobo (2012). Samples collected after the CWs and after ozonation showed a complete absence of toxicity (EC50 = 100%) indicating the detoxification potential of the ARs + hybrid constructed wetlands + O3 system when considering the organisms used in the acute ecotoxicity assays.

The absence of toxicity of the samples after the treatments with CWs and ozonation can be related to the high removal rates of ammonium compounds present in the effluents (above 90%). Diüppont & Lobo (2012) associated the acute and chronic toxicity against D. magna and Ceriodaphnia dubia, respectively, to high N-NH3 values found in domestic wastewater.

Previous studies corroborate the detoxification potential of the CWs planted with H. grumosa. Silveira et al. (2017) observed a complete removal of the toxicity against D. magna after the treatment with vertical CWs. Horn et al. (2014) verified lower reduction of acute ecotoxicity when evaluating a combined system of CWs and photocatalytic ozonation. The authors observed that at the CWs hybrid stage acute effluent ecotoxicity passed from moderately toxic (EC (I) > 50%) to slightly toxic (EC (I) > 75%). No further reductions of the acute ecotoxicity were observed after photocatalytic ozonation.

Although Horn et al. (2014) observed reductions of the acute toxicity against D. magna, the authors also verified that the CWs were not able to reduce the chronic toxicity of the wastewater, which remained highly toxic. For this reason, complementary chronic ecotoxicity assays should be carried out in future studies to confirm the detoxification potential of the system.

**Adjustments to Brazilian and international guidelines**

Table 2 presents the results obtained with the effluent before the treatment by the ARs + hybrid wetlands + O3. The average efficiency of each of the analyzed parameters is only reported in the last monitoring quarter, during which the ozonation process was carried out.

The results were compared to respective emission standards (when applicable) for sanitary effluents according to four different guidelines: one state guideline, the CONSEMA 355/2017 Resolution, which determines the emission standards of urban effluents in surface water in the state of Rio Grande do Sul; the NBR 13969/97, which is the reference of water reuse in Brazil, and two international guidelines, The European guideline for urban wastewater treatment (Directive 1991) and Guidelines for water reuse – EPA/600/R-12/618 – Hawaii (R2 water).

In terms of the reduction of organic matter, the BOD5 values obtained after the treatments are in accordance with the CONSEMA and EPA legislation for emission ceilings and the values are only slightly above the limits established by the European Directive. When considering the removal rates fully attended the emission values established by the CONSEMA 355/2017 Resolution and European guidelines.

Regarding the eutrophying load, the integrated system presented good efficiency for the reduction of the N-NH3 levels, demonstrating compliance well beyond the requirements of control references UWTD 91/27/EEC and CONSEMA/RS Resolution 355/2017 (Q < 200 m3d⁻¹). However, the treatments failed to satisfactorily reduce the P levels.

An important aspect that must be pointed out is the disinfection potential of the system, since the obtained results showed reductions of 99.9% for total coliforms and 100% for E. coli. These values fully comply with the CONSEMA resolution and Guidelines for water reuse – EPA/600/R-12/618 – Hawaii/Water R2. Finally, the wastewater presents a complete absence of acute toxicity against D. magna, which is in accordance with the CONSEMA resolution.

When considering water reuse, the treated effluent can be classified as class 2 (with the exception of residual chlorine) according to NBR 13969/97. The purposes for this class of reuse are ‘floor washing, garden irrigation, maintenance and canals for landscaping purposes’ (NBR – 13969 1997). Furthermore, based on the data presented in Table 1 the treated wastewater is, according to the Guidelines for water reuse – EPA/600/R-12/618 – Hawaii Water R2 (EPA 2012), suitable to be reused in toilets.

**CONCLUSIONS**

The present study investigated the efficiency of a combined system for the treatment of wastewater produced at a university campus. Results of the characterization analysis showed high pollution loadings of the raw effluents, with several parameters exceeding the maximum values allowed by Brazilian and international guidelines for the release of wastewater into water bodies.

In this sense, the performance obtained by the innovative system combining AR + hybrid wetlands + O3 presented high removal rates for organic matter, color and turbidity, as well as being a significant reduction of...
eutrophic potential through the removal of nitrogen and phosphorus. However, it is necessary to add another system to remove P (such as chemical precipitation, a filter with adsorbent material), since the substrate already showed signs of saturation.

The combined system also showed potential for disinfection (through tests with thermotolerant coliforms and E. coli) and detoxification in terms of acute ecotoxicity (with D. magna assays). The FMF system with expanded polyethylene demonstrated durability in terms of resistance to deformations and buoyancy, although some seedlings of H. grumosa died and were replaced during the monitoring.

Some continuity aspects for the study are the introduction of more resistant macrophytes in the FMF, as well as the systematic maintenance of floating debris and macrophytes and more frequent pruning (for example quarterly), since improvements were observed in several parameters from pruning.

The application of the ozonation process promoted the discoloration of the effluent, a factor of extreme importance when considering the reuse of water. From the combined system, it was possible to achieve the effluent emission standards recommended by the current environmental legislation, as well as the class 2 reuse water classification according to NBR 13969/97. However, if applied in a home, it is recommended to use this water as a reuse class 3 (discharges of sanitary vessels), for greater safety and because less monitoring of effluent quality is required.

Thus, the developed system presented potential as a decentralized unit for the treatment and reuse of urban effluents, mainly in rural regions or in condominiums with low population density, due to the area needed for CWs.

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