Does Duckweed Ponds Used for Wastewater Treatment Emit or Sequester Greenhouse Gases (GHG)?


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ABSTRACT
Considering the problem of eutrophication in receiving water bodies, the duckweed ponds have shown a great potential for removing nutrients (N and P) from domestic and agro-industrial effluents. On the other hand, conventional treatment systems are also responsible for greenhouse gases (GHG) emission such as CO$_2$ and CH$_4$. Alternatively, duckweed ponds have been successfully used for polishing effluent and have become more popular in recent years because to the large capacity of these plants in nutrient removal and CO$_2$ fixation. The present study aimed to evaluate the fluxes of greenhouse gases (GHG) emissions and carbon dioxide fixation by duckweed ponds applied to treat wastewater. To this, two pilot duckweeds ponds in series were operated with real wastewater with a flow rate of 200L.day$^{-1}$. Beyond the common physicochemical parameters, the gases emissions from pond surface were measures by using a flux chamber according to method OM-8 by USEPA. The concentrations of CO$_2$ and CH$_4$ inside the chamber were measured by infrared probes. As results, during 123 days of monitoring, there was an important nutrient removal efficiency (TN = 92.6 % from 72.5 mg.L$^{-1}$ and TP = 84% from f 8.1 mg.L$^{-1}$), as well as organic matter (COD = 79% from 156.9 mg.L$^{-1}$). The CO$_2$ emission rate ranged from 3048 to 6017 mgCO$_2$.m$^{-2}$.d$^{-1}$ and fixation rate ranged from 19592 to 42052 mgCO$_2$.m$^{-2}$.d$^{-1}$. Methane emission was not detected (less than 0,1%) during the whole study. The results showed that in presented conditions duckweeds ponds could fix at least three times more CO$_2$ than emit.

KEYWORDS
Duckweed ponds, wastewater treatment, GHG emissions.

INTRODUCTION
For almost two decades the problematic of greenhouse gases emission on climate changes has driven the society development to move to different way. For adapt to this scenario the researches groups, professionals and companies that works with wastewater treatment plants (WWTP) have facing up a new challenge. It means that only watch over the quality of wastewater treated is not enough to help the environmental conservation but also the reduction of GHG emission by WWTP is necessary. This fact becomes evident because of the high amount of GHG produced during organic matter degradation by both biological ways aerobic and anaerobic. Anaerobic systems are known due methane emissions which could be 21 times higher than CO$_2$ regarding global warming potential. However, aerobic and facultative systems could also release high amount of CO$_2$ and N$_2$O (nitrous oxide) which could reach carbon equivalent about 300eq. To this reason wastewater treatment plants (WWTPs) are recognized as one of the larger secondary sources of GHG emissions (USEPA, 1997). Moreover, the estimation of GHG emissions by WWTPs has been based on the on-site emissions only, without considering the
off-site or upstream emissions associated with the plant operation (Shahabadi et al., 2009). Thus, Shahabadi et al. (2009) pointed that hybrid systems (aerobic/anaerobic) could release about 3.3 kgCO₂e/kgBOD, considering both on-site and off-site level. For GHG generated by biological activity only (on-site), Cakir and Stenstrom (2005) developed a model indicating a net release of 0.95 kgCO₂/ kgBOD treated for carbon respiration (including endogenous respiration) and 0.67 kgCO₂/ kg BOD with energy recovery from denitrification. These results highlighted that off-site emissions could reach over than a half of total. For WWTP based on stabilization ponds (SP), the total GHG emissions could be lower due the lower energy requirement comparing with activated sludge for example. However, SP forms a wide anaerobic layer in the bottom creating a large area for methane production mainly in tropical countries. In this sense, Paniagua et al. (2014) conclude that stabilization ponds could emits at least three times more GHG than aerobic activated sludge systems, depending on the surface loading rate.

Duckweed ponds (DWP) has been successfully applied to treat wastewater and producing a valuable biomass as by-product which could be used for biofuel and animal feed. Commonly DWP technology is placed as polish stage with focus on nutrient removal receiving therefore an influent with low C:N ratio. In spite of low carbon load applied (comparing with facultative ponds) Sims and colleagues (2013) pointed a high methane production reaching 1900 mg CH₄ m⁻²d⁻¹ in lab- scale duckweed ponds. By contrast, due the high growth rate (the higher between vascular plants) duckweed could perform a high CO₂ fixation. Moreover when compared with microalgae that commonly growth in stabilization ponds, duckweed could be easily harvested extracting the carbon fixed from the water.

Concerning this issue no data was found in literature that consider the balance of carbon fixed and emitted by duckweed ponds used to sanitary wastewater treatment. Thus a question arises: Does duckweed ponds used for wastewater treatment emit or sequester Greenhouse Gases (GHG)? Intending to answer this question the present work aimed to evaluate the flux of carbon in duckweed pond surface in open field pilot system operating with real domestic wastewater.

**MATERIAL AND METHODS**

**Duckweed treatment pond design and operation.**

The experiment was developed in a pilot-system at Federal University of Santa Catarina (27°35'46.74" S; 48°30’58.64” W, under a sub-temperate climate), located in Florianópolis City, in south of Brazil. Two experimental pilot ponds were made of fiberglass both ponds have dimensions of 4.40x2.40x1.00m. The useful surface and water depth are 8m² and 0.40m respectively. The system was operated in batch. Two duckweed ponds (DWP1 and DWP2) were made of fiberglass with dimension of 4.2 (length) x 2.4(width) x 1m (depth) and 0.42m of water column. The ponds were interconnected by PVC pipes (50mm) and were designed from ammonia loading rate of 15 kg NH₃.ha⁻¹.dia⁻¹. The treatment ponds were run in series in a continuous flow rate of 200L.d⁻¹ with hydraulic retention time (HRT) of 17 days together. The domestic wastewater, applied in the pilot system ponds came from a residential condominium being stored in equalization thanks simulating the pretreatment. The duckweed population from species *Landolitia punctata* (a native species) was adapted in this system working along five years.

**Wastewater treatment monitoring**

Effluent samples were collected once a week in each pond during 123 days and forwarded to a laboratory for analysis. The parameters selected for performance evaluation were pH,
temperature, ORP and dissolved oxygen (DO), by electronic probes \textit{(in situ)} and chemical oxygen demand (COD), biochemical oxygen demand (COB), dissolved organic carbon (DOC), total phosphorus (TP), P-PO₄, total nitrogen (TN), N-NH₃, NO₃, NO₂ were determined according to Standard Methods (APHA, 2005).

**Biomass productivity**

According Landesman \textit{et al.} (2005) the evaluation of duckweed biomass productivity is determined by specific growth rate (g.g⁻¹.day⁻¹) and relative growth rate (g.m².day⁻¹). To determine specific growth rate is necessary to find the produced biomass density (Mohedano, 2012). Biomass density was sampled trough a floating plastic square with internal area of 0.25 m², which was released randomly on the pond surface and the imprisoned biomass was collected, dried and weighed providing biomass weight per area. The specific growth rate (SGR) and relative growth rate (RGR) were obtained from the relation between the average density (g.m⁻²) and total biomass harvested.

**Greenhouse gas emission rate measurement**

Methane and carbon dioxide gas emissions were estimated using the static chamber technique according to OM-8 method from USEPA. This chamber consists of an acrylic cap with 40 cm in diameter of dome, covering a surface area of 0.1257 m² and useful volume of 30 L (Kimbush, 1986). The chamber was placed at the DWP1 submersed 2 cm in liquid keeping suspended by strings.

For CO₂ determination, a multi-function analyzer Testo® (model testo 435-4) coupled with probe QAI was used. The campaigns were carried at least once a month totaling six along the experimental period. To proceed the analyses the probe was fixed inside the chamber and programmed to collect data every 5 min during 24 h. Is important to keep in mind that CO₂ fixation occurs only during light phase of photosynthesis, thus daily cycle monitoring is mandatory. Also a negative control was performed by removing duckweed from inside the chamber and measuring gases without duckweed influence. The dynamics of CO₂ emission and fixation, which occurs alternately between the periods of sunset and sunrise, is usually evidenced in ascending and descending curves in charts. Thus, through the angular coefficient of the tendency line given by gradients can establish a comparison between the velocities of emission and CO₂ fixation. The emission/fixation rate was calculated by the equation 1.

\[
E \ and \ F = \frac{B \times 30 \times 1.96}{t \times A \times 100}
\]

Where:

E and F: Emission and fixation rate (gCO₂.m².d⁻¹);

$t$: time (d);

A: surface area (m²);

30: Volume of chamber (L);

B: Concentration of CO₂ (%) (difference between maximum and minimum);

1.96: Conversion from volume to mass.

In addition, the carbon fixed was also determined based on total biomass yield multiplied by total organic carbon content in this biomass. Thus, biomass samples were collected once a month (from June to November – winter/spring) and analyzed by using oxidation catalytic
combustion method, performed by TOC -L analyzer Shimadzu with accessory for solid samples SSM- 5000A.
Methane concentration was also measured inside the flux chamber by infrared sensor in a portable analyzer (LANDTEC® GEM 2000) with a measuring range of 0-100 % with accuracies ranging from 0-5 % ± 0.3 % and typical flow of 0.3 L.min⁻¹. The campaigns were carried once a month where the gas inside the chamber was sampling by pumping (5min), first with 5 min of between intervals of one hour during 24 hours.

RESULTS AND DISCUSSION
During the whole experimental period the duckweed ponds pilot system performs a satisfactory efficiency with 93, 84 and 79% for TN, TP and COD respectively (Table 1). This suitable efficiency for nutrient removal is expected in duckweeds ponds operating under presented conditions (Mohedano et al., 2012, El-Shafai et al., 2007). Considering the nutrient removal mechanisms based on autotrophic ways (plant absorption) the low C:N:P ratio do not hinders the efficiency. Earlier studies showed that the percentage of nitrogen assimilation by duckweed is the main way of removal in these systems (Cheng e Stomp, 2009; Muradov et al., 2014) The low organic loading rate of 39.2 kgCOD.ha⁻¹.d⁻¹ and high HRT could contribute to this high performance, however, recent studies also showed efficiencies of COD removal ranging from 76-80% (Adhikari et al., 2014; Allam et al., 2014). The concentration of influent and effluent COD and DOC of DWP are show in figure 1.

Figure 1. COD and DOC concentration during the experimental period (DWP1- Duckweed pond 1; DWP2 - Duckweed pond 2).
Table 1. Mean values and standard deviation of the concentration of variables in the effluent to all stages of the treatment system.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>influent DWP1</th>
<th>effluent DWP1</th>
<th>effluent EDWP2</th>
<th>Efficiency (%)</th>
<th>Loading rate (kg.ha⁻¹.d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.9±0.2</td>
<td>6.9±0.2</td>
<td>6.5±0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DO (mg.L⁻¹)</td>
<td>0.7±0.8</td>
<td>0.8±0.7</td>
<td>0.6±0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>COD (mg.L⁻¹)</td>
<td>156.9±58.6</td>
<td>48.1±8.3</td>
<td>32.9±6.1</td>
<td>79.0</td>
<td>39.2</td>
</tr>
<tr>
<td>COB (mg.L⁻¹)</td>
<td>71.4±33.0</td>
<td>24.7±10.3</td>
<td>12.9±7.1</td>
<td>81.8</td>
<td>17.8</td>
</tr>
<tr>
<td>DOC (mg.L⁻¹)</td>
<td>31.5±8.0</td>
<td>12.1±1.8</td>
<td>8.5±1.6</td>
<td>72.9</td>
<td>7.8</td>
</tr>
<tr>
<td>TN (mg.L⁻¹)</td>
<td>72.5±20.9</td>
<td>25.5±7.1</td>
<td>5.3±2.3</td>
<td>92.6</td>
<td>18.1</td>
</tr>
<tr>
<td>N-NH₄ (mg.L⁻¹)</td>
<td>58.8±21.7</td>
<td>21.4±6.6</td>
<td>3.2±1.8</td>
<td>94.6</td>
<td>14.7</td>
</tr>
<tr>
<td>N-NO₂ (mg.L⁻¹)</td>
<td>-</td>
<td>0.06±0.1</td>
<td>0.09±0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-NO₃ (mg.L⁻¹)</td>
<td>-</td>
<td>0.1±0.2</td>
<td>0.7±0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TP (mg.L⁻¹)</td>
<td>8.1±1.6</td>
<td>3.2±0.9</td>
<td>1.2±0.4</td>
<td>84.0</td>
<td>2.0</td>
</tr>
<tr>
<td>P-PO₄ (mg.L⁻¹)</td>
<td>5.1±1.7</td>
<td>2.1±0.5</td>
<td>0.9±0.2</td>
<td>82.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The total nitrogen concentration in final effluent reached 5.3 mg/L, fitting in more restrictive standards to environmental control worldwide, for example UE legislation 91/271/EEC of 15 May 1991.

Other particularity of the presented system was the excellent removal efficiencies of phosphorus obtained. Thus, considering the effluent of first pond alone, the TP concentration could fit on regional legislation (less than 4 mg.L⁻¹) According to Farrel (2012) duckweed have more phosphorus in their biomass than other aquatic macrophytes. As nitrogen, phosphorus is assimilated by the plant (principal form is PO₄) and removed from the system exclusively by harvesting. Öbek and Hasar (2002) observed that with harvesting the efficiency of phosphorus removal growth from 50% to 96.7% from no harvesting to 2 days between each, respectively.

Biomass productivity

The literature relate that biomass management is a subtle and important step for treatment efficiency, because the amount of biomass removed should follow the biomass growth maintaining a constant density. The specific growth rate obtained was 0.13 and 0.062 g.g⁻¹.d⁻¹ for DWP1 and DWP2, respectively, being a low rates when compared with others studies as Mohedano (2012) and Bergman et al. (2000) whom found 0.24 g.g⁻¹.d⁻¹ and 0.3 g.g⁻¹.d⁻¹, respectively, using the same species (Landoltia punctata). Despite the low yield, there was no impairment in treatment efficiency.

Greenhouse gas emission rate measurement

The CO₂ flux behavior was determined by photosynthetic activity and followed therefore the photoperiod. The same behavior was noted during the whole period, that is, after the sunset the CO₂ concentration starts to increase constantly during the dark period, however, along with sunrise the CO₂ is quickly removed. The velocity of CO₂ decrease during the morning was always fast than CO₂ increase in night (Figure 2). Therefore, considering the six campaigns the total amount of carbon fixed was higher than carbon released. CO₂ emission rate ranged from 3.048-6.017 mgCO₂.m⁻².d⁻¹ and fixation rate ranged from 19,592.5-42,052.8
mgCO₂.m⁻².d⁻¹ as could be seen in table 2. The high variation between campaigns could be due environmental changes as luminosity and temperature, as well the organic matter degradation at the moment.

![Campaign 3](image1)

![Campaign 4](image2)

**Figure 2.** Graphs of CO₂ behavior with and without (control) inside the chamber in campaigns 3 and 4.

**Table 2.** Minimum/maximum and emission/fixation rates of each campaign.

<table>
<thead>
<tr>
<th>Campaigns</th>
<th>Min-Max Emission (ppm)</th>
<th>Max-Min Fixation (ppm)</th>
<th>Emission rate (mg.m⁻².d⁻¹)</th>
<th>Fixation rate (mg.m⁻².d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(jul)</td>
<td>184-7365</td>
<td>7365-101</td>
<td>5549</td>
<td>19592</td>
</tr>
<tr>
<td>2-(aug)</td>
<td>204-6738</td>
<td>6738-158</td>
<td>5456</td>
<td>35499</td>
</tr>
<tr>
<td>3-(sep)</td>
<td>115-7893</td>
<td>7893-182</td>
<td>6019</td>
<td>42053</td>
</tr>
<tr>
<td>4- (sep)</td>
<td>200-7820</td>
<td>7820-500</td>
<td>5939</td>
<td>20682</td>
</tr>
<tr>
<td>5- (oct)</td>
<td>240-6473</td>
<td>6473-280</td>
<td>4845</td>
<td>22121</td>
</tr>
<tr>
<td>6- (oct)</td>
<td>150-4055</td>
<td>4055-500</td>
<td>3048</td>
<td>9523</td>
</tr>
</tbody>
</table>

As showed fixation rate was always higher than emission rate even when the climate conditions were not favorable to the plant metabolism as during campaign 6 which was carried under cloudy day (3500±2614 Lux and 25.5±3.52 °C) in morning (8:00-11:00h)
period. Also, a very high temperatures were registered inside the chamber during springer sunny days, because of the glass acrylic that causes a greenhouse effect and resulting in temperatures over 45°C. Certainly, it was a stressor factor to duckweed metabolism, even though the CO₂ content remained lower than normal atmospheric concentration (i.e. about 400ppm) during the whole light period indicating the higher fixation rate than compared with emission rate.

Unfortunately, there are few studies in literature with focus on CO₂ flux in duckweed ponds. Lindeman (1972) observed increase in fixation rate in duckweed cultivated under high CO₂ concentration with no luminosity variation, indicating that the CO₂ accumulated into the chamber during the night could improve duckweed photosynthesis yield. When the environmental conditions are similar, as the case of campaign 4 and 5, the accumulation inside the chamber was not significant to the fixation rate (because in day 21 that had the high accumulation had the low rate between each other). It is important to highlight that if the accumulation was even higher inside the chamber the quantity fixed would be higher as well.

Observing the negative control in Figure 2 (without duckweed) was possible to note that the CO₂ released by heterotrophic metabolism (organic matter degradation) has constant rise until the equilibrium concentration with liquid phase. Indeed without duckweed the CO₂ capture have not occurred. Another important point is that if the organic loading rate were higher probably the emission would be higher as well.

The CO₂ fixation calculated from biomass TOC content (39.7%±0.25) and specific growth rate was 12,713 mgCO₂.m⁻².d⁻¹ being two times less than the mean value comprises the six campaign (24,911 mgCO₂.m⁻².d⁻¹). The values of mass balance obtained from theoretical calculations and from primary data are usually distinct due the difference of methods. However, in this case, the duckweed inside the chamber was exposed to a high CO₂ concentration during the sunrise period and this fact could enhance photosynthesis yield. Indeed the duckweed inside the chamber could fix CO₂ with a higher rates than duckweed in outside.

Methane production was not detected in any campaign carried throughout the study period. This fact could be due the low organic loading applied and the small depth of ponds (40cm) that allows the dissolved oxygen diffusion until the bottom layer. The methanogenic Archaea are obligatory anaerobic and require a reducing environment with redox potential below -150 mV for their growth (Wang et al., 1993). However, the average ORP values inside the ponds were about -3.0mv ±10.4 inhibiting methanogenic organisms. Sims et al. (2012) evaluated CO₂ and CH₄ emission rate in a duckweed pond used for stormwater treatment. For CO₂ the results were 1700-3300 mgCO₂.m⁻².d⁻¹ and for CH₄ were 20000-37000 ppm. The results suggest that methane is predominant in their study when considering his potential as a GHG compared to CO₂.

CONCLUSIONS

The duckweed pond system evaluated showed a high efficiency for nutrient removal from wastewater. Moreover the CO₂ fixed by duckweed through photosynthesis was almost three times higher than CO₂ released by organic matter degradation (played by heterotrophic organisms). Thus CO₂ emission rate ranged from 3,048 to 6,017 mgCO₂.m⁻².d⁻¹ and fixation rate ranged from 19,592 to 42,052 mgCO₂.m⁻².d⁻¹. Methane emission was not detected by the
infrared probe used (with 0.1% of accuracy). In agreement to this, the ORP values measured at the bottom of both ponds were about zero (-3mv ± 10mv) do not supporting a suitable methanogenic metabolism. However in the present study the DWPs received a low organic loads that is usual to wastewater polish stage. Thus is recommended to evaluate the GHG emission under higher organic loads.

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REFERENCES
Farrell J. B. (2012). Duckweed Uptake of Phosphorus and Five Pharmaceuticals: Microcosm and Wastewater Lagoon Studies. All Graduate Theses and Dissertations, Utah State University Merrill-Cazier Library, Logan, Utah.