Evaluation of Temporal Stability of Dissolved Oxygen Conditions in a Small-Scale Phytodepuration System

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***Abstract*— Water regeneration is a top priority to achieve sustainability. Different alternatives to the traditional wastewater treatments that combine nitrification-anammox-denitrification are becoming popular in the last few years. These systems combine bacteria and plants to remove the organic matter and generate harvestable biomass. Despite the importance of oxygen concentration for the degradation of organic matter, no studies analyse the variation of dissolved oxygen in those systems. In this paper, we are going to evaluate the temporal and spatial variability of dissolved oxygen in the ponds of a phytodepuration system composed of *Typha domingensis* to identify if the bottom of the ponds is in anaerobic/anoxic conditions or not. Thus, the concentration of dissolved oxygen in 6 points and at 4 depth are measured during 2 months. Our results indicate that there is a high temporal variability of dissolved oxygen, caused mainly by the entrance of water into the system. The variability was much lower in the bottom of the third (and last) tank. The spatial distribution of the oxygen in normal conditions follows a gradient, having the highest concentration (0.5 mg/L) on the surface of the first pond and the lower concentration at the bottom of the second and third ponds (≈0.1 mg/L). Finally, we relate the system's performance with the produced biomass, 1.8 kg/m^2, and the reduction of total dissolved solids, 80%.**

Keywords-Typha domingensis; spatial variability; anaerobic conditions; wastewater; crops

I. Introduction

The wastewater treatment is an urgent need to accomplish the Sustainable Development Goals and have clean waters. The regenerated waters can be a valuable resource for agriculture since they can be used to irrigate crops. In recent years, the use of regenerated water has increased, and the apparition of alternative systems, such as the phytodepuration, are becoming popular [1]. The phytodepuration consists of the combined use of bacteria and plants to regenerate the water in ponds.

The operation principle of phytodepuration is that different bacteria in the water under anaerobic and aerobiotic conditions are capable of transforming the organic matter into nutrients through the combination of nitrification-anammox-denitrification processes [2]. This process is facilitated thanks to the introduction of small amounts of oxygen in the water by the plants through their roots. On the other hand, the plants consume the excess of nutrients in the water. Thus, the water quality is improved as the plant grows by absorbing these nutrients. The excess of nutrients and organic matter might cause environmental damages in rivers and lakes, known as eutrophication. Moreover, the phytodepuration systems do not produce any sewage sludge.

Recently, a research project to evaluate the use of small-scale phytodepuration to regenerate the wastewater of a small region is started. A phytodepuration system based on three ponds was set up. In this paper, we want to evaluate the variability of Dissolved Oxygen (DO) concentration along the ponds. First of all, we want to assess the generated vertical gradient of DO from the surface to the bottom. The plants inject oxygen into the water in the radicular area and while the bacteria are consuming this DO. On the other hand, and considering that the system will not work as a stationary system with a fixed and determined flow, we have to evaluate if this operation principle is functional. The system is injecting wastewater into the phytodepuration according to the water level of the reception tank.

This phytodepuration system aims to combine the regeneration of wastewater with the cropping of vegetals which can be later used for different purposes. This method is very convenient for rural areas with small villages and agroindustry, which produce wastewater with very high organic matter levels. Furthermore, the phytodepuration might involve a new source of income by harvesting the cropped plants for the rural population. Specifically, in our system, we have southern cattails as a crop. It has several applications, mainly as livestock feed [3]. Even so, recent research has proved their use for bioenergy production [4].

Although several authors pointed out the relevance of DO in converting organic matter into nutrients, no one paper shows the evaluation of its variability (temporal nor spatial) and its range in those systems. The inclusion of sensing technologies, or at least periodic monitorisation with probes, must be considered to evaluate the system's performance. Despite the importance of monitorisation in agriculture, where we find several monitoring systems [5], the monitorisation of phytodepuration is still in an early development stage.

In this paper, we analyse the variability of DO in our system. We will evaluate its temporal and spatial variability for 6 measuring points (at the entrance and exit of each pond) and 4 different depth per point (from the surface to the bottom). We will compare the stability of conditions in each pond for two months to define if they are reaching nearly anoxic conditions, which are required in the bottom of the ponds. A commercial optical probe was used to measure the DO. Data was gathered, as average, once per week. Thus, we will present the harvested biomass, which is growing over the water and injecting oxygen at the end of the study, in kg and kg/m^2. Furthermore, the rate of total dissolved solids removal is given.

The rest of the paper is structured as follows; Section 2 outlines the related work. Section 3 describes the materials and methods. The obtained results are analysed in Section 4, highlighting the implications of DO on the system's performance. Finally, Section 5 summarises the conclusions and future work.

II. RELATED WORK

In this section, we are going to summarise the studied DO characteristics in phytodepuration and similar processes.

In [6], Borges et al. studied the variability of several parameters on constructed wetland system to treat water from the Corumbataí River. Their system consists of 4 reactors on continuous water flow. The water input is the river which is considered reactor 1. In reactor 2, they have macrophytes (*Eichhornia crassipes* (Mart.) Solms), covering 80% of the water surface. Water was sampled at the end of each reactor. In their case, the initial DO level of water at the entrance of reactor 2 was 9 mg/L, and the DO after reactor 2 8.9 mg/L. Thus, they do not found any extra relevant oxygenation by the macrophytes (the water was fully oxygenated from the beginning). Their data indicates a reduction of total suspended solids of 54.5%. The authors also found an increase in the chemical demand of oxygen (CDO) of 26.7% after reactor 2.

Caselles-Osorio et al. [7] evaluated the modification of studied parameters on a horizontal subsurface-flow constructed wetland using *Cyperus articulatus* L. Authors pumped the wastewater from a septic tank to a set of 4 tanks, 2 of them with plants and the other 2 vertical perforated pipes. Their study had a duration of 3 months with a total of 43 measurements. The water exit from the septic tank with a DO concentration of 0.17 mg/L. The DO in the tanks with the plants reaches 2.1 (±1.2) mg/L. On the other hand, the tanks with the perforated pipes only went to 1.7(±1.2) mg/L. After finishing the experiment, the harvested biomass was 5 kg/m2 in the tanks with plants.

García-Perez et al. in [8] evaluate the temporal variability of DO in a recirculating vertical flow constructed wetland. In their system, they pump the water from a septic tank. Their pond has 6 x 6 x 1.2 m. Gravel was added to cover 50% of the volume of the wetland. Thus, 25% of the wetland were uncovered. As plants for consuming the nutrients, the authors used maize. The authors measured the DO in 6 periods over the 220 days of the experiment. The water input had an average DO of 1.2 mg/L. Their results indicate that the wetland was in aerobic conditions with average values of 5.3 mg/L (maximum and minimum values of 7.1 and 34 mg/L). Nonetheless, the authors do not specify precisely where are the water samples were taken (surface or bottom).

A final example can be found in which Palta-Prado and Morales-Velasco used different Poaceae for domestic wastewater phytodepuration [9]. The authors pumped the wastewater from a reduced region into 4 treatments. The wastewater had an average DO of 5.67 mg/L. The different treatments consist of diverse plant species. Their results indicate that none of the treatments increased the DO values in water. Average DO values for each treatment were 5.5 to 5.16 mg/L. Thus, CDO was nearly halved in all the cases.

As far as we know, no one paper has analysed the distribution of DO in the phytodepuration system and their trend along with the system or depth. Furthermore, it has not been studied for constructed wetlands or natural wetlands used for remediation regardless of their soil/bottom characteristics. Thus, it is necessary to have a preliminary approximation before performing an exhaustive analysis or studying its variation when the flow is modified or before adding a more complex (polluted) water input.

III. MATERIALS AND METHODS

In this section, we are going to detail the characteristics of the phytodepuration system and the measuring process.

A. Description of the phytodepuration system.

The system has been built in "IMIDRA - El Encin" facilities at Alcalá de Henares (Spain). The phytodepuration system consists of an initial tank of 40 m^3. This tank has a coarse grating grid, acting as a primary decanter. The first tank receives the wastewater from the "El Encín" facilities, and it is estimated that 20% of the CDO is removed. Then, water reaches the rectangular ponds, which contain the floating plants. The phytodepuration system comprises three pools of 9.6 x 3.6 x 0.8 m each (27.6 m^3 per pool), with a volume of 83 m^3 (Figure 1). Based on previous experiences, a depth of the water layer of 80 cm has been established, considering a maximum root length of 50 cm. Thus, a lower zone of 25-30 cm remains for the decomposition of the sludge in which anoxic conditions are required.

B. Description of the phytodepuration system.

The concrete structure, consisting of three ponds, was finished in the middle of January of 2020, see Figure 2 (a). The selected plant was *Typha domingensis* Pers. An autochthonous plant is well adapted to eutrophic conditions and used widely for wastewater treatment [10][11]. The *T. domingensis* was located over floating plates. The floating plates have the function of keeping the plants on the water surface but also preventing the entrance of DO from the atmosphere to the water. Thus, it facilitates the generation of the required anoxic conditions for the bacteria in the bottom.

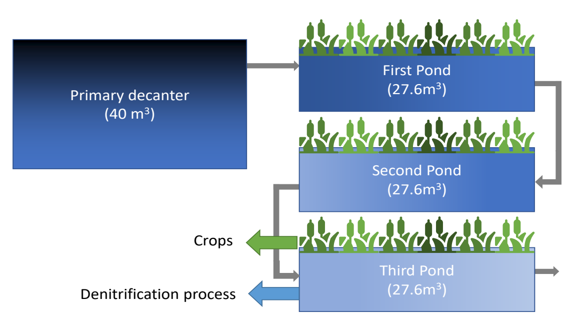

Figure 1. Scheme of phytodepuration system.

The placement of floating plates and plants began on 25/06/2020 (16 plants/m2). The *T. domingensis* came from the "Viveros Forestales La Dehesa SL", a specialised aquatic plant nursery for phytodepuration. The plants come from an alveolus tray, with a height of 15 cm in the aerial part and roots of 10 cm in length. They were kept in the shade under irrigation for seven days before their installation. Once the plant reached 1.67 m height and 35 cm root (02/09/2020), the study period starts. The studied period finished on 11/11/2020 when autumn tones began to appear, and the plants were harvested. Pictures of the different moments are shown in Figure 2. The dates of the photographs are Figure 2 (a) 18/05/2020, Figure 2 (b) 09/07/2020, and Figure 2 (c) 02/09/2020.

C. Measurement of DO in the ponds

Measurements of DO had been taken at two points per pond, one at the entrance and one at the exit of each pond. Thus, we have a total of 6 measurement points. In each point, DO was measured at four depth (6, 25, 50 and 75 cm). The measurements started on the water surface and finished at the bottom.

DO was measured with a probe HI98198 optical meter (HANNA [12]). The probe was calibrated with a 0 mg/L oxygen solution prior to data collection. For each measure, we wait until the value of DO is stabilised. A picture of the data gathering process and the probe is in Figure 2 (d).

IV. RESULTS

In this section, we analyse the variability of measured DO in the ponds. First of all, a general overview of the gathered data focusing on the temporal variations between points is shown. Then, the spatial variability along with the depth and the ponds is analysed. Following, we statistically analyze the data. Finally, we discuss the limitations of our study and the implication of the gathered data.

A. General overview of DO and its temporal distribution

The first step in order to analyse the variability of the DO is to have a general overview of the obtained values along the studied period. We can see the mean value of DO for each pond and each depth in Figure 3 (a) to (d). The different letters represent different depth from 6 cm to 75 cm, respectively. We can see that in general terms, the DO values are low. They never exceed the 2.6 mg/L. Although in some days, the DO increases, the detected increments are smaller for more profound points of the pond.

Concerning the temporal variability, we can identify mainly two peaks in the DO concentration. The first peak is found in the second measured day (7 days after starting the measurements). This peak increases the DO at all depth, but it only has an adverse effect (DO higher than 1mg/L) on the first pond. The second and third ponds have DO concentrations lower than 1 mg/L at all the depth. In fact, the DO values of the third pond are almost the same as in the previous measures. Meanwhile, in the second pond, we only found differences in the most superficial point. The second DO peak is found in two consecutive days, 41 and 49 days after starting the study. On day 43, the maximum DO concentration was found in the second pond with an average concentration of 1.44 mg/L at the most shallow depth. Meanwhile, the day 49, the DO concentration reached 2.47mg/L. On day 43, the second pond was the one with the higher concentration at all depth. Nonetheless, for day 49, the third pond had a higher concentration than the other at 50 and 75 cm depth. This trend might be explained by an accidental introduction of oxygen in the system at some moment before day 43.

B. Spatial distribution of DO in the ponds and their depth

After outlying the temporal distribution of DO concentration, we are going to focus on spatial variability. In Figure 4 (a) to (d), we represent the spatial distribution of the four measured points, entrance and exit of the first pond, and exit of the second and third pond, in the horizontal axis, and the four depth in the vertical axis. Figure 4 (a) to (c) displays the aspects of the ponds at different days, being Figure 4 (a), (b), and (d) the representation of normal distribution and Figure 4 (c) the spatial distribution in non-regular conditions. The different colours indicate the DO concentration (higher in blue tones and lower in green tones).

Focusing on the system's data in regular conditions, we can see how the DO decrease from the surface to the bottom of the pond and along with the ponds (from measured point 1 to 4). Comparing both days, the DO levels are higher in (a) than in (b) since that day there was a peak in the OD. Nonetheless, the spatial distribution is expected in the system. Figure 3 (c) shows that the aforementioned trend is not followed in unstable conditions.

(a) (b) (c) (d)

Figure 2. Images of the phytodepuration system and the data gathering. Figure (a) shown the structure of the system Figures (b) and (c) show the growth of the crop in two months, and Figure (d) presents the used probe.

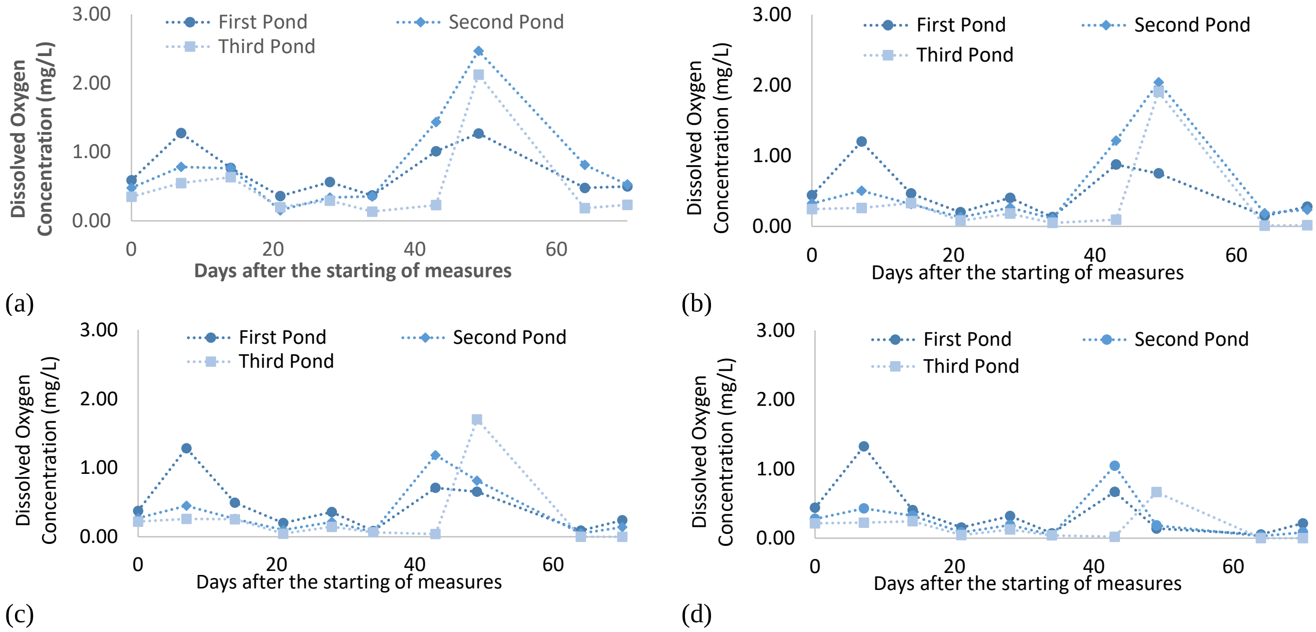

Figure 3. Overview of DO concentration at different depths (a) 6 cm, (b) 25 cm, (c) 50 cm, and (d) 75 cm.

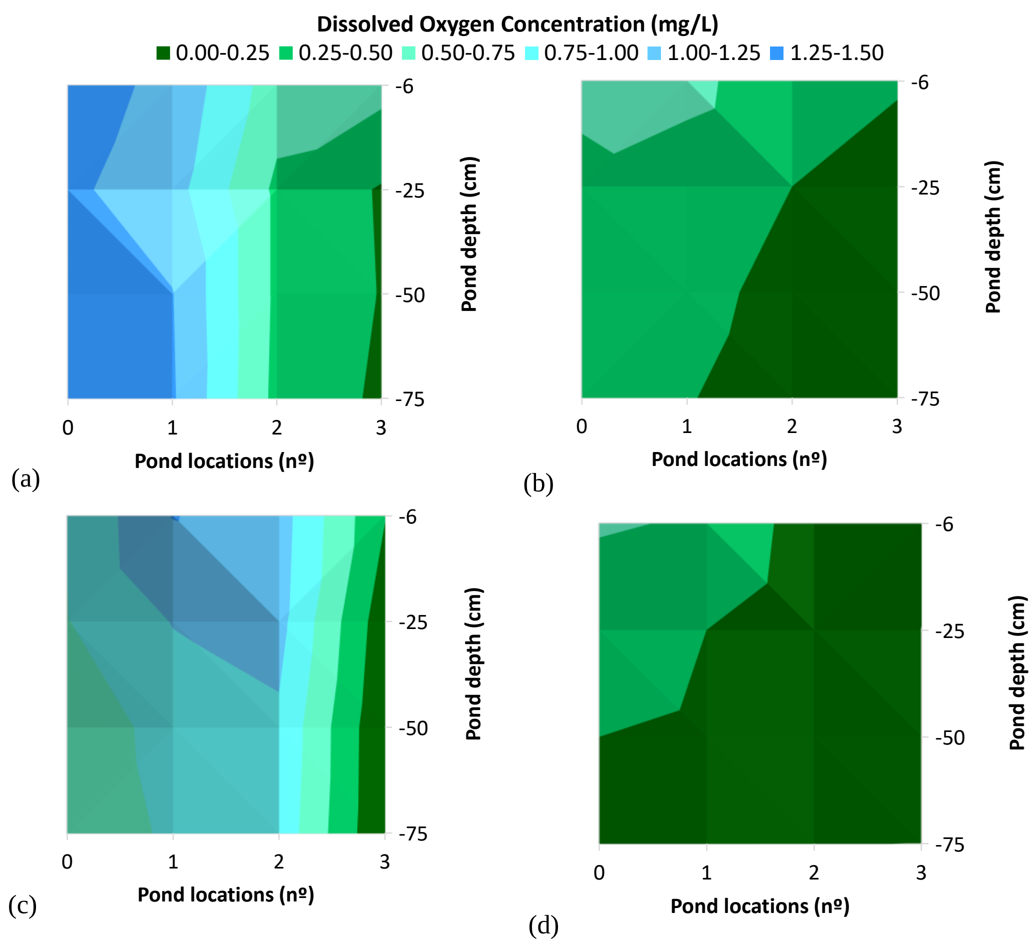

Figure 4. Spatial distribution of DO in different days, (a) 09/092020, (b) 30/09/2021, (c) 15/10/2020, and (d) 11/11/2021

In (c), we can identify a point with having a higher DO concentration than the previously measured point. This point is the measurement at the exit of the first pond. As detailed before, a possible explanation is that an accidental introduction of oxygen happened in the system, caused by diverse conditions of water input or an increased flow.

Concerning the area with low DO (nearly anoxic or anoxic-dark green colour), we see that in regular conditions, we can see that in Figure 4 (a) and (c), most of the ponds present DO concentrations of up to 0.75 mg/L. Thus, only some points of pond 3 present the optimal conditions for the operation of the bacteria.

On the other hand, focusing on Figure 4 (b) and (d), the whole volume of the ponds has a concentration of DO lower than 0.75 mg/L. Thus, up to 50% of the ponds are in a nearly anoxic condition in Figure 4 (d) and up to ¾ in Figure 4 (b).

C. Temporal variability in each measured point

Following, we are going to focus on the temporal variability in each one of the measured points. In this case, we are going to represent all the measured points, including the beginning and end of each pond (6 measured points).

The average value of DO for each sampling point and each depth can be seen in Figure 4, represented in vertical coloured bars. Meanwhile, the black error bars indicate the standard deviation of gathered data. In general terms, we can see that the average values follow the trend of decreasing DO along with the measured points and the depth. Nevertheless, there is a point that does not follow this trend. Measured point 3 has a higher DO concentration than measured point 2. The peak causes this detected on 15/10/2020; this data can be considered as an outlier value. The average values at the bottom are almost identical for the two measured points of the third point (point 5 and 6).

With regards to the standard deviation, we can see that the higher variation is found for measured points 3 and 4 (depth of 6 and 25 cm). Again, this is caused by the peak of DO on 15/10/2020. For measured points 1 and 2, the standard deviation values are between 0.41 and 0.34 (relatively stable along the pond and the depth). Concerning pond 3 (measured points 5 and 6), the standard deviation is much lower in the bottom (0.25 and 0.16 mg/L) than in the surface (0.71 and 0.48 mg/L). Thus, we can affirm that we can find more stable conditions in the third point and less DO than in the previous ponds. This is explained by the characteristics of pumped water in the ponds. The pumped water might suppose an input of oxygen in the system. Moreover, the included oxygen is consumed by the bacterial during the denitrification process.

D. Limitations of the study and evaluation of phytodepuration performance

In this section, we are going to analyse the limitations of the presented research and the implications of the DO concentration on the performance of the phytodepuration.

First of all, the main limitation of our study is the limited number of measurements. Therefore, it will be more accurate to have an in-situ sensor with continuous measurements. Nonetheless, considering the scope of phytodepuration, it is not feasible to bear the costs of the acquisition of this monitoring technology. Therefore, punctual samples are required to evaluate the operation of the whole system.

In addition, there is a limitation in our study considering the input of water. In our case, the pumped water into the system comes from a small area, including the work centre of a research area and a small amount of agroindustry wastewater. Nonetheless, no control has been done regarding the water flow. Thus, we cannot know the flow in our system since it varies over the days, and we cannot estimate a fixed retention time. Moreover, we have performed the study during a rainy period in which abrupt changes in water flow are expected. Finally, we do not know the water conditions in the water reception tank.

Finally, we will evaluate the effect of DO concentrations on the performance of the process. Considering that the majority of the denitrification happened in the bottom of the ponds, we are going the classify if the denitrification process is optimal (nearly anoxic conditions 0 to 0.5 mg/L), good (0.5 to 1 mg/L) or not good (>1 mg/L), see Figure 6. For the first measured point, the entrance of pond 1, in 70% of the measures, the conditions are nearly anoxic; only in 1 case, we find a condition characterised as not good for the bacteria. Similar results are found for the ending of the first pond. Focusing on the second pond, only 10% of the time operates in non-optimal conditions. Lastly, the third pond is always optimal (90%) or good (10%) conditions.

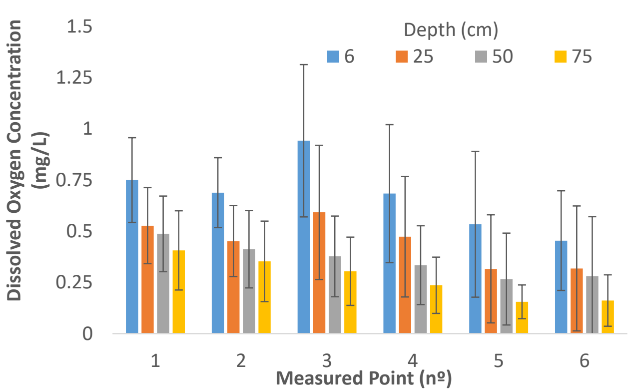

Figure 5. Mean and standard deviation of measured DO concentration along with the ponds.

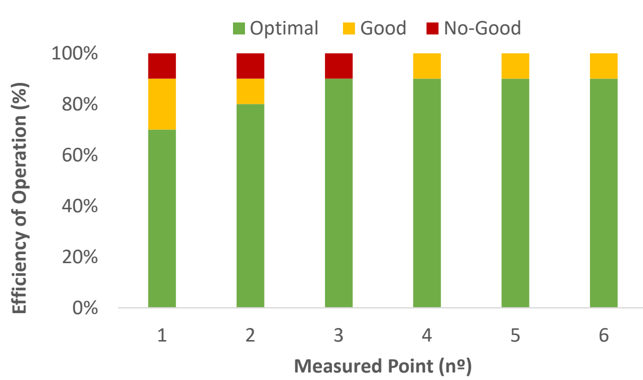

Figure 6. Operational conditions of the ponds in the studied period.

Although the system has been operating without any restriction or condition in the water flow, we can confirm its proper operation based on water quality values and the DO values. In all the analysed period, both the input and the output of the phytodepuration were periodically measured. The data of total suspended solids confirms that there is a reduction of 80% as average. However, CDO values are relatively low (at the entrance and exit), and the effect of phytodepuration on the reduction or increase of CDO is not yet apparent. Although in some cases CDO decreased, in other cases, it increases. Thus, more research is needed. Concerning the system's productivity, a total of 187.88 kg of typha biomass was harvested on 11/11/2020. It supposes average productivity of 1.8 kg/m^2.

According to our data, the *Typha domingensis* injects a minimal amount of oxygen in the water and has nearly no impact on the measured DO. In similar papers [7], authors have shown how other species have much higher oxygen transference rates. Although the low DO concentration, the combined nitrification-anammox-denitrification process is operating well, and the crops have grown.

V. Conclusions

In this paper, the temporal variability of DO in phytodepuration ponds is analysed. In phytodepuration, we need a stable and low DO in the bottom to allow denitrification, which converts the organic matter into valuable nutrients for the plants above the water. Thus, the optimal operation of bacteria ensures the cleaning of the water and the growth of the crops.

Our results indicate that the DO is low in the three ponds in general terms, mainly due to the water input and a decreasing DO concentration along with the ponds. The DO also follows a negative gradient along with the depth of the pond. Thus, we have confirmed that our design of a small-scale phytodepuration system with no control on the flow accomplishes DO's expected values and variability.

As future work, we want to evaluate the denitrification process in each pond. We will include low-cost water quality sensors measuring the turbidity and the organic matter developed in [13]. On the other hand, we will study the crop performance in each pond to evaluate if the different amounts of nutrients in the ponds affect crop performance.

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