

Article

Physiological Investigations of the Plants Involved in Air Biofiltration: Study Case

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Abstract: In this study, the behavior of an aerial plant (*Tillandsia xerographica*) during air biofiltration was investigated by monitoring the trend of the CO₂ concentration in the processed air as a response to a change in the environmental conditions. In this regard, a botanical biofilter equipped with *T. xerographica* was continuously operated with ambient air for about three weeks under different light intensity, air flow rate, ambient temperature, and relative humidity. The plant was able to decrease the CO₂ concentration in the processed gas in both the presence/absence of light, as long as a regular alternate day/night regime was kept, this behavior being attributed to its specific plant metabolism. Overall, plant physiology under the influence of the above mentioned factors is pointed out, which in turn reveals the plant potential in urban air biofiltration, with the possibility to further address not only the carbon dioxide removal but also other trace gaseous contaminants in ambient air as well, improving the air quality and reducing the health risks associated with exposure to polluted air. Therefore, further modeling and optimization of this process, along with the investigation of the plant’s response under different contaminated environments, is expected to significantly contribute to the development of new such versatile biofilters for air treatment.

Keywords: air biofiltration; aerial plants; CO₂ capture; plant physiology; biofilter; plant metabolism



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1. Introduction

Addressing plant physiology-related challenges play an important role in botanical biofilter development for air treatment. In this study, a versatile aerial plant (*Tillandsia xerographica*) with high potential in air biofiltration is undertaken for investigation. It is already known that botanical biofiltration can improve the air quality both indoors and outdoors and can reduce the health risks associated with the exposure to the gaseous contaminants.

In contrast with the terrestrial plants (i.e., soil/substrate-based) that are usually involved in air biofiltration studies, where the aerial part along with the roots and their rhizosphere microorganisms act symbiotically for the gaseous pollutants removal during the air biofiltration process [1], the leaves of *T. xerographica* (a soil/substrate-free plant) play a central role in the plant architecture and gas uptake, as its roots are poorly developed—a common aspect for the *Tillandsia* group—thus, because the roots of *Tillandsia* do not have an anatomical structure adapted to absorb water from substrate, this epiphyte presents certain characteristics, such as the peltate trichomes (scales) formed by a stem directly connected to the internal tissues of the leaf, a multicellular shield, and the peripheral wing [2]. The trichomes are located on the epidermis and are specialized in the absorption of water and nutrients from the atmosphere. The indumentum of intricate trichomes is also involved in plant temperature regulation, light reflection, and the distribution of water by capillarity on the plant surface [3,4], permitting the plants development in significant

stressful environments [2,5,6], as are the xeric microhabitats. It was included in aerophytes category [7], being able to colonize trees and shrub canopies as well as rocks and other supports [8]. The plants reproduction process is by seeds (sexual) or by production of sprouts (vegetative, asexual). Due to its ornamental qualities, it is used as an indoor and outdoor decorative plant.

Despite the plants widespread popularity in Mexico, Guatemala, El Salvador, Honduras, and a part of USA, where it often grows in the upper branches of tall trees, the species is considered threatened because of commercial exploitation. Consequently, the plant is intensively cultivated in nurseries [9].

Like most of the species within the Neotropical genus *Tillandsia* (subfamily *Tillandsioideae* within family *Bromeliaceae*), *Tillandsia xerographica* seems to exhibit [10,11] a crassulacean acid metabolism (CAM). This photosynthetic pathway, restricted to the genus *Tillandsia* (especially the epiphytic life-form), has a wide physiological plasticity, high water use efficiency, and a significant advantage in conditions of limited (atmospheric) moisture. Basically, the CAM mechanism allows the nocturnal fixation of CO₂ in malic acid facilitated by phosphoenolpyruvate carboxylase, followed, in the light period, by its release, via decarboxylation and assimilation in the Calvin cycle [12–14]. Thus, in the light period there is an increase in the internal CO₂ concentration causing the stomata closure, minimizing water loss through transpiration and optimizing water use efficiency [15].

Light intensity, environmental temperature, water, and nutrient availability are among the main environmental factors influencing the CO₂ assimilation in the CAM plant species [16]. Martin et al. [17] highlighted that the epiphytic CAM bromeliads as *Tillandsia ionantha* can adapt to both high and low light levels but are more efficient in the utilization of lower irradiances, allowing colonization of the shaded microhabitats. Also, the capacity for dissipation of excess light energy was found to be beneficial for the plants developed in full sun and exposed to drought stress [17], while a study on *Tillandsia usneoides* [18] highlighted that under constant illumination the CO₂ exchange was similar to day/night conditions. A comparison of the relationships between CO₂ gas exchange and leaf trichomes cover for the 12 species of *Tillandsia* [5] found a positive correlation between the maximum rates of night CO₂ uptake and leaf trichome cover, but also a low-level diurnal CO₂ loss by diffusion, probably via trichome stalks which have a low resistance compared to the leaf cuticle.

A great number of studies have highlighted that plants can remove a wide spectrum of air pollutants, with direct effect on human health and energy consumption [8,19,20]. Among that, a constant objective was represented by a reduction in CO₂ footprint [21,22], which can be achieved by using plants photosynthetic capabilities in biofilters. Although this particular species was never investigated in respect to CO₂ exchange patterns, the capacity of removing CO₂ in absence of light would make from *Tillandsia xerographica* a very promising solution (perhaps in combination with C3 plant species) for the selection of the appropriate plant species for CO₂ removal in biofilters. Exploring this process aspect will contribute to a better understanding of the removal mechanisms and of biofilter functionality, depending on specific environmental conditions. For instance, coupling some C3 and CAM plants grown on a solid substrate resulted in a lower CO₂ emission during some VOCs (volatile organic compounds) biofiltration [23,24]. From an implementation point of view, the use of substrate-free aerial plants such as *T. xerographica* (not limited to) is an affordable option, as they are residue free and are subject to easy transport and maintenance.

The plants ability to take up and transport a certain contaminant from the air to different plant parts, to then metabolize or store it in vacuoles or cell walls, depends on plant morphology/structure and contaminant characteristics, along with plant capacity to cope with any induced oxidative stress or effect that can influence their photosynthesis and respiration through various enzymatic and non-enzymatic mechanisms such as antioxidant systems, heat shock proteins, and phytochelatin [25–28]. In the case of soil-based plants, there is a continuous interaction between plants and their rhizosphere microorganisms through chemical and physical signals that favor the contaminant removal; particularly,

microorganisms can influence plant physiology by producing hormones, enzymes, or toxins [29–31]. As introduced earlier, the environmental conditions (e.g., abiotic factors such as temperature, relative humidity, light, water availability, carbon dioxide and/or other pollutants, etc.) can influence the metabolism and plant performance, thus an important attention should be paid to the plant physiological behavior in respect to these aspects, for assuring an appropriate plant response and enhanced process performance [32]. Thus, physiological investigations of the plants involved in air biofiltration are important to understand the factors that influence their performance.

The performance of plants in air biofiltration processes strongly depend on the plant's physiology, taking into consideration the above mentioned aspects. The aim of this study is to evaluate the behavior change of *Tillandsia xerographica* during air biofiltration, by monitoring CO₂ concentration in the processed air as a response to a change in the environmental conditions. Aspects related to illumination conditions, gas contact time, irrigation, relative humidity, and temperature are particularly addressed. The obtained results are presented and discussed, allowing the pointing out of the plant physiology under different factors influencing and depicting the plant metabolism based on the light-dependent CO₂ capture.

2. Materials and Methods

A transparent column-biofilter (7 L volume), made from plexiglass (Poly(methyl methacrylate)), was equipped with three *Tillandsia xerographica* plants (commercially available, 3–4 years old, with high axis of 21–24 cm considering an elliptical plant shape without extension of leaves). The plants were previously immersed in regular distilled water for about half an hour and then the excess water was removed from their surface before use. The biofilter (Figure 1) was continuously operated for about three weeks (period I–XI, Figure 2) during the summer period with ambient air (conditioned air and fresh air mixture), being most of the time (period IV–IX, Figure 2) exposed to moderate natural illumination under day/night regime. Prior to the first day of continuous biofilter monitoring, the biofilter was subject to an acclimatization period under dynamic regime (0.25 L/min air flowrate) and continuous (artificial) illumination (12 kLux light intensity) for about four days, which allowed the *Tillandsia* individuals to adapt in the microclimate of the column-biofilter. Carbon dioxide concentration, relative humidity, and temperature in inlet and outlet gas were continuously monitored, along with light intensity (usually, 1 min sampling frequency) by using specific sensors (NDIR sensor for CO₂, capacitive polymer sensor for relative humidity, NTC thermistor for temperature, silicon photodiode sensor for light intensity) coupled to the LabQuest 2 stand-alone interface (Vernier, Beaverton, OR, USA), used to collect sensor data, acting as a data logger as well, with its built-in graphics and analysis application (integral/derivative function, etc.). The high-resolution touchscreen of this device facilitated fast and easy collection, analysis and sharing of the acquired data via wireless connectivity.

An AQ-Expert multifunctional indoor air monitor (E-Instruments, Langhorne, PA, USA), an Extech Thermometer and Humidity Meter RH101, and an Extech LT300 digital Lux meter (Nashua, NH, USA) were additionally used for regular control of these measured parameters. No other relevant gaseous contaminants or products were detected in the processed air. According to the the flowsheet diagram of the experimental setup presented in Figure 1, the processed air was fed at the bottom of the bioreactor by using an air pump at a controlled flow rate. The loading rate of the biofilter over the course of the monitoring period ranged between $38 \div 198 \text{ g CO}_2/(\text{m}^3 \cdot \text{d})$ (depending on the air flowrate and CO₂ concentration in inlet air), considering the biomass packing bed volume (consisting in the studied plants) of about 6 L including void spaces. In addition, the influence of plant irrigation was investigated at a certain moment by feeding regular distilled water (100 mL, with electrical conductivity of 3 $\mu\text{S}/\text{cm}$ and pH 6.5) at the top of the bioreactor (except this, no plant irrigation was performed over the course of the test period). Although in this study, distilled water was used as it is not expected to induce any possible influence on the physiological behavior of this plant type, on longer term it might be necessary considering

a small nutrient supply [33]. The treated air was evacuated at the top of the reactor, while the wastewater was collected and evacuated at the bottom. Also, in addition to the natural light, which contains all of the visible spectra, the influence of the artificial lighting was occasionally investigated by illumination of the bioreactor with two LED-array lamps in a visible spectrum (characterized by a spectral range between 440 and 720 nm and a maximum absorption peak located at 580 nm) oppositely located at a distance from the reactor (in this case, the experimental installation was covered by an aluminum folium). The operating ranges of all these parameters are specified in Figure 2. A similar control reactor was also parallelly running, in order to check the process time to time (discontinuously), with a roughly similar response. The day/night cycles, respectively, the natural light/dark cycles, were approximately equal over the course of the test period ranging between day 4.5 and day 16.5, containing alternative cycles of about 15 h of daily light and 9 h of dark with a small tendency of day time decreasing. Also, it can be observed from the Figure 2, the period of artificial illumination during the monitored period (days 0–1, 2.5–4 and 16.5–19).

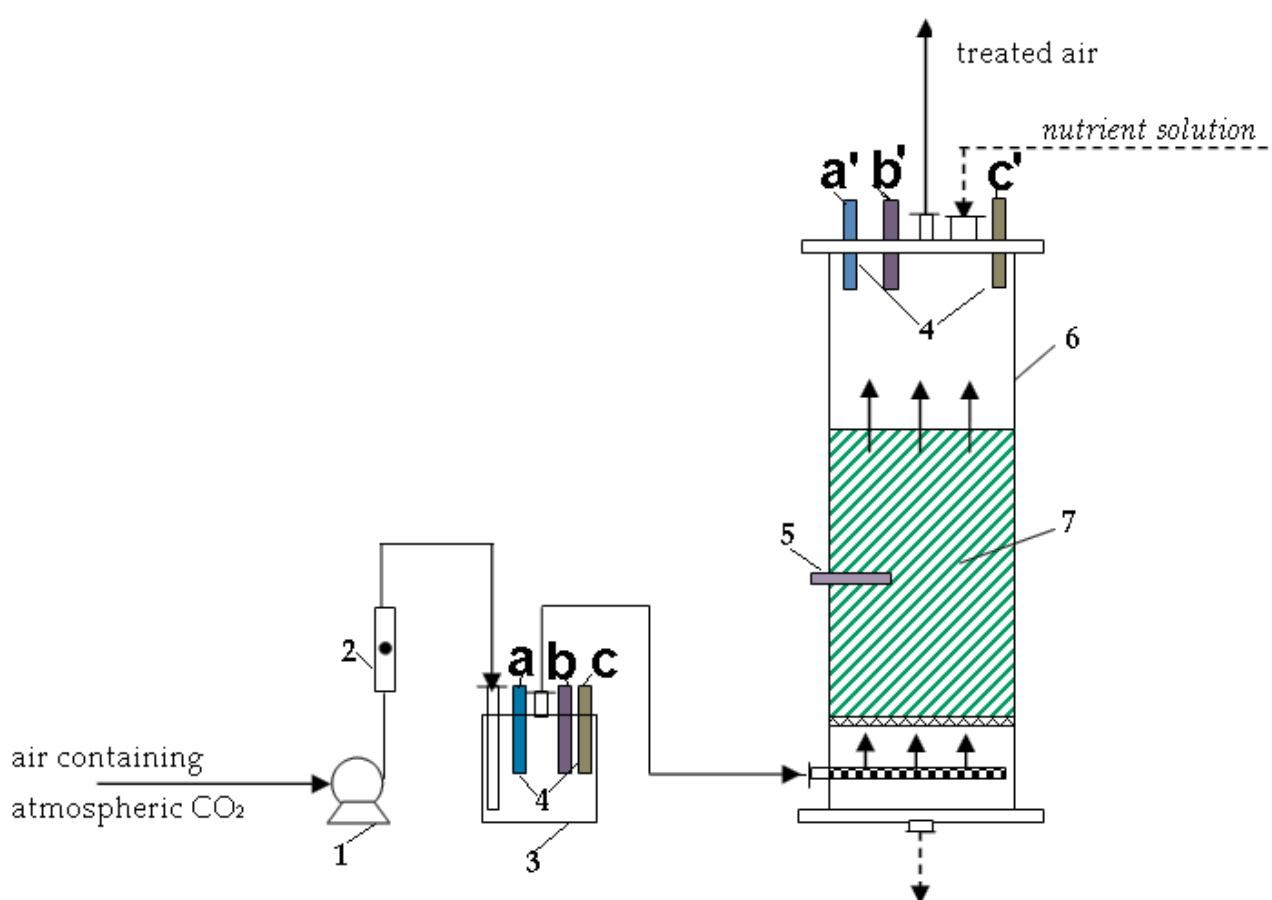


Figure 1. Flowsheet diagram of the experimental setup. 1—air pump; 2—flow meter; 3—inlet monitoring unit; 4—sensors (a&a'—IR CO₂, b&b'—RH, c&c'—T); 5—external light sensor; 6—column; 7—“packed bed” based plants.

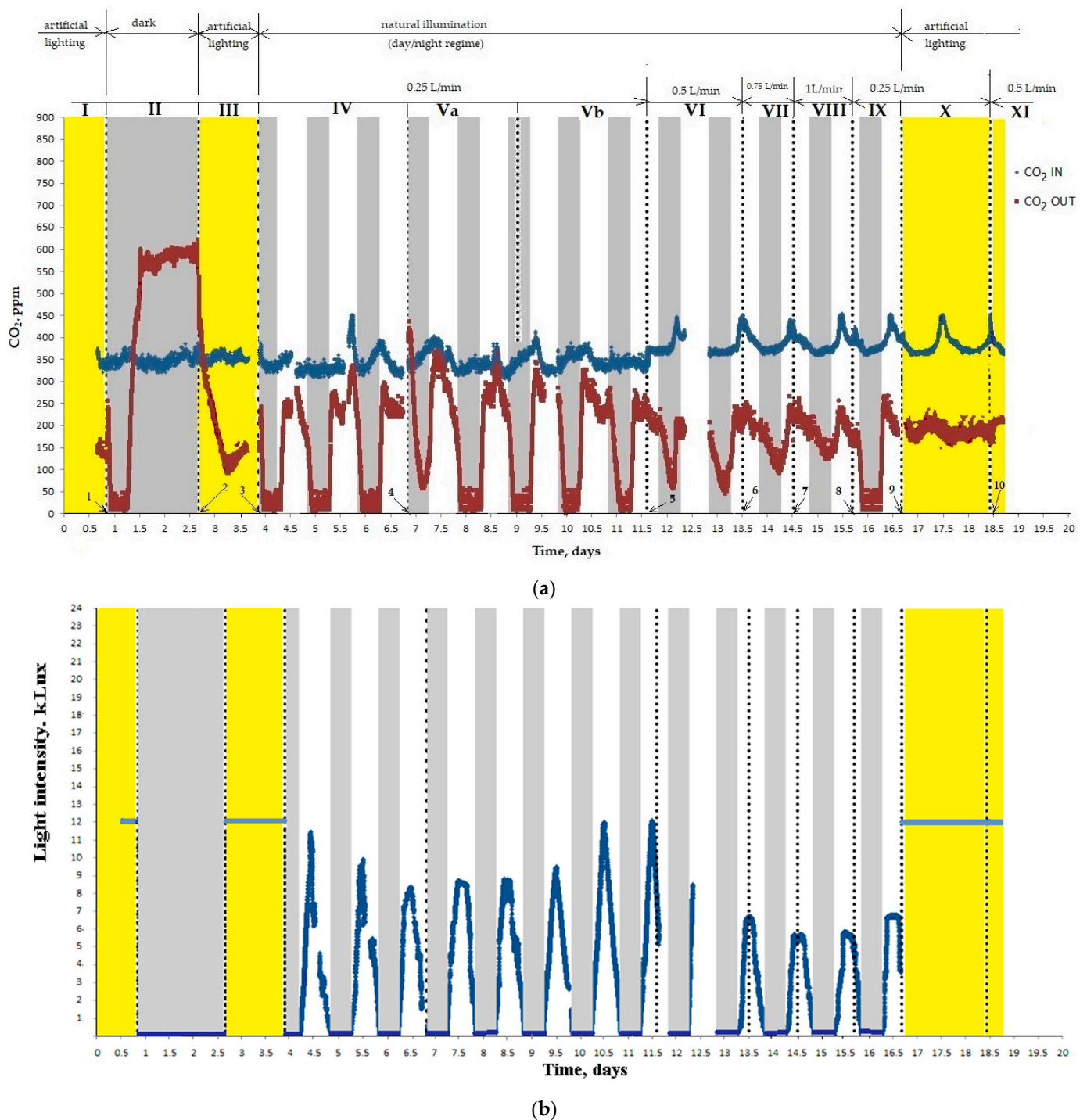


Figure 2. (a) Variation of CO₂ concentration over the course of the test period. (1—stop artificial lighting; 2—start artificial lighting; 3—start natural illumination regime (day/night cycle); 4—irrigation (distilled water supply); 5, 6, 7—air flow rate increase; 8—air flow rate decrease; 9—start artificial lighting; 10—flow rate increase; yellow color—artificial light; grey color—dark (II)/nighttime; transparent color—daytime with natural light). (b) Variation of the light intensity over the course of the test period.

3. Results

3.1. Investigation of Plant Response to Light Conditions

Figure 2a shows the CO₂ variation over the course of the test period under different environmental conditions. As it can be seen, the level of the outlet CO₂ concentration was

strongly influenced by the illumination conditions (Figure 2b). First of all, the plant was able to decrease the CO₂ concentration in the processed air in both the presence/absence of light, as long as a regular alternate day/night regime was maintained (region IV–IX, Figure 2a). In contrast, operating the system under continuous dark conditions (region II) does not sustain this dual performance and results in an increase in CO₂ concentration as result of CO₂ production. A similar trend was observed in Soreanu et al. [34], where CO₂ production in continuous dark conditions occurred; a similar behavior resulting in CO₂ loss under continuous darkness conditions was observed by other authors for other CAM plants as well [18]. Switching from continuous dark regime (region II) to lighting regime (region III) allowed the biosystem to recover and return to the initial CO₂ removal performance (region I). Therefore, in order to overcome the above mentioned drawback associated with excessive CO₂ production, the biosystem should be periodically exposed to light. On the other hand, under the day/night operating regime in the actual study, the CO₂ decrease was significantly more pronounced during the night than during the day, which might suggest the presence of a concurrent CO₂ production phenomenon during the day, simultaneously with CO₂ uptake. Overall, the plant capacity to perform both diurnal and nocturnal CO₂ uptake clearly confirms the facultative CAM-driving plant performance, which occurs as illustrated in this study. The reversible switches between these metabolic pathways are specific to facultative CAM plants [35].

Moreover, it can be noted that the CO₂ decrease during the light presence, at the same air flow rate, is more pronounced under constant light intensity (artificial illumination) (region I, III) than under fluctuating light intensity (natural illumination) (region IV, Vb). This suggests that under constant light intensity, the concurrent CO₂ production is diminished, likely due to the plant switching for a classical photosynthesis (C3 type). These results show that operating the biosystem under natural illumination (day/night regime) without any artificial lamps, can assure a continuous CO₂ removal at different rates (according to a cyclic pseudo-steady state determined by the day/night cycle), which could be temporarily compromised by cloudy days. In contrast, operating the biosystem at constant light intensity assures a steady-state CO₂ capture performance and better process control. Different technological configurations can be further adapted to benefit from both operating modes.

3.2. Influence of Different Operating Parameters

3.2.1. Influence of the Light Intensity and Illumination Type

In addition to the earlier mentioned aspects, it can be mentioned that for the day/night regime (region IV–IX, Figure 2a), the light intensity influence was directly related to CAM behavior. Generally speaking, the CO₂ removal performance was higher during the nocturnal operation when compared with the diurnal trends, revealing a faster CO₂ metabolic rate in the first case. During the artificial illumination, for the same air flow rate (0.25 L/min), a slightly more pronounced decrease in outlet CO₂ concentration is observed in the early stages (region I, III) than later (region X), which might be subject to the change/interactions in other environmental conditions as well (e.g., relative humidity and/or temperature). However, similar performances in terms of CO₂ concentration decrease are observed for roughly similar light intensities at the same flow rate under diurnal/night regime (e.g., regions IV and Vb), which demonstrates a stable performance over the long term operation and biosystem reproducibility.

In addition, the light spectrum in the visible area of the artificial source has a dominant peak at 580 nm, similar to the natural light, which suggests a similar effect on the plant behavior, without relevant aspects that could be due to illumination with different wavelengths.

3.2.2. Influence of Water Supply and Relative Humidity (RH)

Water supply is associated with an increase in RH in the outlet air (region V, Figure 3). As can be seen in Figure 3, the outlet RH values tend to decrease over time in the absence

of plant irrigation (regions I–IV and VI–XI), this effect being amplified by the air flow rate increase (regions VI–VIII). However, this RH decrease did not appear to affect the plant performance. This aspect was also observed for an obligatory CAM plant (*Tillandsia flabellate*) in the study of Hu et al. [13]. According to Figures 2 and 3, under day/night regime at the same air flow rate (regions IV and Vb), the CO₂ decrease in the outlet gas was not significantly influenced by the RH decrease, which indicates that the biosystem was not affected in the absence of a water supply. The decrease in RH in the outlet gas is a consequence of the plant evapotranspiration decreasing, as a form of protection against water loss in the absence of irrigation, e.g., by closing stomata during the day and its opening during the night [13,15]. However, it might be possible that the inlet RH increasing during the night to have a favorable contribution to the nocturnal CO₂ uptake [18]. In addition, no relevant dependence between the inlet RH and outlet CO₂ concentration is observed, when separately referring to day/light or night/dark period (Figures 2a and 3).

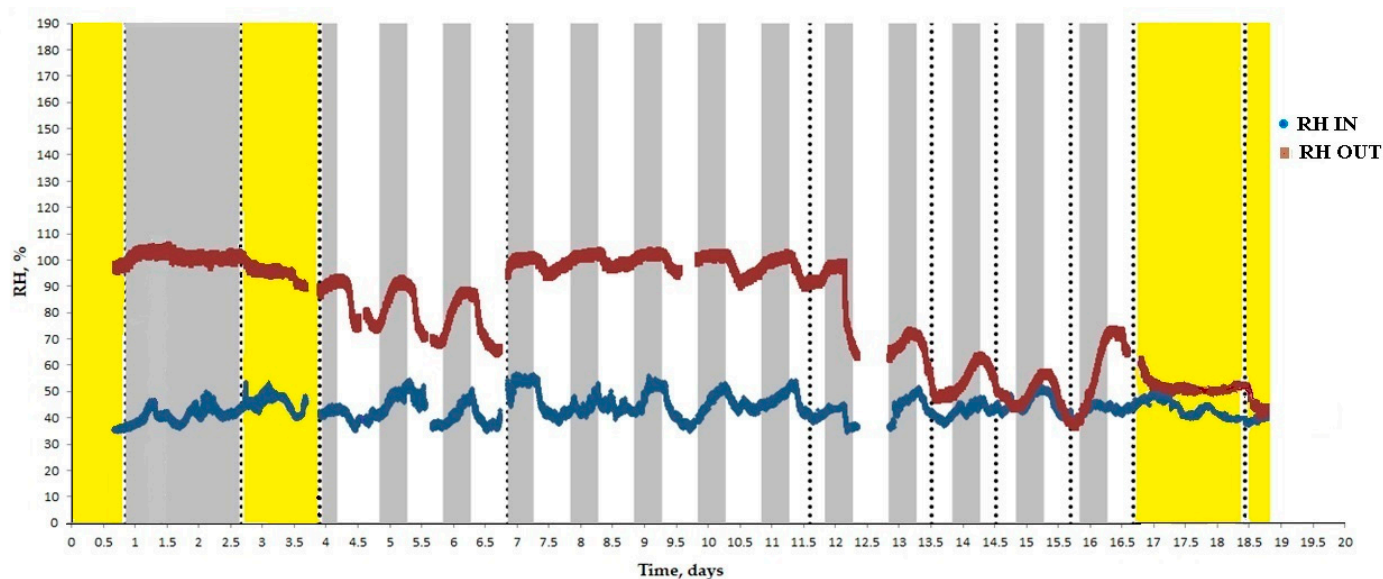


Figure 3. Variation of the relative humidity (RH, %) over the course of the monitoring period.

On the other hand, the plant performance was affected by the water supply (region V), when a temporary increase in the outlet CO₂ concentration was observed for about 1 day, after which recovered back to normal. This observation is in agreement with the previous study [34], where a similar effect was observed. Tissue wetting was also a cause for CO₂ loss in some studies performed by Martin and Siedow, when using *Tillandsia usneoides* as CAM plant [18]. More detailed aspects concerning the response of some other facultative CAM plants to water stress are reviewed by Winter and Holtum [35].

3.2.3. Influence of the Air Flow Rate

Generally speaking, an increase in the air flow rate is often accompanied by a decrease in pollutant removal due to the lower phases contact at higher air flow rates. In the actual study, this effect is clearly observed during the nocturnal operation, when the increase in the air flow rate from 0.25 to 1 L/min determines the apparition of higher CO₂ levels in the treated gas (regions VI–VIII versus Vb and IX, Figure 2a). In contrast, the outlet CO₂ concentration during the diurnal operation seems to not be significantly affected by the air flow rate increase (e.g., regions VI–VIII versus Vb and IX, Figure 2a), possibly due to a dilution effect of the concurrently emitted CO₂ during the day. On the other hand, under artificial illumination, the increase in the air flow rate (e.g., from 0.25 to 0.5 L/min, region X–XI) slightly affected the CO₂ removal, due to the lower contact time of gas–solid phases, where the solid phase is represented by the entire plant surface. From this behavior, it can be deduced that the CO₂ emissions in the presence of light are associated with

“CAM operation mode” (day/night cycle), being inhibited during “C3 operation mode” (continuous illumination).

3.2.4. Influence of the Temperature

As can be seen in Figure 4, the output temperature was higher than the input temperature, this difference usually ranging between 1 and 3 °C. This aspect could be due to a possible greenhouse effect (amplified by the CO₂) and other metabolic processes. Moreover, the high-input-temperature fluctuations (e.g., sometimes by 7–9 degrees) did not affect the plants capability to uptake CO₂ already observed (e.g., region V versus IX). Although CAM is well expressed at constant temperatures, the differences between diurnal and nocturnal temperatures are favorable to CAM likely through favoring the acid accumulation involved in plant metabolism [36,37]. Overall, according to the actual results, the plant is able to adequately perform between 22 and 33 °C. The interaction between temperature and air humidity may influence the relationship between temperature and stomatal opening responsible for CO₂ and water management [37].

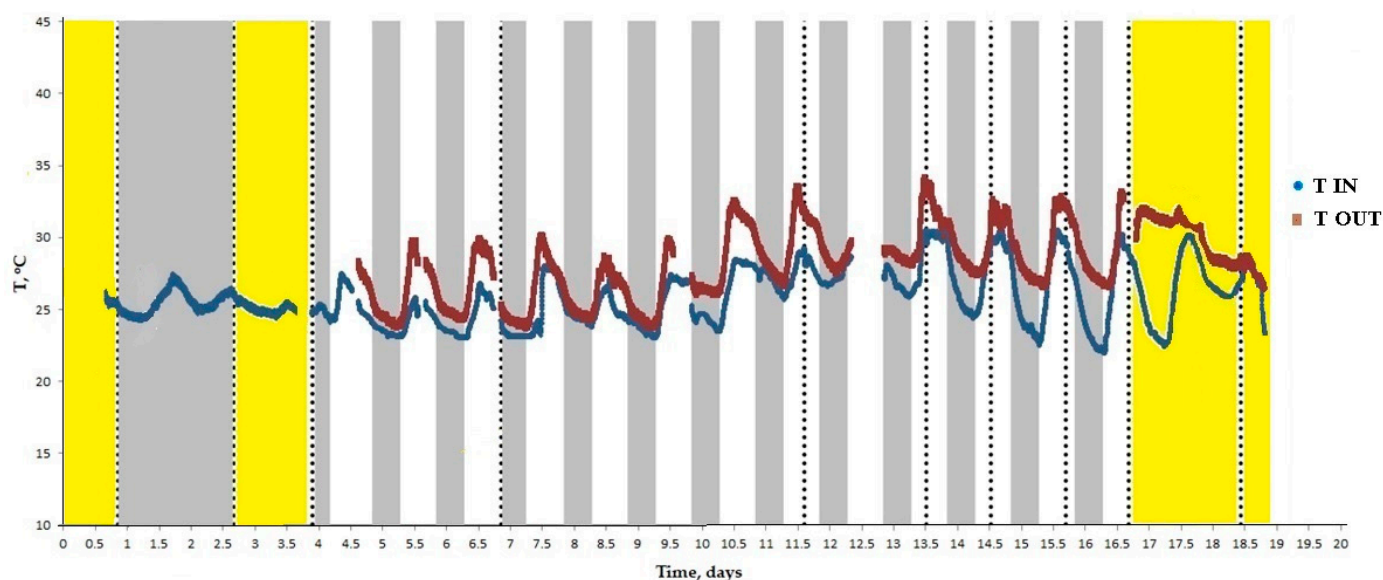


Figure 4. Variation of the temperature over the course of the test period.

No phytotoxic effects (e.g., chlorosis, necrosis, wilting, leaf and stem deformations) have been visually observed in the frame of the mentioned experimental conditions.

3.3. Challenges and Perspectives

Green infrastructure (and other related nature-based strategies) involves plant-based architectures with a role in botanical air biofiltration, climate control, wellbeing, and health, being an important contributor to urban sustainability [38–42]. Overall, carbon-neutrality is considered as the long-term goal of this urban sustainability concept [43]. Urban air quality, which plays a key role in urban sustainability, is influenced by industry, transportation, and other specific local sources [42,44]. Moreover, the closed or semi-closed spaces (associated with the indoor environment) are more prone to pollutant accumulation than the open spaces (the outdoor environment) [44]. Particularly, botanical biofilters represent a sustainable option for the reduction of CO₂ in indoor ambience compared to classical ventilation systems. Although they are important for particulate matter removal, most of these systems are based on mechanical air filtration and are unable to remove the gaseous pollutants (pollutant concentration is reduced by dilution with outdoor air) while they are consuming a significant quantity of energy [45].

Using plants in order to remove indoor air pollution was demonstrated to be potentially more cost effective and energy efficient [45,46]. Different plant species presents

different efficiencies of CO₂ removal via photosynthesis, mainly depending on their requirements for light but also on other environmental factors [24,46,47]. One of the most important controlling factors of photosynthesis is the light intensity. Moreover, under low-light intensities the CO₂ can be emitted to the environment by both plants and system microorganisms through respiration. Thus, in indoor environments with alternate day/night conditions, the CAM plants should represent a benefit, as they are adapted to capture CO₂ under dark conditions. A study of Treesubstorn and Thiravetyan (2018) [24] suggested the construction of botanical biofilters using CAM and C3 plants in order to regulate the level of CO₂ emission from plants under low light or dark conditions.

It has been shown that in the genus *Tillandsia* some species exhibited obligate C3 photosynthesis (e.g., *T. deppeana*, *T. anceps* Loddiges, *T. complanata* Benth, *T. australis*, etc.), while others were obligate CAM [48–52]. It was highlighted that speciation in the *Bromeliaceae* family was facilitated (among other factors) by the shift from C3 to CAM photosynthetic pathway. Specific research has indicated, in *Tillandsia* sp., C3 as ancestral and CAM as derived, as well as multiple repeated transitions between them. These shifts present shared gene expression patterns explaining the existence of some species capable of performing both photosynthesis types [52]. Some of these epiphyte species require almost no substratum, exhibiting both CAM and C3 photosynthesis, and also the potentially intermediate species are among the best possible plants candidates for CO₂ removal biofilters. Our results are in concordance with those of Goode et al. [49], indicating that some *Tillandsia* species are CAM species able to assimilate CO₂ through the C3 pathway in particular environmental conditions. Also, *Tillandsia xerographica* exhibited a CO₂ exchange pattern under the day/night regime, a behavior being highlighted by Martin and Siedow [18] as well for *Tillandsia usneoides*. Concomitant CAM and C3 photosynthetic pathways were also emphasized for other plant species [53]. These findings corroborated with no significant effect of the air relative humidity on CO₂ uptake, when separately referring to day/light or night/dark period and resilience to water deficit reflect the high potential of this species in air biofiltration.

At the end of the experiment, *Tillandsia xerographica* kept its initial trend in CO₂ removal and physical appearance, which demonstrates its suitability to be involved in air biofiltration systems. Along with other benefits (residue-free (no solid substrate is required), robustness, versatility in adapting to environmental stress, easy maintenance, minimum nutrients/water, aesthetical role), this plant can assure a better process control and less biowaste generation. Integration of this plant within air biofiltration systems for urban air biofiltration (e.g., the treatment of the contaminated air issued from the underground auto parking, but not limited to) represents an interesting environmental application and different technological configurations could be adapted in this sense. As can be seen from the presented results, the approached biosystem is complex, involving interconnected phenomena and interfering factors. Physiological investigations of the plants involved in air biofiltration can provide valuable information for optimizing the design and operation of air biofiltration systems. They can also help to select the most suitable plant species and cultivars for different environments and pollutant scenarios [29]. Further process modeling and optimization of this botanical biofiltration process is expected to provide useful information for better understanding of the plants response under different factors influence and develop new versatile biofilters for air treatment. Taking into consideration the dynamic character of the investigated biosystem, artificial intelligence-based algorithms are specifically envisaged to be used in this regard (subject to other papers). Moreover, further examination of the plants response under different contaminated environments is expected to provide new insights into the plants capability in the frame of environmental applications. Furthermore, an investigation of the plants structural and morphological characteristics could contribute to a better understanding of the mechanisms involved in these processes [5,22,35]. Last but not least, as also highlighted by Winter and Holtum [34], further investigations to highlight the enzymatic mechanisms for this facultative CAM aerial plant, correlated with expression of genes that play a crucial role in stress tolerance and

adaptation, as transcription factors, protein kinases, phosphatases and microRNAs [54–57], would bring new contributions regarding the physiological mechanisms of species from the *Bromeliaceae* Family.

4. Conclusions

Addressing plant physiology-related challenges play an important role in botanical biofilter development for air treatment. In this study, a versatile plant (*Tillandsia xerographica*) with high potential in air biofiltration is undertaken for an investigation in this regard. The obtained results show that *T. xerographica* belongs to the facultative CAM plants, being able to perform both nocturnal and diurnal CO₂ capture. Moreover, the those plants versatility to adapt and switch between different metabolisms is revealed, which allows the biosystem to be operated in both “CAM mode” (day/night cycle) and “C3 mode” (continuous illumination). Primary aspects related to the plant’s behavior in respect to these metabolic pathways, pointing out the plant changes in response to different environmental conditions, are depicted within this study. Process investigation through artificial intelligence algorithms is further envisaged for more in-depth exploring of interconnected phenomena and interfering factors involved in plant performance. Further examination of plant structural and morphological characteristics in response to different environmental factors, along with its biochemical and genetic investigation, would contribute to better understanding of plant mechanisms involved in air biofiltration processes.

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