Influence of operational conditions on the efficiency of Wolffia brasiliensis for polishing domestic wastewater of facultative stabilization ponds

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Abstract

Stabilization ponds are widely used for wastewater treatment in developing countries but have limitations in removing certain pollutants, necessitating polishing processes to enhance effluent quality and mitigate environmental impacts. Aquatic macrophytes, particularly *Lemnaceae*, offer a low-cost, efficient alternative due to their rapid reproduction and high nutrient absorption. This study evaluated the effects of operational conditions on *Wolffia brasiliensis* development and its efficiency in polishing wastewater from facultative stabilization ponds. The parameters assessed were chemical oxygen demand (COD), biochemical oxygen demand ($BOD_{5,20}$), total nitrogen (TN), and total phosphorus (TP). Three cultivation conditions were tested: untreated effluent, recirculated effluent (6×10^{-3} L s^{-1} flow rate), and 10% diluted effluent. Experiments were conducted indoors (21,818 lux, 24°C ± 1.3°C) and outdoors (natural conditions, 31°C ± 3.5°C). Biofilm formation hindered macrophyte growth and contaminant removal in outdoor units. Indoor conditions yielded higher biomass (85.87 ± 11.9 g m^{-2} d^{-1}) than outdoor ones (31.35 ± 9.3 g m^{-2} d^{-1}). Recirculated effluent led to the highest growth rates (42.06 ± 33.1 g m^{-2} d^{-1} indoor, 15.70 ± 11.7 g m^{-2} d^{-1} outdoor). All *W. brasiliensis* units significantly improved pollutant removal compared to controls. These findings highlight *W. brasiliensis* as an effective, sustainable solution for polishing effluents from facultative stabilization ponds, particularly for $BOD_{5,20}$, COD, TN, and TP removal, reinforcing its potential for wastewater management in developing countries.

1. Introduction

Stabilization ponds are among the simplest methods for wastewater treatment because they are characterized by low costs of implementation, maintenance, and operation (Arthur 1983; Von Sperling 2002). Such systems are particularly well-suited for applications in developing countries from tropical and subtropical regions, where land areas are generally available at relatively low prices and climatic conditions such as high temperatures and year-round solar radiation favor the biodegradation process (Arthur 1983; Mahapatra et al. 2022). According to the Brazilian Water Agency (ANA), in 2020, stabilization ponds accounted for 32% of the 3,600 sewage treatment plants (STPs) under operation in Brazil, serving as the primary treatment technology (ANA 2020). However, while such ponds promote a satisfactory removal of organic matter, their efficiency for nutrient removal, mainly regarding nitrogen and phosphorus, is limited (Fujioka et al. 2020), resulting in the eutrophication of receiving water bodies and overall water quality decrease (Miwa et al. 2007). Hence, effluent polishing technologies that offer both high efficiency and low installation costs are often required and have seen increasing adoption. Aquatic macrophytes have emerged as a prominent strategy for nutrient and organic matter abatement from wastewater, being recognized as a versatile and scalable intervention that can be adapted to diverse regional and operational contexts (Herzog and Rozado 2010; Uysal 2013).

Among aquatic macrophyte species, floating plants of the Lemnaceae family are particularly notable for their simple structural organization and predominance of photosynthetic tissues (Wolverton and McDonald 1980; Ziegler et al. 2016; Chakrabarti et al. 2018). They are tolerant to environmental pollutants, including metals (Cheng and Stomp 2009; Ziegler et al. 2016), and show potential for removing suspended solids and biochemical oxygen demand (Mohedano et al. 2014). Due to such advantageous traits, Lemnaceae species have been increasingly employed in wastewater treatments and post-treatment processes (Ziegler et al. 2017; Toyama et al. 2018). Macrophytes of the genus *Wolffia* - recognized as the smallest macrophytes in Lemnaceae - are distinguished by their rapid reproduction and growth rates (Suppadit et al. 2008). Kumar et al. (2022) evaluated the nutrient removal capacity of *Wolffia globosa* under controlled laboratory conditions and reported an above 99% efficiency for removing total inorganic nitrogen and orthophosphate.

Soda et al. (2013) evaluated the nutrient removal capacity of *Wolffia arrhiza* under controlled laboratory mesocosms supplied with synthetic wastewater and the findings revealed nitrogen and phosphorus removal rates of 0.03–0.18 mg N m^{-2} d^{-1} and 0.023–0.079 mg P m^{-2} d^{-1}, respectively. Similarly, Zhang et al. (2011) investigated the potential of *W. arrhiza* for polishing effluents from stabilization ponds, reporting removal efficiencies of 84% for total phosphorus (TP), 46% for total nitrogen (TN), 76% for biochemical oxygen demand (BOD_5,20), and 69% for chemical oxygen demand (COD). Numerous studies have underscored the effectiveness of Lemnaceae species in wastewater treatment, particularly in nutrient removal. The most extensively studied genera include *Lemna* (Valderrama et al. 2002; Uysal 2013; Chen et al. 2018; Toyama et al. 2018), *Landoltia* (Garcia 2015; Chen et al. 2018; Toyama et al. 2018; Zanetoni Filho 2019; Cerqueira 2021), and *Spirodela* (Cheng and Stomp 2009; Chen et al. 2018; Toyama et al. 2018). In contrast, research on the genus *Wolffia* remains relatively scarce (Landolt and Kandeler 1987; Kotowska et al. 2018). To date, most investigations on *Wolffia* have been conducted in continental (Kotowska et al. 2018) and temperate climates (Zhang et al. 2011; Soda et al. 2013; Kumar et al. 2022), with limited data available on its performance under tropical climatic conditions.

This study evaluated the polishing process of previously treated wastewater from a Wastewater Treatment Plant (WWTP) located in Ilha Solteira (São Paulo State, Brazil) using *W. brasiliensis* macrophyte. Three distinct operational conditions, namely, undiluted effluent without recirculation (CW), effluent diluted to 10% without recirculation (ED), and undiluted effluent with recirculation (CR) facilitated by a submersible pump were assessed. The study focused on the influence of operational conditions on the relative growth rate of the macrophyte and the removal efficiency of key contaminants, including chemical oxygen demand (COD), biochemical oxygen demand ($BOD_{5,20}$), total nitrogen (TN), and total phosphorus (TP). The findings offer valuable insights for the design and construction of wetlands, supporting the development of more sustainable solutions in environmental management.

2. Materials and Methods

The study was conducted at the Sanitation Laboratory of the Civil Engineering Department (20°25'42.4" S 51°20'29.9" W), at São Paulo State University “Júlio de Mesquita Filho” (UNESP), Ilha Solteira campus, São Paulo, Brazil.

2.1. Characteristics of the studied wastewater

The effluent utilized was collected from the outlet of a primary facultative pond at the Ilha Solteira Wastewater Treatment Plant (WWTP), which includes a preliminary treatment system comprising a screening unit and a sand trap, followed by two primary facultative ponds of 458 m length, 105 m width, and 1.5 m depth (Costa 2015). The initial concentrations of chemical oxygen demand (COD), biochemical oxygen demand ($BOD_{5,20}$), total nitrogen (TN), total phosphorus (TP), turbidity, total solids (TS), total suspended solids (TSS), and dissolved solids (DS) in the effluent were analyzed according to APHA (2017) methods (Table 1).

Table 1
Initial characterization of the studied effluent used in the experimental polishing units

Parameters	Mean ± SD
COD (mg L^{-1})	371.00 ± 1.53
$BOD_{5,20}$ (mg L^{-1})	73.94 ± 0.93
TN (mg L^{-1} N)	7.05 ± 0.05
TP (mg L^{-1} P)	3.26 ± 0.17
Turbidity (NTU)	220.00 ± 1.73
TS (mg L^{-1})	680.00 ± 15.58
TSS (mg L^{-1})	170.00 ± 11.00
DS (mg L^{-1})	510.00 ± 5.00
Note. SD: Standard deviation obtained by three analytical replications.	

COD: Chemical Oxygen Demand; $BOD_{5,20}$: Biochemical Oxygen Demand; TN: Total Nitrogen; TP: Total Phosphorus; TS: Total Solids; TSS: Total Suspended Solids; DS: Dissolved Solids.

2.2. Plant material, microscopic analysis and species identification

The macrophyte was collected from the Wastewater Treatment Plant (WWTP) at the Provisional Detention Center of São José do Rio Preto (20°41'17" S 49°20'17" W), São Paulo, Brazil. The WWTP comprises a primary anaerobic pond, a secondary facultative pond, and two maturation ponds in series. *Wolffia* was found to develop spontaneously at all stages of the treatment process; however, the specimens used in this study were collected from the secondary facultative pond. Following collection, the macrophyte was cultivated and maintained in a 200 L polyethylene tank in an outdoor area adjacent to the laboratory. The tank received natural light and was supplied with the domestic wastewater used in the experimental trials.

An analysis under a binocular optical microscope (50x magnification) confirmed the macrophyte species selected, whose morphological characteristics, including presence of blackened cells and occurrence of papillae on the upper surface of the plant, were observed. According to Landolt (1994), *W. brasiliensis* is distinguished from other species by the presence of darkened cells and fronds of 0.3 to 1.4 mm length. Such features were identified in the specimens used in this study (Fig. 1).

2.3. Experimental design

The assessment of the growth potential of the macrophyte under varying conditions involved analyses of three distinct cultivation scenarios, namely, undiluted effluent without recirculation (CW), effluent diluted to 10% without recirculation (ED), and undiluted effluent with recirculation facilitated by a 2.5 W submersible pump operating at 6×10^{-3} L s^{-1} continuous flow rate (CR). Additionally, control units containing only effluent (C) were included for evaluations of the self-purification capacity of the effluent and the natural decay of the studied parameters (Fig. 2). The experiment was conducted in two distinct environments. In an indoor one, 12 experimental polyethylene units containing 3 L of effluent each were arranged randomly within a wooden box (Fig. 2). The setup was illuminated by two tubular LED lamps (3000 K, 18 W, and 1.20 m length) connected to an analog photoperiod timer set to a 12 h light: 12 h dark cycle.

The second environment was an outdoor area adjacent to the laboratory, where 12 experimental units were randomly arranged within a greenhouse of 3 m width, 3 m length, and 2.78 m height. The initial density of fresh *Wolffia* biomass in all experimental units was standardized at 200 g m^{-2}. The volume of evaporated effluent in each unit was replenished weekly towards maintaining the experimental conditions. The evaporated volume was systematically monitored and recorded, with corrections applied for accurate calculations of the removal efficiencies of the evaluated parameters.

2.4. Growth rate

Wolffia growth was quantified in each experimental unit with the use of Relative Growth Rate (RGR) (g m^{-2} d^{-1}) based on dry matter, as described by Verma and Suthar (2014), and calculated by Eq. 1.

$$RGR = \frac{DM}{N.\,A} \qquad [1]$$

where DM = dry matter during the growing season (g), N = number of growing days, and A = surface area of the growing section (m^2).

2.5. Monitoring parameters and statistical analysis

The water quality parameters used for evaluations of the efficiency of the effluent polishing process, namely biochemical oxygen demand ($BOD_{5,20}$), total phosphorus (TP), total nitrogen (TN), and chemical oxygen demand (COD), were analyzed weekly over a 35-day period, according to the Standard Methods for the Examination of Water and Wastewater (APHA 2017). The ambient temperature was monitored daily by two digital thermohygrometers that recorded maximum and minimum values, while pH was measured weekly by a portable digital pHmeter from Hanna Instruments, following the guidelines provided by APHA (2017). The determination of the reduction efficiencies of $BOD_{5,20}$, COD, TP, and TN was based on the quantification limits specified by APHA (2017). A data analysis was conducted using a completely randomized design, with factor A representing type of treatment, factor B representing the environment (internal or external), and time as a subdivided plot. A statistical analysis was performed with the use of Sisvar software, applying analysis of variance (ANOVA) and Scott-Knott mean clustering test, both with a 5% significance level. The approach enables assessing the significance of the environment (internal versus external) and the growing conditions within each environment (diluted effluent, undiluted effluent, and effluent with recirculation). All the graphs used in this work were generated using Origin® 2023 software.

3. Results and discussion

3.1 Monitoring parameters

The external temperature was significantly higher ($p < 0.05$) compared to the internal one, with averages of 24°C indoor and 31°C outdoor (Fig. 3). A greater standard deviation was observed in external temperature, which can be attributed to the natural variability of outdoor environments, The optimal temperature range for *Wolffia* cultivation is between 17°C-30°C and 17.5°C-34°C according to Skillicorn et al. (1993) and Hasan and Chakrabarti (2009), respectively. The indoor environment showed higher growth rates for *W. brasiliensis* compared to the outdoor one, which frequently exceeded the ideal temperature range outlined by Skillicorn et al. (1993) and Hasan and Chakrabarti (2009). Kumar et al. (2022) and Chakrabarti et al. (2018) successfully cultivated *W. globosa* under controlled laboratory conditions at 31°C using synthetic effluent and Lemna minor under outdoor conditions at 25–28°C, respectively, both similar to the conditions observed in this study.

Overall, regarding the pH parameter, no significant differences were detected among operation conditions within the same environment on the 7th, 21st, and 28th days (Fig. 4). However, experimental units in outdoor environments showed significantly ($p < 0.05$) higher pH values (9.81 ± 1.3) compared to indoor ones (7.83 ± 0.4) from the 7th day of the experiment (Fig. 4), highlighting the influence of environmental factors such as temperature and light on pH levels. Skillicorn et al. (1993) defined the optimal pH range for *Wolffia* development as 7.00–8.00, while Hasan and Chakrabarti (2009) reported that *Lemnaceae* species can tolerate pH levels between 5 and 9. Thus, the pH values recorded under indoor laboratory conditions were considered viable and associated with higher macrophyte growth rates. In contrast, lower growth rates coincided with high outdoor temperatures and elevated pH levels. The indoor pH values in this study align with those reported by Kotowska et al. (2018) for *W. arrhiza* (7.60–7.90), which supported satisfactory growth, and with those found by Sirirustananun and Jongput (2021) (7.50–7.79) for the same species. Kumar et al. (2022) observed optimal pH values when cultivating *W. globosa* but reported an increase to 10.3 by the end of the culture, a trend consistent with the present study's findings for outdoor experimental units.

3.2 Relative growth rate

In both environments, CR showed significantly higher biomass and growth rate values ($p < 0.05$) compared to CW and ED (Fig. 5), which can be attributed to the recirculation of nutrients within the system. Conversely, ED showed significantly lower values ($p < 0.05$) relative to CW and CR, due to reduced nutrient concentrations caused by the dilution process. The maximum relative growth rates for all experimental units were recorded on the 7th day of cultivation, i.e., 85.0 g m^{-2} d^{-1} for indoor CW unit, 32.38 g m^{-2} d^{-1} for outdoor CW unit, 74.04 g m^{-2} d^{-1} for indoor ED unit, and 26.19 g m^{-2} d^{-1} for outdoor ED unit, as well as 98.21 g m^{-2} d^{-1} for indoor CR unit and 35.47 g m^{-2} d^{-1} for outdoor CR unit.

Said et al. (2022) analyzed the relative growth rate of *W. globosa* using hydroponic fertilizer water. The growth rates in the outdoor experiments ranged from 97.76 to 176.66 g m^{-2} d^{-1} after five days of cultivation. Similarly, Verma and Suthar (2014) treated urban wastewater using *Lemna gibba* and achieved a maximum relative growth rate of 117.75 g m^{-2} d^{-1}.

The indoor units showed significantly higher ($p < 0.05$) growth rates and biomass values from the 7th day compared to the outdoor ones, which can be attributed to the controlled environmental conditions provided to the macrophytes (e.g., regulated temperature and light). Additionally, a biofilm possibly composed of microalgae and bacteria (Cavinatto and Paganini 2007) dispersed through the air, particularly in warm and dry environments, was developed in the outdoor experimental units. Such a phenomenon was not observed in the indoor units, as illustrated in Fig. 6. The spontaneous formation of biofilms in wastewater is a natural process that occurs when environmental conditions favor the colonization of surfaces by microorganisms, representing a critical mechanism for microbial proliferation (Butler & Boltz, 2014). Factors such as high nutrient concentrations (Rochex and Lebeault 2007; Sehar and Naz 2016; Volk and LeChevallier 1999; Frias et al. 2001), which are often present in effluents, elevated temperature (Adetunji and Odetokun 2012), light intensity (for algae), oxygen availability (Chang et al. 2015), and pH (Agarwal et al. 2011) promote biofilm formation.

3.3 Effluent polishing

Starting from the 7th day, the outdoor units exhibited significantly higher values ($p < 0.05$) for COD and $BOD_{5,20}$ parameters, compared to the indoor units. Moreover, the values of these parameters were significantly higher ($p < 0.05$) in the units used as controls compared to the units containing *W. brasiliensis*, underscoring their capacity to reduce COD and $BOD_{5,20}$ levels (Fig. 7).

Contrarily to the observations for $BOD_{5,20}$ and COD, the external units exhibited significantly lower TN and TP concentrations ($p < 0.05$) compared to the internal ones (Fig. 8). According to Su et al. (2011), biofilms formed by microbial consortia, such as microalgae and bacteria, can achieve a high nutrient reduction efficiency, including TN and TP, reaching up to 93%. Rajasulochana and Preethy (2016) claimed microalgae require nutrients present in wastewater for their growth.

The cultivation units with *Wolffia* showed significantly higher efficiency ($p < 0.05$) for all parameters analyzed and in both environments, compared to the control units, highlighting the feasibility of using macrophyte as polishing (Fig. 9). The highest efficiencies for $BOD_{5,20}$ and COD were achieved in the indoor units, whereas the outdoor units showed the best results for TN and TP. The high nutrient removal efficiency in the outdoor units can be attributed to the development of biofilm, which probably hindered macrophyte growth and led to partial plant mortality. The process resulted in the release of decomposing organic matter into the system, contributing to increased concentrations of $BOD_{5,20}$ and COD in the outdoor units due to intensified organic degradation.

The highest efficiency values for $BOD_{5,20}$ and COD were 91% and 76%, respectively, for the internal CW unit. Zanetoni Filho (2019) applied *L. punctata* for the polishing of domestic effluent and reported maximum removal efficiencies of 66.35% for $BOD_{5,20}$ and 59.08% for COD. Kotowska et al. (2018) compared the potential of *W. arrhiza* and *L. minor* for treating urban wastewater and reported maximum efficiencies of 91% and 81% for $BOD_{5,20}$ removal and 90% and 88% for COD removal, respectively. Garcia (2015) investigated *L. punctata* for polishing effluents from WWTPs and observed maximum efficiencies of 88.12% for COD and 91.14% for $BOD_{5,20}$. Zhang et al. (2011) applied *W. arrhiza* in a wastewater tank and observed maximum removal efficiencies of 76.9% for $BOD_{5,20}$ and 69.1% for COD. Cerqueira (2021) analyzed *L. punctata* in polishing aquaculture recirculation system effluent and achieved a maximum COD removal efficiency of 64.5%.

The highest phosphorus removal efficiency (TP) observed in this study exceeded 99%. Garcia (2015) reported a maximum TP removal efficiency of 66.18% using *L. punctata* in WWTP effluent polishing and Valderrama et al. (2002) treated ethanol production wastewater with an integrated culture of *Wolffia sp.* and *Chlorella vulgaris*, achieving a maximum TP removal of 28%. Zanetoni Filho (2019) observed a maximum TP removal efficiency of 6.85% and Kotowska et al. (2018) recorded maximum TP removal efficiencies of 83% for *W. arrhiza* and 77% for *L. minor* in urban wastewater. Zhang et al. (2011) achieved a maximum TP removal efficiency of 84.3%, whereas Cerqueira (2021) reported 47%. Kumar et al. (2022) analyzed *W. globosa* cultivated in synthetic wastewater with varying nitrogen concentrations, reporting maximum removal efficiencies of 99.57% for TN and 100% for TP.

The highest nitrogen removal efficiency (TN) in the present study exceeded 98%. Xu and Shen (2011) analyzed the cultivation of *Spirodela oligorrhiza* in swine effluent, achieving a maximum TN removal efficiency of 83.7% and Chen et al. (2018) tested *L. punctata* in wetland system wastewater and observed an approximately 90% maximum TN removal efficiency. Zanetoni Filho (2019) achieved a maximum TN removal efficiency of 26.76%, whereas Cerqueira (2021) reported 65% for *L. punctata*. Kotowska et al. (2018) reported maximum TN removal efficiencies of 90% and 78% for *W. arrhiza* and *L. minor*, respectively. On the other hand, Zhang et al. (2011) reported a maximum TN removal efficiency of 46.1% with *W. arrhiza* in a wastewater tank.

4. Conclusions

This study assessed the polishing performance of a tropical macrophyte under different environmental conditions for effluent treatment. *W. brasiliensis* showed high potential for the polishing of effluents from stabilization ponds, particularly under controlled indoor cultivation conditions, with temperature and light regulation favoring macrophyte growth and efficient pollutant removal. The growth rate of *W. brasiliensis* was strongly influenced by cultivation conditions (particularly nutrient availability in the effluent). In units containing 10% diluted effluent, the growth rate was considerably lower compared to units with undiluted or recirculated effluent, which can be attributed to reduced concentrations of essential nutrients, such as nitrogen and phosphorus, due to dilution. Conversely, in units with undiluted and recirculated effluent, higher nutrient concentrations supported a faster and more efficient growth, resulting in a significantly greater biomass production. The effluent recirculation maintained a continuous and balanced nutrient supply, enhancing both macrophyte growth and pollutant removal efficiency. Biofilm formation in external units can affect macrophyte growth, and this phenomenon underscores a need for strategies that control biofilm growth in macrophyte-based treatment systems towards maximizing the benefits of plant use in effluent polishing. The results obtained in this study indicated that the efficiency in reducing the parameters of COD, $BOD_{5,20}$, TN and TP was similar to that reported in studies conducted in temperate climate regions. However, the observed growth rate was slightly lower, possibly due to differences in local environmental conditions, which may influence the metabolism and adaptation of the organisms involved in the process. Future studies should be developed on a larger scale and under real-world conditions, considering seasonal variability and precipitation influences, for deepening the understanding of *W. brasiliensis*' efficiency throughout the year.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contributions

All authors contributed to the study conception and design. Tsunao Matsumoto conceived the ideas for the study; material preparation, data collection and analysis were performed by Ana Beatriz Laluce Vaz. The first draft of the manuscript was written by Ana Beatriz Laluce Vaz, Davi Fernandes Gasparini Cunha and Gleyson Borges Castro and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

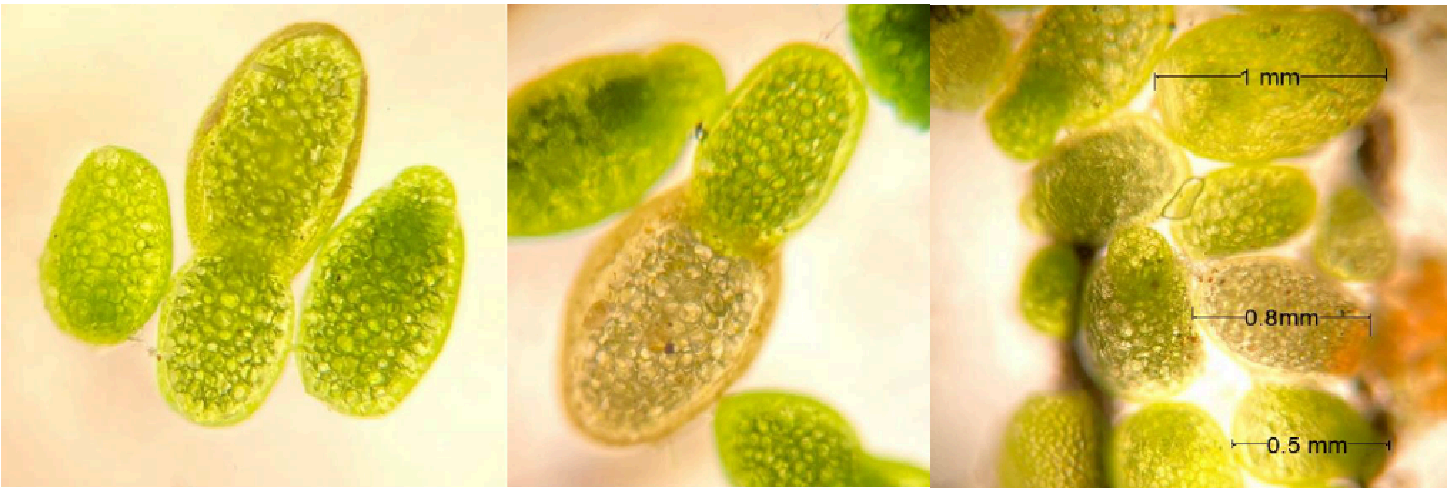


Figure 1

Microscopic view of *Wolffia brasiliensis* at 50x magnification

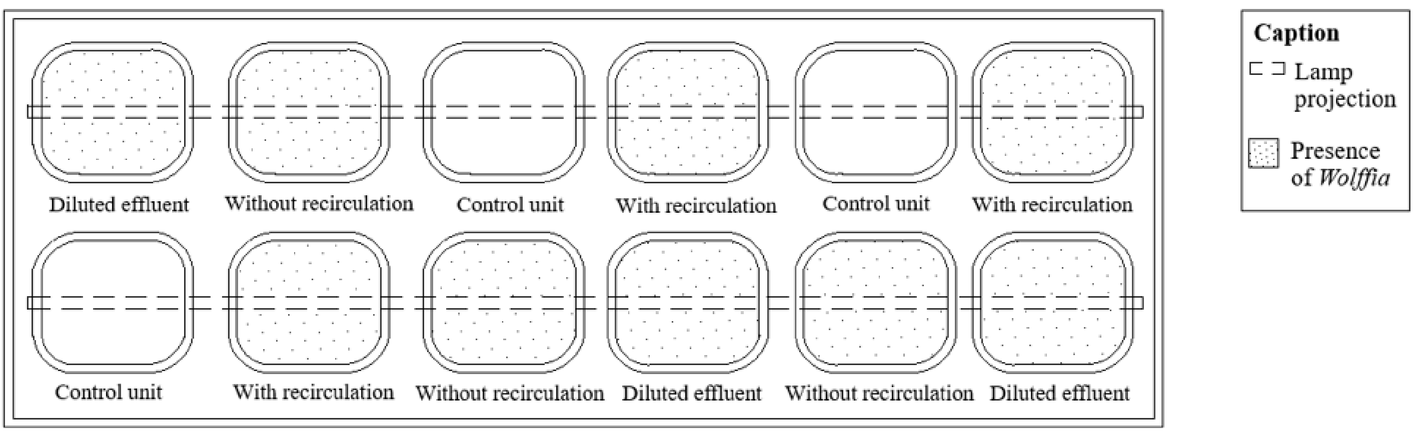
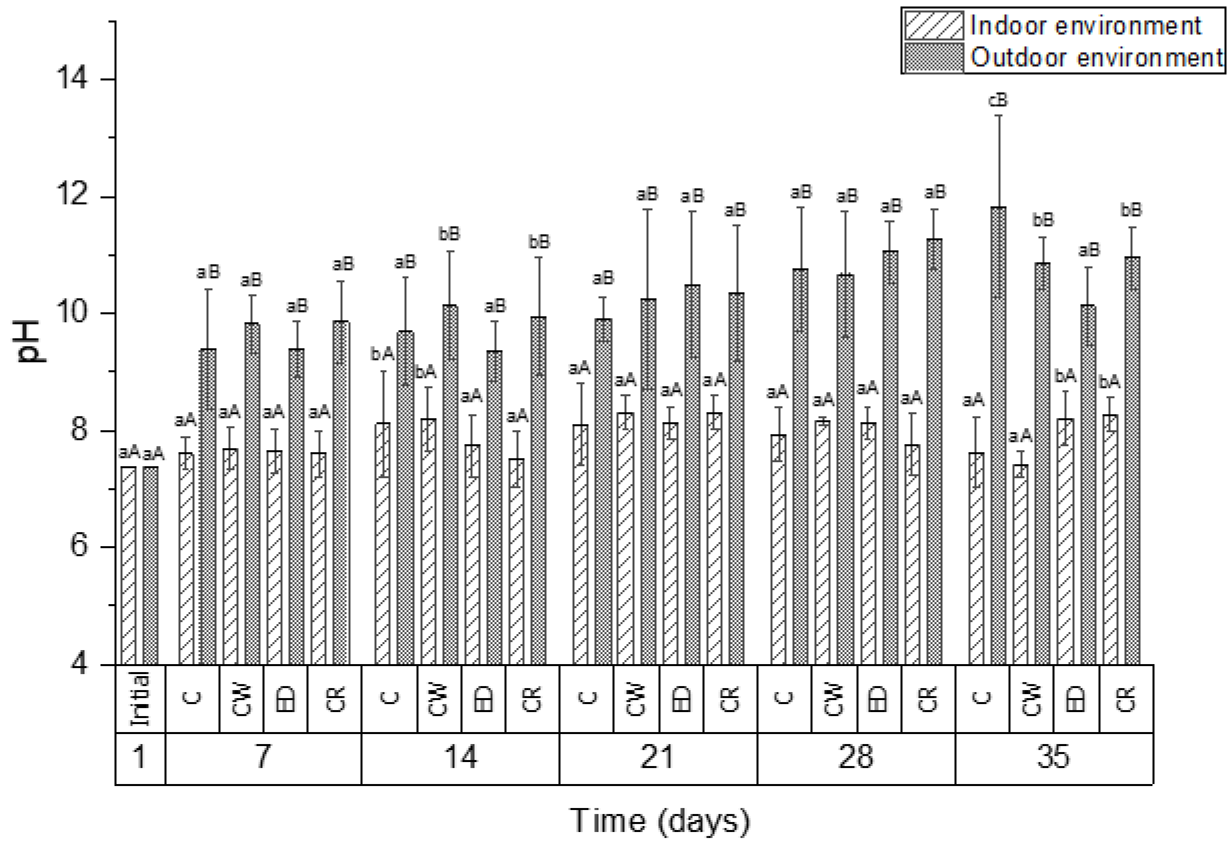


Figure 2

Details of the internal cultivation units arranged in the laboratory

Figure 3

Air temperature values for indoor and outdoor environments



C: control unit; ED: unit with diluted effluent;
CW: unit containing only *Wolffia*; CR: unit with recirculation.

Note: Means followed by a same letter do not differ from each other; lowercase refers to different growing conditions and uppercase refers to environment (Scott-Knott at 5%).

Figure 4

pH values for 35 days of cultivation for indoor and outdoor environments

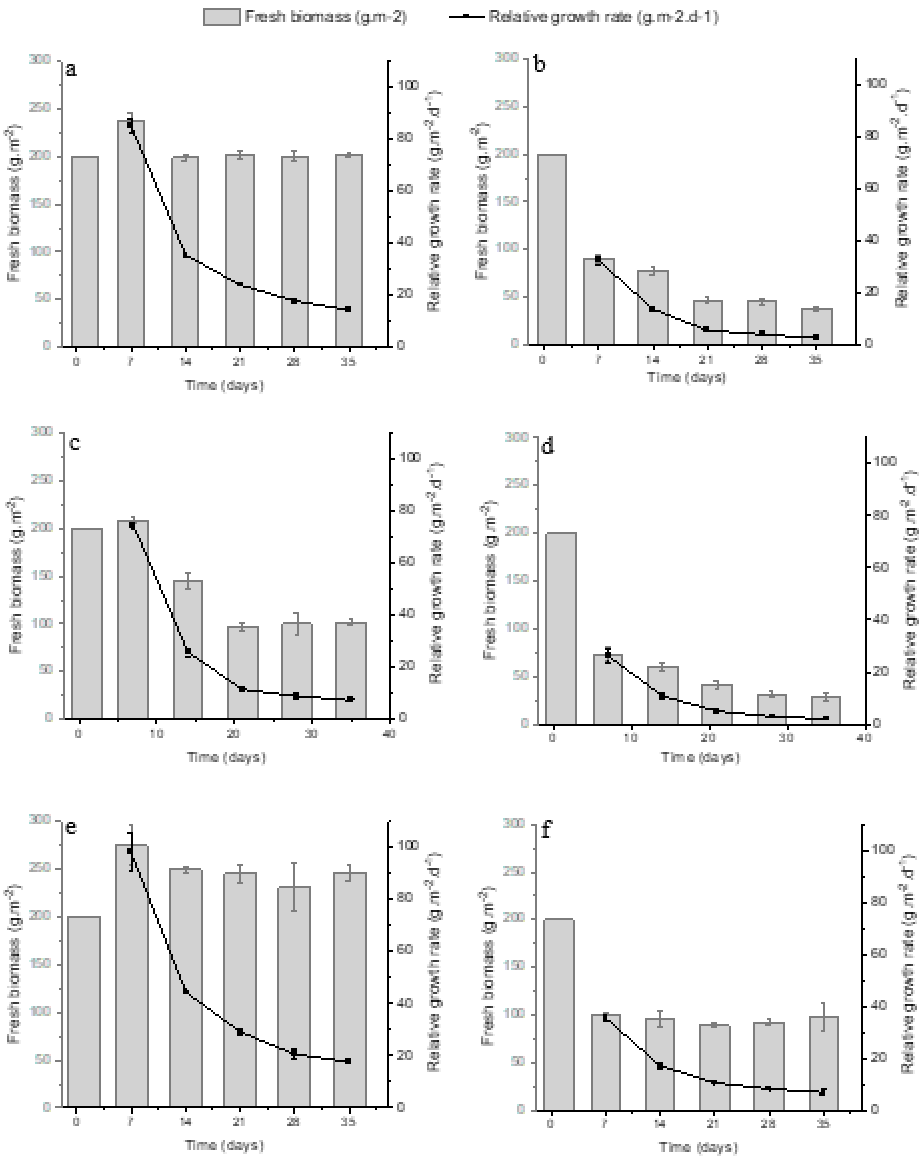


Figure 5

Relative growth rate and biomass of *W. brasiliensis*under different cultivation conditions (a) results for CW unit (containing only *Wolffia*) in the indoor environment; (b) results for CW unit (containing only *Wolffia*) in the outdoor environment; (c) results for ED unit (containing diluted effluent) in the indoor environment; (d) results for ED unit (containing diluted effluent) in the outdoor environment; (e) results for CR unit (containing nutrient recirculation) in the indoor environment; and (f) results for CR unit (containing nutrient recirculation) in the outdoor environment

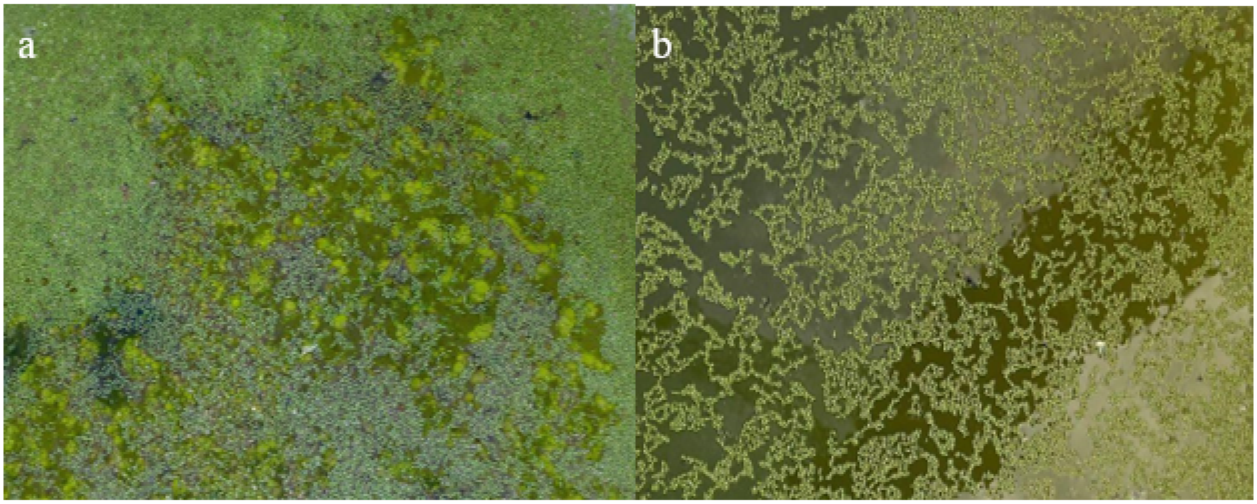
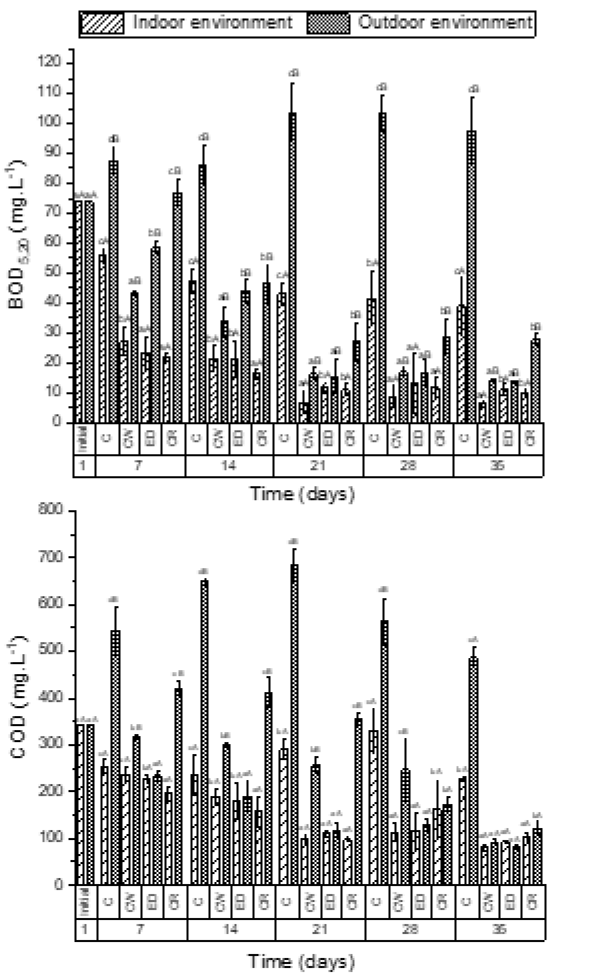


Figure 6

Biofilm formation in (a) outdoor units and *Wolffia* cultivation in (b) indoor units

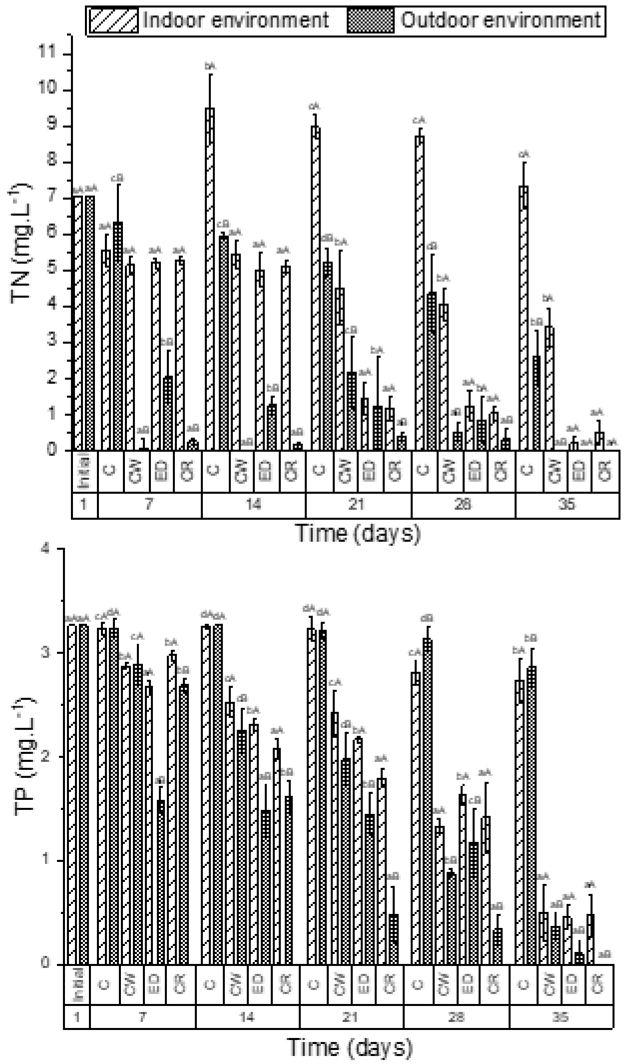


C: control unit; ED: unit with diluted effluent;
CW: unit containing only *Wolffia*; CR: unit with recirculation.

Note: Means followed by a same letter do not differ from each other; lowercase refers to different growing conditions and uppercase refers to environment (Scott-Knott at 5%).

Figure 7

Dynamics of COD and $BOD_{5,20}$ in the experimental units

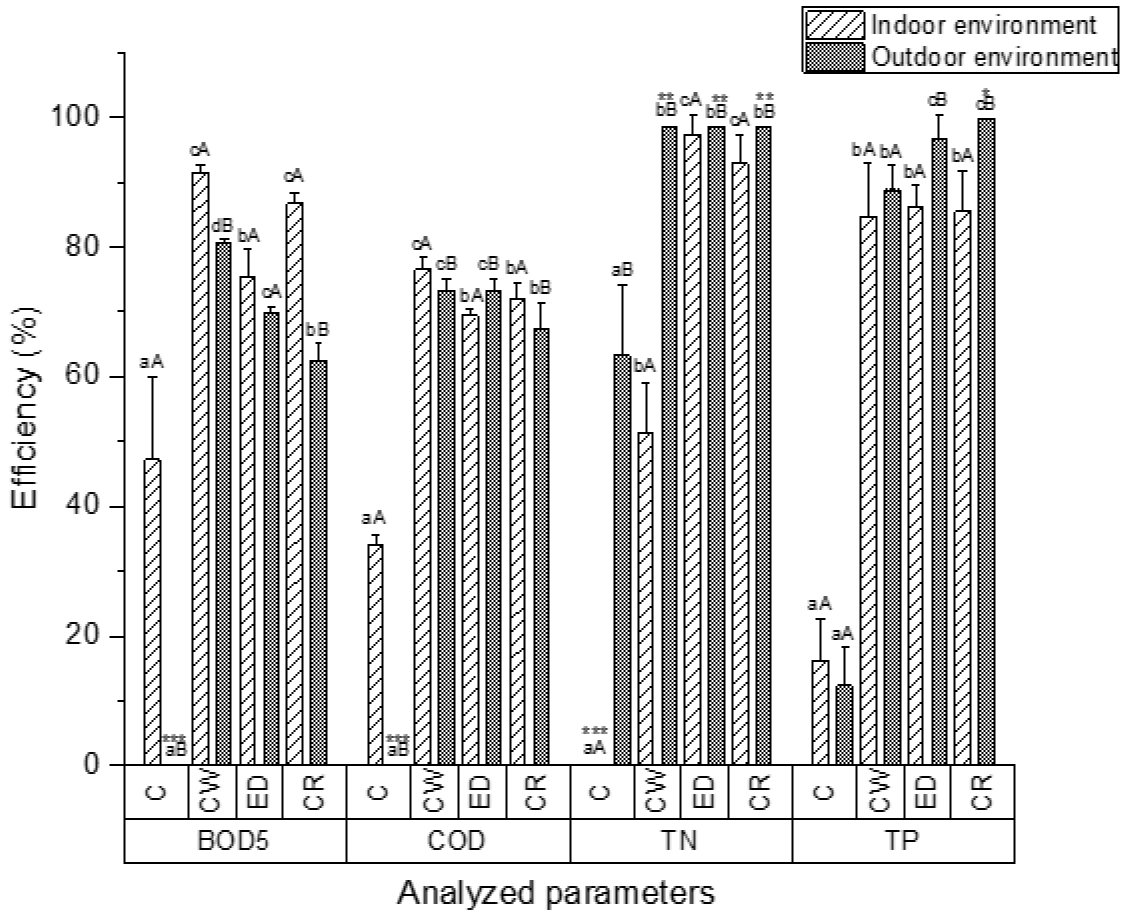


C: control unit; ED: unit with diluted effluent;
CW: unit containing only *Wolffia*; CR: unit with recirculation.

Note: Means followed by a same letter do not differ from each other; lowercase refers to different growing conditions and uppercase refers to environment (Scott-Knott at 5%).

Figure 8

Dynamics of Total Nitrogen and Total Phosphorus in the experimental units



C: control unit;	ED: unit with diluted effluent;
CW: unit containing only *Wolffia*;	CR: unit with recirculation.

Note: Means followed by a same letter do not differ from each other; lowercase refers to different growing conditions and uppercase refers to environment (Scott-Knott at 5%). * Efficiency > 99%; ** Efficiency > 98%; *** Negative reduction efficiency for the parameter, indicating an increase in concentration over the polishing

Figure 9

Efficiency analysis for all experimental units considering $BOD_{5,20}$, COD, TN, and TP