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Evaluation of UASB effluent post-treatment in pilot-scale by microalgae HRP and macrophytes pond for nutrient recovery

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ABSTRACT

The partially treated effluents discharge into water resources impacts water quality and living beings. These effluents contain nutrients such as phosphorus and nitrogen, which can promote the eutrophication of water sources and, consequently, the development of microalgae and macrophytes at levels above the environmental balance. However, the control of these photosynthetic beings can reduce and even prevent the eutrophication. The present work aims to evaluate the removal and recovery of nutrients from effluent treatment through high rate microalgae ponds (AP) and treatment ponds with the presence of macrophytes (MP) in two steps. In step I, the ponds were operated individually (parallel period), in step II they were operated in sequence, where the effluent from the AP to directed to the MP (series period). On the two steps, the performance evaluation regarding the climatic conditions to divide into two periods: warm (summer and spring) and cold (autumn and winter) and the HDT of 2.2, 3.3, and 4.1 days in each pond to evaluate the removal and recovery of nutrients from Algae Pond (AP) and Macrophyte Pond (MP), cultivation ponds fed with sanitary effluent pretreated by an Upflow Anaerobic Sludge Blanket reactor (UASB). The main results for nitrogen and phosphorus removal and recovery were for 4.1 days Hydraulic Detention Time in the warm period for the series system, reaching values below 1.0 mg/L for phosphorus and close to zero for ammonia nitrogen; non-detection by analysis method. For nutrient recovery, the maximum values found were 0.12 $g/m^2 \cdot day$ for phosphorus and 0.79 $g/m^2 \cdot day$ for nitrogen.

1. Introduction

The partially treated effluents discharge into water resources impacts water quality and living beings. These effluents contain nutrients such as phosphorus and nitrogen, which can promote the eutrophication of water sources and, consequently, the development of microalgae and macrophytes floating at levels above the environmental balance. However, the control of these photosynthetic beings in nutrient removal and recovery systems can reduce and even prevent the eutrophication of water bodies (Barroso Júnior, 2020).

The effluent treatment ponds used to purify sanitary and industrial sewage have several variants coming from the stabilization ponds. Microalgae cultivation ponds (High Rate Ponds – HRP) and ponds with macrophytes. HRP are open channels, with a water depth of around 0.2 m–1.0 m, having a mixing system, usually mechanized (Park et al., 2011; Barroso Júnior et al., 2021), which can be substituted for the facultative and maturation ponds advantageously (Benemann et al., 1977). In macrophytes ponds, it is possible to remove and recover nutrients from the liquid phase and fix atmospheric CO_2 for growth via photosynthesis. The development of microalgae and macrophytes is influenced by solar radiation, availability of nutrients, temperature, pH, among others. (Nasr et al., 2009; Mohedano et al., 2014).

The HRP option promises to reduce the costs of cultivating microalgae and the energy required for the treatment of effluents, allowing the remove and recovery of nutrients operating with HDT values between 3 and 6 days (Craggs et al., 2014; Barroso Júnior et al., 2021), which can last up to 21 days (Militão et al., 2019). In treatment ponds with microalgae, phosphorus and nitrogen removal/recovery is possible.

Abbreviations: AP, Algae Pond; MP, Macrophyte Pond; UASB, Upflow Anaerobic Sludge Blanket.

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When there is no removal of microalgae from the liquid, low removal of phosphorus is reported, reaching values between 25 and 55% of removal, and total nitrogen between 40 and 90% (Tsolcha et al., 2017; Benítez et al., 2018; Barroso Júnior et al., 2021).

Ponds with macrophyte cultivation are a variation of stabilization ponds with a layer of floating plants to assist the treatment process. Floating macrophytes, such as the genus Lemna, or duckweed as they are popularly known, belong to the subfamily Lemnoideae (formerly Lemnaceae family) with the highest growth rate among higher plants (Mohedano, 2010).

Most studies regarding macrophytes ponds present the generation of energy biomass and nutrient removal and recovery as a principal vantage. Nitrogen (N) and phosphorus (P) removal present values around 15.0–84.0 and 24.0–72.5%, respectively, when using *Lemna gibba* (Greenaway and Woolley, 2001), *Spirodela intermedia* (Basílico et al., 2017), *Spirodela polyrrhiza* (Cheng and Stomp, 2009; Li et al., 2016; Singh et al., 2016) and *Wolffia* sp. (Valderrama et al., 2002).

The removal of nutrients in floating macrophyte cultivation ponds for sanitary effluents to also influence by solar radiation, pH, temperature, HDT value of the operation of the pond, and others. The HDT values may vary for each experiment, using values between 2.2 and 30.0 days, obtaining lower values of nutrients in the effluent for higher values of HDT (El-Shafai et al., 2007; Suppadit, 2011; Teles, 2016; Barroso Júnior, 2020).

The use of these ponds in series can present high removal and recovery of nutrients from the interaction between microalgae, floating macrophytes (lemnas), and microorganisms, along with the alternation between aerobic and anaerobic, and anoxic environments. Through the ponds with impeller blades, more significant contact between microorganisms and pollutants is possible, in addition to the ease of removal of lemnas using automated systems, allowing better density control and more excellent recovery of biomass and nutrients as final effluent with low concentrations of pollutants. In addition to the possibility of removing nutrients and producing energy biomass, this type of treatment system can be used in small and medium communities, as its maintenance is simple and low-cost.

Therefore, this work aims to evaluate the removal and recovery of nutrients from effluent treatment through high rate microalgae ponds (AP) and treatment ponds with the presence of macrophytes (MP) in two steps: In step I, the ponds were operated individually (parallel period), in step II they were operated in sequence, where the effluent from the AP to directed to the MP (series period). On the two steps, the performance evaluation regarding the climatic conditions to divide into two periods: warm (summer and spring) and cold (autumn and winter) and the HDT of 2.2, 3.3, and 4.1 days in each pond.

2. Materials and methods

The experiment was conducted at the experimental plant located in a Sewage Treatment Station (STS) in Porto Alegre – Rio Grande do Sul – Brazil – with a humid subtropical climate. According to INMET (2020), the annual precipitation in this area is 1397 mm, well distributed throughout the year with higher peaks between June to September, with high ambient temperatures in summer and spring (25.1 °C ± 5.3) and low in the winter and autumn (17.3 °C ± 5.9). The influence of rains precipitations would not evaluate, because, in the subtropical clime, the precipitations and evapotranspiration are similar, not contributing to the dilution of pollutants, microorganisms, and algae in the treatment system.

For microalgae, the calculation of phosphorus and nitrogen recovery is only possible when removing the microalgae from the liquid effluent can be calculated from the characterization of the removed biomass, and microalgae removal is not the focus of this work, which did not have discoursed. Indeed, microalgae have not been removed from the liquid at the experiment, so measuring the microalgae nutrient recovery is impossible. However, the microalgae pond is part of the treatment system, mainly in the parallel system, so it participates in nutrient recovery, including aiding in nitrification during the aerobic period of the pond.

The present study was to be carried out through a pilot-scale experiment composed of a lung tank, followed by an anaerobic reactor type UASB designed in fiberglass and with a valuable volume of 18.3 m^3 and a height of 4.0 m at the end of the process, two ponds of effluent treatment, one with the presence of algae (AP) and the other with the presence of floating macrophytes, also known as lemnas (MP). The ponds have a length of 40 m, a width of 10 m, a depth of 0.75 m, and slopes with an incline of 45°, operated with a water depth of 0.3 m, resulting in a volume operation of 80 m^3. Impulse paddles have been installed at two ponds to promote flow rates between 20 and 30 m/s at the liquid, preventing algae sedimentation and allowing better mixing between the influent, microorganisms, and algae.

The operation treatment system is divided into two steps, as shown in Fig. 1. In step I, the effluent from the UASB reactor was divided equally into each of the ponds, one operated with microalgae (mixed cultivation) and the other with the presence of floating macrophytes, in a parallel system, where the ponds are independent of each other. In this condition, the system was operated between September 2015 and September 2016. The final effluent from the MP and AP were directed to the final collection box of the treatment process, created allowing the collection of samples and measurement of the outflow discharge.

In step II, a condition operated from January 2017 to July 2018, the change only occurred in effluent disposal from the ponds. They were connected by a 150 mm diameter PVC tube, allowing the AP effluent to direct the MP. The final effluent from the MP to direct to the final collection box of the treatment process. The AP preceding the MP aims to promote the removal/partial conversion of ammonia nitrogen based on high pH values (>9.5) due to the high photosynthetic activity of the algae present. The MP served as the final polishing of the effluent since the layer of floating macrophytes that occupies the pond's entire surface prevents the penetration of sunlight and favors the control and removal of the mass of algae generated in the AP.

Fig. 2a shows the top view of the experimental facilities at the STS before an operation, and Fig. 2b shows both ponds in the entire operation.

The species cultivated in the ponds appeared spontaneously, developing naturally in the study region without inoculation. Several genera to found in the microalgae pond with high variation for the predominant ones: *Nitzschia, Chlorella, Euglena, Desmodesmus, Scenedesmus, Gomphonema, Lepocinclis, Trachelomonas, Coelastrum, Tetrastrum, and Phacus*. In the macrophyte pond, the predominant species were *Lemna minor, Spirodela Intermedia, Spirodela polyrhiza, and Wolffia columbiana.*

Lemnoidaee macrophytes were specified by retired UFRGS professor, limnologist Dr. Albano Schwarzbold and confirmed by the laboratory specialized in plant composition, the Soils Analysis Laboratory located at the Faculty of Agronomy - Department of Soils - Federal University of Rio Grande do Sul - UFRGS, where the percentages of nitrogen, phosphorus, potassium, calcium, sulfur among others did identify. The identification and quantification analysis of microalgae did conduct by the taxonomist M.Sc. Ronaldo dos Santos Padilha with extensive experience in this area.

To treatment system performance evaluations were carried out quantifying physical and chemical analyses of the organic matter and nutrients, carried out once a week between September 2015 and July 2018, shown in Table 1. They were divided into 12 operational periods, differentiated from the combination of a Hydraulic Detention Time (HDT) of 2.2, 3.3 and 4.1 days, in each pond, the climatic condition (warm or cold period), and the disposal of the treatment by the ponds (series and parallel). The warm period presents ambient temperatures of 25.1 °C ± 5.3, and the cold period to 17.3 °C ± 5.9.

In the period for series ponds, it should note that each pond operates with an HDT of 2.2, 3.3, and 4.1 days, totaling 4.4 HDT; 6.6 and 8.2 days in both ponds, operating both ponds always with the same HDT value.

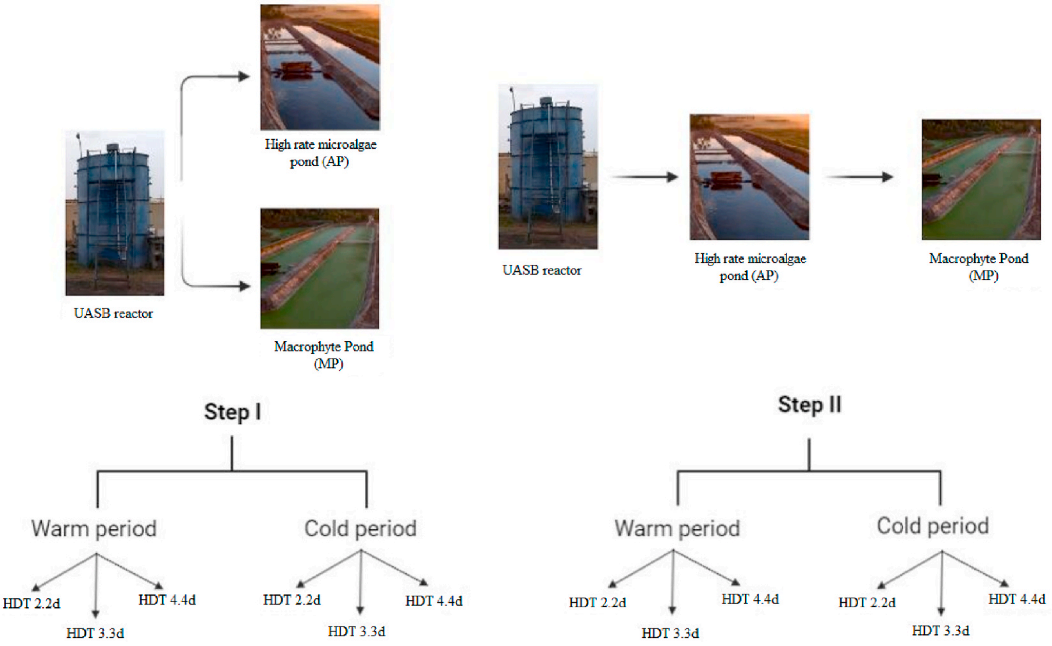

Fig. 1. Pilot plant of effluent treatments representation.

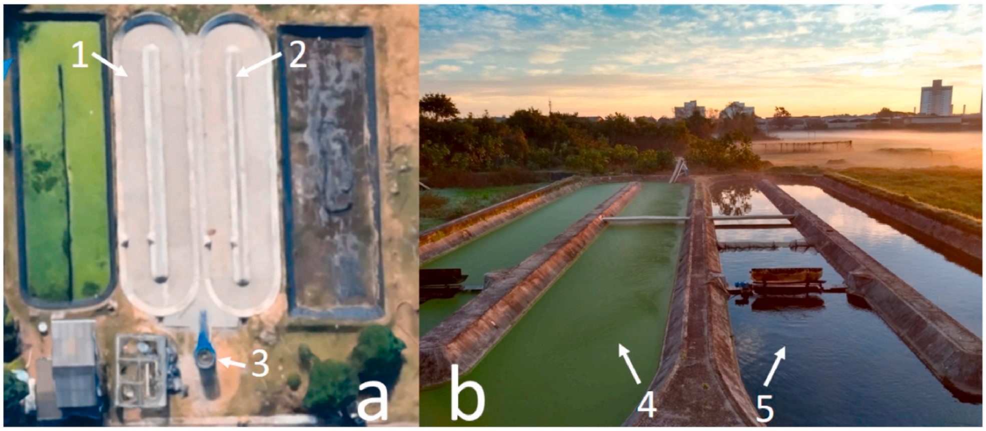

Fig. 2. a) Ponds before the start of the operation (1) Microalgae pond; (2) Macrophyte pond; (3) UASB reactor and b) Ponds after the start of the operation: (4) Microalgae pond and (5) Macrophyte pond.

The operating conditions of the treatment system in series, parallel, warm and cold resulted in a series of abbreviations being P-parallel, S-series, W- warm, and C-cold, generating acronyms such as AP2.2 PW being: AP – algae ponds; 2.2–2.2 days HDT; P – parallel period and W – warm weather condition. The macrophyte pond generates acronyms such as MP4.1SC being: MP – macrophyte pond; 4.1–4.1 days HDT; S – series period and C – cold weather condition.

To characterize the effluents have been 10 (ten) collections carried out for each operational period. As shown in Table 1, solar radiation data are provided by INMET (2020).

2.1. *Unit performance evaluation parameters*

The system treatment performance has been analyzed from the characterization of the influent and effluent of each treatment step, based on physical, chemical, and biological analyses, according to methodologies described in the Standard Methods of Examination of Water and Wastewater (APHA, 2005): DBO5 (5210B); COD (5220 D); volatile suspended solids (SSV) (2540 D and E); Kjeldahl total nitrogen (NTK) (4500 B and C); ammonia nitrogen (4500B); nitrite and nitrate (4110B); total phosphorus (4500 B); phosphate (4110B); thermotolerant coliforms (9223 A); alkalinity (2320 A); turbidity (2130B); total organic carbon (TOC) (5310 C); pH (4500B); dissolved oxygen (OD) (4500 O H). In the effluent of the ponds, Chlorophyll – a was also analyzed, according to Wetzel and Likens (2000).

In addition to the analyses, measurements to carry out three times a week for essential operational control are verified: pH, DO, turbidity, solar radiation, pond temperature, UASB affluent temperature, and air temperature. These measurements always took place between 8:00–9:00 in the morning.

The production of Lemnaceae to evaluate by removing the excess mass of macrophytes depends on the production of macrophytes in the pond (density control). The excess removed was weighed by collecting a homogeneous sample to determine humidity, carried out in triplicate in an oven at controlled temperature.

Table 1

Effluent treatment steps in the pilot pond system according to HDT, climatic condition, and operational condition of the ponds.

HDT for each pond (days)	**N° of samples**	**Rate (m^3/h)**	**Period**	**Climate condition**	**Solar Radiation (W/m^2)**
Step I – Parallel Ponds					
2.2	10	1.52	Sep–Nov/2015	Warm	587,8 ± 146,2
3.3	10	1.01	Nov/2015–Jan/2016	Warm	521,2 ± 132,6
4.1	10	0.81	Jan–Mar/2016	Warm	626,1 ± 166,4
2.2	10	1.52	Aug-Set/2016	Cold	509,5 ± 113,5
3.3	10	1.01	Jun–Jul/2016	Cold	439,4 ± 106,1
4.1	10	0.81	Apr–May/2016	Cold	690,2 ± 155,8
Step II – Series Ponds					
2.2	10	1.52	Jan–Mar/2017	Warm	591,6 ± 133,4
3.3	10	1.01	Mar–May/2017	Warm	566,1 ± 128,5
4.1	10	0.81	Oct/2017–Mar/2018	Warm	632,9 ± 143,2
2.2	10	1.52	Aug–Oct/2017	Cold	441,6 ± 118,6
3.3	10	1.01	May–Jun/2017	Cold	437,4 ± 114,7
4.1	10	0.81	Jul/2017 e Jun–Jul/2018	Cold	654,7 ± 134,4

2.2. *Macrophytes density control*

The density of floating macrophytes is measured at six different randomized points in the pond, performed with the aid of a 0.5 m × 0.5 m sampler composed of a mesh with approximately 0.1 mm holes, which allows detention of macrophytes. Density maintains between 30 and 60 g/m^2 (dry basis), with sample collection in triplicate, carried to an oven at controlled temperature (105 °C) for 24 h.

The density was chosen based on work developed by Gómez et al. (2016), so that the macrophyte blanket fills the surface of the pond, but without their overlapping, which can cause the death of the Lemnaceae, damaging the production of biomass and the liquid effluent at the end of the process. Excess biomass production is to remove by an automated treadmill controlled by a frequency inverter and timer, which adjust according to the production of macrophytes.

2.3. *Nutrient recovery calculation*

The estimation of nutrient recovery was performed from the macrophyte pond mass balance, taking into account the pond effluent and effluent concentrations for the desired nutrient, knowing that the difference between the two would be the maximum recovery of nutrients.

For the calculation of nutrient recovery, the analysis of plant composition of biomass was used, with values of nitrogen, phosphorus, calcium, and other nutrients, as used by Barroso Júnior (2020). Nitrogen and phosphorus recovery to estimate by the percentages of nutrients present in the macrophyte biomass and the amount of biomass removed from the pond. Calculation of the nutrient recovery also considered the difference in densities between the density measured at the end of the current removal of macrophytes and measured at the last removal. The latter value can be positive or negative. Positive when the current density is higher than the density in the last withdrawal and negative when the opposite occurs. The mass of nutrients can be calculated by Equation (1).

$$MN = [c * Bc \pm (da - db)] * A \qquad \text{Eq. 1}$$

where MN: mass of nutrients (g); Bc: biomass removed ($g/m^2 \cdot day$); da: density measured in the pond after macrophyte removal ($g/m^2 \cdot day$); db: density measured in the pond in the last macrophyte harvest ($g/m^2 \cdot day$); A: surface area of the pond (m^2); c: nutrient concentration (g/m^3). The macrophytes nutrient composition has been realized by the Soil Analysis Laboratory located at the Faculty of Agronomy - Department of Soils - Federal University of Rio Grande do Sul – UFRGS, as cited before.

For microalgae, the calculation of phosphorus and nitrogen recovery is only possible when they remove from the liquid effluent, and it can have calculated from the characterization of the removed biomass, and microalgae removal is not the focus of this work, which did not have discoursed. Indeed, we do not remove microalgae from the liquid at the experiment, so we do not measure the microalgae nutrient recovery. However, the microalgae pond is part of the treatment system, mainly in the parallel system, so it participates in nutrient recovery, including aiding in nitrification during the aerobic period of the pond.

2.4. *Statistical analyses*

Statistical analysis of the data was performed using the significance test, according to ANOVA by the Tukey method, with a 95% confidence interval using the R software. Letters are used to assistance classifying the values found. The letter "a" is used for the highest value found, followed by the letter "b" for the second-highest, and so on. Values with the same letter do not present a statistically significant difference, and values with different letters present a significant difference according to the statistical method used.

3. Results and discussion

3.1. *Temperature and solar radiation*

The effluent temperature values from the ponds and the incident solar radiation at the site to presented in Tables 2 and 3:

Table 2 presents the data referring to Step I, for the hot and cold periods. The temperature values for the warm period are higher ($p < 0.05$) when compared to the cold period. It is also possible to assess that solar radiation was higher for the period the ponds operated with 4.1 days of HDT for the hot and cold periods.

In this work, maximum values of 18.9 g/m^2day were observed for the

Table 2

Ponds temperatures in the warm and cold periods during Step I.

Temperature (°C) – Warm Period						
	HDT = 2.2 days		HDT = 3.3 days		HDT = 4.1 days	
	MP	**AP**	**MP**	**AP**	**MP**	**AP**
Maximum	30.6	32.4	31.5	31.9	35.1	35.5
Average	28.1 ± 4.5^a	28.9 ± 4.9 ^{a}	29.2 ± 3.2 ^{a}	29.7 ± 4.1 ^{a}	30.1 ± 4.6 ^{a}	30.5 ± 5.2 ^{a}
Minimum	24.2	24.6	25.2	26.0	26.4	27.0
Solar Radiation (W/m^2)						
Average	587.8 ± 146.2 ^{b}		521.2 ± 132.6 ^{b}		626.1 ± 166.4 ^{a}	
Temperature (°C) – Cold Period						
	HDT = 2.2 days		**HDT = 3.3 days**		**HDT = 4.1 days**	
	MP	**AP**	**MP**	**AP**	**MP**	**AP**
Maximum	20.1	19.7	19.0	21.1	22.8	23.4
Average	16.2 ± 2.3 ^{b}	16.4 ± 1.5 ^{b}	16.0 ± 1.8 ^{b}	16.6 ± 2.1^b	17.6 ± 2.9 ^{b}	19.1 ± 2.8 ^{b}
Minimum	11.6	14.6	13.6	13.5	13.0	13.6
Solar Radiation (W/m^2)						
Average	509.5 ± 113.5 ^{b}		439.4 ± 106.1 ^{c}		690.2 ± 155.8 ^{a}	

Table 3
Ponds temperatures in the warm and cold periods during Step II.

Temperature (°C) – Warm Period	HDT = 2.2 days		HDT = 3.3 days		HDT = 4.1 days	
	MP	AP	MP	AP	MP	AP
Maximum	33.6	33.1	33.3	33.8	35.4	35.6
Average	29.2 ± 5.3^a	29.5 ± 5.2^a	29.6 ± 4.6^a	29.9 ± 4.5^a	30.6 ± 4.8^a	30.8 ± 5.4^a
Minimum	25.7	26.0	26.8	26.9	27.1	27.4
Solar Radiation (W/m^2)						
Average	591.6 ± 133.4 ^{b}		566.1 ± 128.5 ^{b}		632.9 ± 143.2 ^{a}	
Temperature (°C) – Cold Period	**HDT = 2.2 days**		**HDT = 3.3 days**		**HDT = 4.1 days**	
	MP	**AP**	**MP**	**AP**	**MP**	**AP**
Maximum	22.5	22.8	19.9	20.7	23.2	23.6
Average	15.9 ± 2.8^b	16.1 ± 2.5^b	15.7 ± 2.4^b	15.9 ± 2.8^b	17.9 ± 2.9^b	19.5 ± 3.2^b
Minimum	10.8	12.5	11.3	11.6	12.0	12.1
Solar Radiation (W/m^2)						
Average	441.6 ± 118.6 ^{c}		437.4 ± 114.7 ^{c}		654.7 ± 134.4 ^{a}	

hot period and 11.1 g/m^{2}day for the cold period, following the same trend presented by the bibliography. According to Nascimento (2001) and Perreira et al. (2012), the highest production of microalgae is achieved when there are higher temperature values, so there is greater productivity of microalgae biomass for the hot period. Macrophytes present higher values of biomass production for the warm period. However, in addition to temperature, solar radiation and nutrients influence biomass production, as evaluated by Stadtlander et al. (2019) and Strzałek et al. (2019).

Table 3 presents the data referring to Step II, for hot and cold periods. The evaluated temperature values show a significant difference between the hot and cold periods. However, they do not present significant differences for the same climatic condition when the HDT values are modified, and there is also no significant difference between the parallel and series period for the same condition climate.

The solar radiation values were higher for the 4.1 days HDT, with no significant difference between the hot and cold periods and the series and parallel system for this HDT.

The transfer of microorganisms between lakes in step II occurs from the AP to the MP. The UASB effluent is directed to AP, where microalgae production occurs, and a part of these can be transferred to MP. Macrophytes do not transfer to the AP due to macrophytes flotation and the effluent transfer tube being located at the bottom of the ponds.

In MP, shading occurs due to floating macrophytes. Therefore, solar radiation does not reach the microalgae. Consequently, algae mortality results in the clearing of the affluent (Park et al., 2010), and the release of nutrients that the macrophytes and microorganisms located at the water and roots of macrophytes can sorb.

3.2. Nutrients

The nutrient evaluation divides into ammonia, NTK, nitrite, nitrate, phosphoric, phosphate, and total phosphorus.

The raw sanitary sewage is pretreated by the anaerobic reactor (UASB), where biodegradable organic matter converts into gases such as CH_4 and CO_2 and releases nutrients such as phosphate and ammonium ion.

3.2.1. Nitrogen analyze

Ammonia nitrogen (ammonia) concentrations increase in the UASB reactor due to ammonification reactions. According to Bueno (2011), there are breaks in organic chains (proteins, amino acids) releasing ammonium ions into the liquid, converting organic nitrogen into ammoniacal. The same happens with phosphate, which is also released when breaking down organic chains, raising levels in the UASB reactor.

For the parallel period (Step I) warm, the ammonia nitrogen concentrations in AP showed low variation in the HDT tested. When evaluating the concentrations between AP and MP in the warm parallel period, there was a significant difference for ammonia nitrogen concentrations ($p < 0.05$), with a lower concentration for AP for the HDT values of 2.2 and 4.1 days, as shown in Fig. 3.

For the period in warm series, the influence of HDT to reduce ammonia nitrogen concentrations is noticeable. The combined treatment of AP and MP provide values close to zero for the operating period with HDT of 4.1, the lowest values recorded in the study.

According to Bueno (2011) and Tchobanoglous et al. (2015), there are several ways and factors to remove ammonia nitrogen from the liquid effluent. In this work, four factors described by the authors as mentioned above are mainly applied, as shown in Fig. 4. The first (Figs. 4–1) is related to the realization of photosynthesis by microalgae, which raises the pH of the pond. The pH values recorded in AP vary between 6.7 and 10.7, with high values during the day when solar radiation occurs. With the high pH (>9.5), it can occur due to photosynthesis by microalgae which raises the pH of the pond, reaching values above 9.5, and thus, a portion of the ammoniacal nitrogen can be converted into gas and detach from the liquid (Pereira et al., 2012).

The second factor (Figs. 4–2) may be associated with nitrification reactions, which require dissolved oxygen (DO) levels above 1.5 mg/L, temperatures in the mesophilic range, and sufficient time for the development of bacteria nitrifiers (Nitrosomonas and Nitrobacter) (Tchobanoglous et al., 2015). In AP, the DO reaches values above 22 mg/L during the day, and for MP, the DO values reach up to 1.8 mg/L. The evaluation of the alkalinity parameter also supports this hypothesis since the nitrification process consumes the alkalinity of the medium (Winkler and Straka, 2019), reducing its concentration in the pond effluent. The alkalinity reduction values and increased nitrate concentration in AP effluent confirm that hypothesis, with the mean alkalinity values in mg/L in the UASB reactor being 226.8 ± 36.4 and for the AP and MP 72.8 ± 10.5 and 101.8 ± 16.3, respectively for the series period.

In the third factor (Figs. 4–3), nitrite and nitrate resulting from ammonia nitrification can be converted into gaseous nitrogen by denitrification and assimilated by microalgae, transforming into organic nitrogen (Winkler and Straka, 2019). In addition, it is also worth highlighting the portion present in the form of dissolved ions in the final effluent of the ponds.

The last factor (Fig. 4–), nitrite and nitrate, can be sorbed by the lemna and the microorganisms contained in the roots, removing the pollutant from the liquid.

In MP, nitrification is possible because, during the day, the DO levels are above 1.5 mg/L. However, the alkalinity consumption is relatively low for the parallel period, obtaining total alkalinity values, in mg/L, in the UASB reactor of 224.7 ± 31.6; at AP 83.2 ± 15.3; and in the MP of

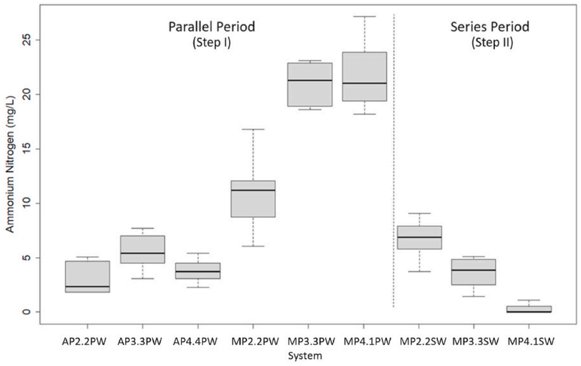

Fig. 3. Ammonia nitrogen concentrations in ponds on warm period according to the type of treatment.

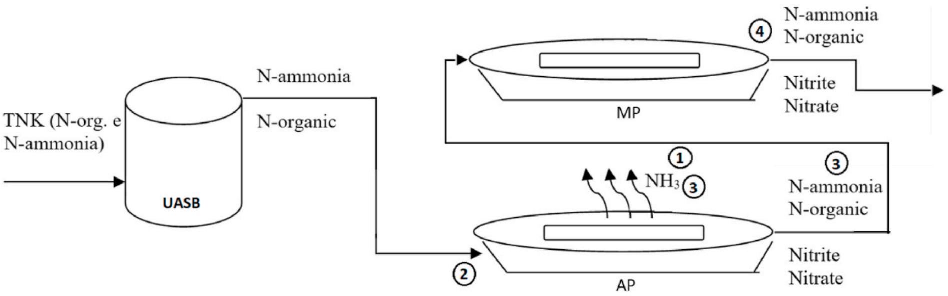

Fig. 4. Illustration of the nitrogen cycle in the treatment system, adapted from Barroso Júnior (2020).

196.2 ± 31.1 for the parallel period, in which the low alkalinity consumption of the MP can to evaluate in comparison with AP.

In the series period, there is an increase in alkalinity between AP and MP, which indicates the occurrence of reduced nitrification, or due to lower values of ammonia nitrogen input and possibly also due to denitrification. This condition was also reported by Racchetti et al. (2017) and Raeisossadati et al. (2019). In Table 4, the nitrate values are to be present.

The highest nitrate values registered in the AP for the warm period in all tested HDT and the parallel cold period with 2.2-day HDT, significant differences for the other values evaluated ($p < 0.05$). These high values may be related to nitrification activity likely to occur in the ponds, especially for the higher HDT (Raeisossadati et al., 2019).

In the cold period, the lowest values of ammonia nitrogen to obtain for the series system with HDT of 3.3 and 4.1 days, with no significant difference between these periods ($p > 0.05$), but these values are statistically lower than the others ($p < 0.05$), shown in Fig. 5. In this period, there is the more effective removal of ammonia nitrogen for the series system when compared to the hot period, showing attractiveness for the system in a series of treatments.

Bouali et al. (2012) reported ammonia nitrogen removal rates of about 70.6% using a pilot study of constructed wetlands for tertiary sewage treatment using duckweed and immobilized microalgae, recorded 14.0 mg/L of ammonia nitrogen.

Table 4
Nitrate values for the parallel period and series in warm and cold operating conditions.

Nitrate concentration (mg/L)				
Sample	DS – Domestic Sewage	UASB	AP	MP
Parallel Period Warm (Step I)				
HDT 2.2 days	0.22 ± 0.12	0.22 ± 0.09	3.69 ± 1.62	0.76 ± 0.44
HDT 3.3 days	0.16 ± 0.11	0.13 ± 0.05	3.80 ± 1.23	0.23 ± 0.15
HDT 4.4 days	0.33 ± 0.04	0.13 ± 0.05	3.29 ± 1.19	0.22 ± 0.07
Parallel Period Cold (Step I)				
HDT 2.2 days	0.53 ± 0.06	0.47 ± 0.04	3.19 ± 0.92	0.73 ± 0.07
HDT 3.3 days	0.51 ± 0.04	0.51 ± 0.06	1.53 ± 0.50	0.58 ± 0.05
HDT 4.4 days	0.51 ± 0.04	0.50 ± 0.06	1.55 ± 0.39	0.51 ± 0.07
Series Period Warm (Step II)				
HDT 2.2 days	0.20 ± 0.06	0.66 ± 0.14	1.09 ± 0.19	1.37 ± 0.21
HDT 3.3 days	0.11 ± 0.07	0.37 ± 0.10	0.76 ± 0.16	0.96 ± 0.11
HDT 4.4 days	0.29 ± 0.09	0.19 ± 0.09	1.29 ± 0.28	0.57 ± 0.10
Series Period Cold (Step II)				
HDT 2.2 days	0.31 ± 0.18	0.23 ± 0.11	0.36 ± 0.10	0.67 ± 0.21
HDT 3.3 days	0.10 ± 0.03	0.55 ± 0.17	1.59 ± 0.27	1.40 ± 0.15
HDT 4.4 days	0.07 ± 0.03	0.12 ± 0.05	1.15 ± 0.39	0.44 ± 0.10

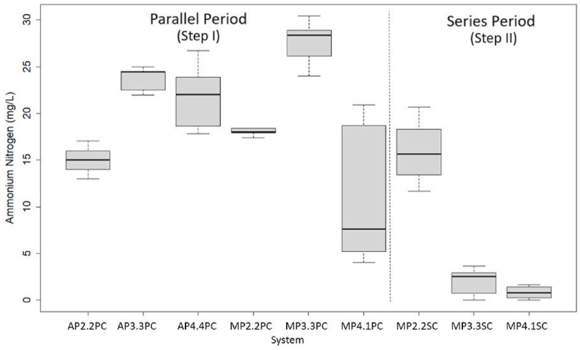

Fig. 5. Ammonia nitrogen concentrations in ponds according to the type of treatment for the cold period.

Denitrification is also possible without solar radiation in ponds, allowing for anoxic reactions. This hypothesis can support the increase in nitrite levels (0.53–0.71 mg/L) and alkalinity (Winkler and Straka, 2019), in addition to the reduction of total nitrogen in the MP. The occurrence of denitrification in AP is also possible since there is a record of an increase in nitrite levels, but as nitrification occurs during the day, further studies of this pathway need more concrete conclusions.

The lowest concentrations of nitrogen variants are presented in the warm period with series ponds and 4.1 days HDT since solar radiation and temperature influence reactions and photosynthetic beings (Huang et al., 2019; Militão et al., 2019; Tsolcha et al., 2018).

The indices of solar radiation and temperatures combined with higher HDT values allow better development of photosynthetic beings and microorganisms attached to plant roots and in water depths, allowing more effective removal of pollutants. These factors provide an environment conducive to removal and longer contact time between pollutants and microorganisms. It is possible to observe the reduction of nitrate concentrations in the series period when analyzing AP and MP, reaffirming that nitrification/denitrification occurs.

3.2.2. *Phosphorus analyze*

Total phosphorus presents a reduction in its concentration throughout the treatment, with more outstanding removals from the ponds (lower concentrations) for the warm period, when higher temperatures catalyze microbial reactions.

The removal of total phosphorus in ponds can occur mainly by 1) assimilation of photosynthetic organisms and microorganisms, 2) binding to other elements, and 3) precipitation, being retained in the ponds (Teles, 2016; Barroso Júnior, 2020). The graph in Fig. 6 shows the phosphorus data in the ponds for the warm period in the parallel and series system.

The values presented by AP during the parallel period do not show

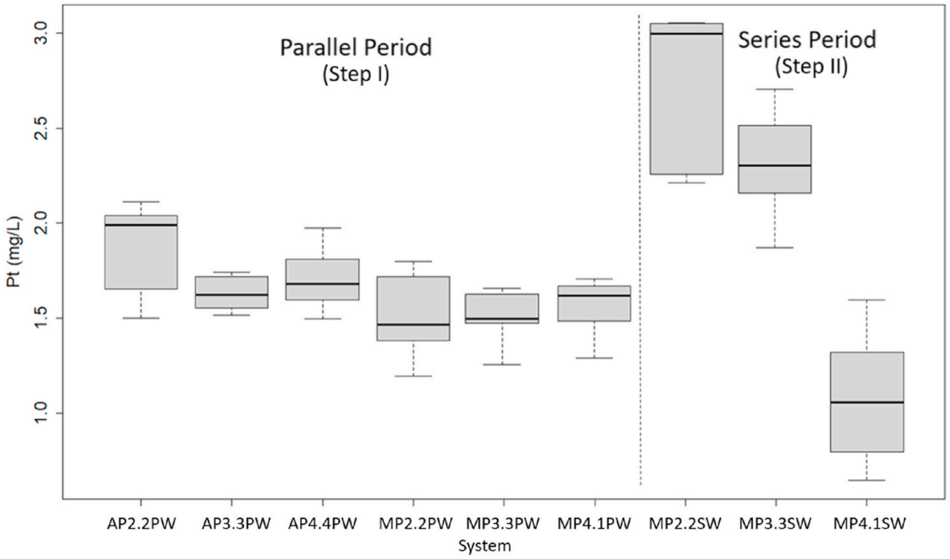

Fig. 6. Phosphorus concentrations in ponds on warm period according to the type of treatment.

high variation between the mean values for the HDT tested. This fact may be due to phosphorus assimilation by microalgae (Putri and Hung, 2020) as the most potent, most effective means of phosphorus removal from the liquid way, and as the algae are not removed from the effluent, the values are statistically equal ($p > 0$ 0.05).

The MP presents statistically constant mean values for the HDT tested in the parallel period ($p > 0.05$). The solar radiation and temperature can significantly influence phosphorus removal (due to biomass productivity) than the HDT values tested in this experiment. Thus further experiments with MP testing values of hydraulic detention times are needed higher.

In the series period, there is a tendency to reduce total phosphorus concentration by increasing HDT values. The combined treatment of the ponds provides a higher HDT. Thus there is a longer contact time between the microorganisms and the phosphorus. The phosphorus can be assimilation, mainly by macrophytes and microorganisms attached to their roots, removing the pollutant from the liquid.

The serial system for total phosphorus removal has values below 1.0 mg/L for the HDT of 4.1 days. In this period, the lowest pollutant values in the treatment are recorded compared to the others ($p < 0.05$). For this period, the average total phosphorus removal efficiency was 70.3%, reaching the maximum removal value of 82.5%.

The total phosphorus concentrations for the cold period in the series system may be due to the raw effluent presenting a higher concentration of total phosphorus, between 5.4 and 7.8 mg/L, compared to the parallel cold period, which presents a concentration between 4.5 and 5.8 mg/L ($p < 0.05$).

Bouali et al. (2012) reported lower removal rates of total phosphorus (30.0%) with constructed wetlands using duckweed and immobilized microalgae.

Fig. 7 shows the total phosphorus concentrations for the cold period operating the treatment system in series and parallel.

In the cold period, in parallel, the highest concentrations in AP are notorious when compared to the warm period, with significant differences between these two periods ($p < 0.05$), since solar radiation and temperature strongly influence the development of microalgae (Huang et al., 2019).

In the parallel cold period, there is a trend of higher values for AP for higher HDT. The pond loses efficiency in removing total phosphorus, with 2.2 days HDT being more suitable for this operational condition.

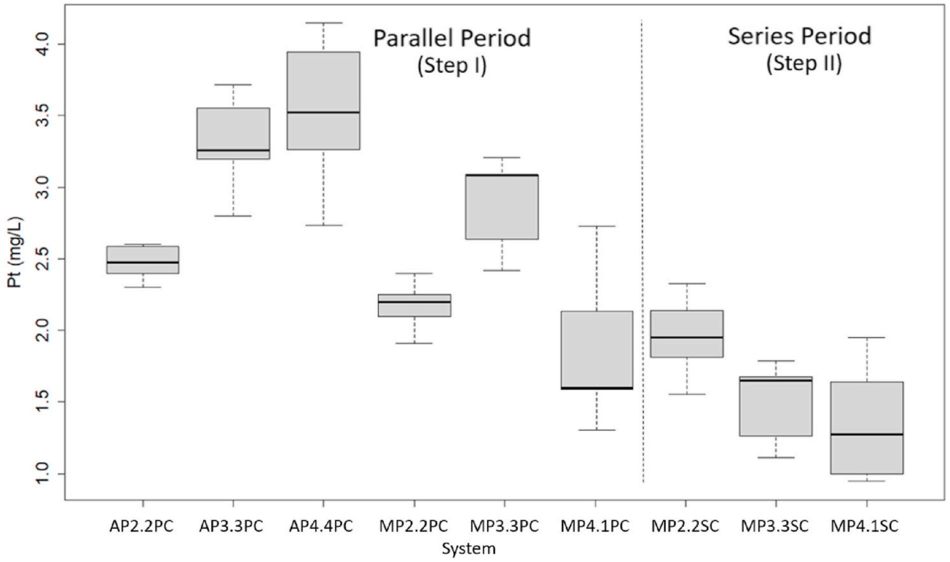

Fig. 7. Phosphorus concentrations in ponds on cold period according to the type of treatment.

The temperature and solar radiation may be exercised to a more significant influence than HDT, as also evaluated by Sukačová et al. (2015). It may also be related to the N/P ratio as evaluated by Benítez et al. (2018), or due to the species of microalgae and microorganisms developed under these operating conditions can provide an environment capable of achieving higher removal values.

The MP in the parallel cold period has a lower mean value of total phosphorus for the 4.1 days HDT ($p < 0.05$), possibly due to the longer contact time between the microorganisms and pollutants, different from the hot parallel period, where there was no direct influence of HDT for total phosphorus removal in MP.

In the serial period, the lowest values recorded by the treatment presented for the serial ponds and 4.1 days HDT ($p < 0.05$), with values below 1.0 mg/L of phosphorus in some collections. It also emphasizes the removal of nitrogen in the system in series, which is more constant and with the direct influence of HDT to remove pollutants, being more suitable and reliable than the system in parallel.

The lower values of phosphorus concentration presented in the MP can be explained by the removal of excess floating macrophytes, which need nutrients (N, P, K, among others) that are sorbed from the liquid, reducing the concentration in the effluent. Along with macrophytes, colonies of microorganisms attached to the roots can be removed, which also need nutrients to develop. Thus, it is to believe that the most considerable portion of phosphorus removal occurs through the absorption of lemnas and bacteria adhered to their roots, reported by other authors such as Yin et al. (2015) and Teles (2016).

The biofilm adhered to the slopes of the ponds can absorb nutrients (phosphorus and nitrogen) to develop new cells, which can contribute to the reduction in the concentration of nutrients in the final effluent, also observed by Brugnago (2014) and Teles (2016).

The best alternative to reach mean values below 1.0 mg/L is to raise the HDT of the MP since the lowest values are obtained for the HDT of 4.1 days in the MP, whereas for the AP, there is no high difference between the phosphorus concentrations for different HDT values. The higher HDT in the MP makes it possible to have a longer contact time between microorganisms, pollutants, and macrophytes, reducing the total phosphorus concentrations in the final effluent.

3.2.3. *Phosphorus recovery by MP*

Nutrient recovery can perform from the removal of cultivated photosynthetic biomass, and these will be proportional to the biomass mass removed from the pond and the characteristics of its plant tissue. The macrophyte biomass analysis was performed, covering 6 (six) samples throughout the experimental period, shown at the Table 5.

The biomass of macrophytes presented, in step I, 5.70 ± 0.70% of nitrogen and 0.75 ± 0.20% of phosphorus. In step II, considering dry weight, 5.30 ± 0.60% of total nitrogen and 0.80 ± 0.10% of total phosphorus. The percentage of phosphorus for macrophytes found in the bibliography presents values between 0.70 and 1.40% (Stadtlander et al., 2019; Strzałek et al., 2019), being close to values found in this work.

Phosphorus recovery on MP was calculated by removal of the lemna, as these must remove to control the surface density of the MP. Thus, by removing the lemna, the phosphorus is recovered through biomass. The recovered portion is a percentage of phosphorus absorbed by the lemnas and microorganisms attached to the roots of these macrophytes, which remove phosphorus along with them.

The biomass production of macrophytes in this work was 9.7–13.9 g/m^2·day for step I and 7.1–14.5 g/m^2·day for step II. The values were of production of lemnas biomass cited in the literature are in the range of 8.0 g/m^2·day (Ennabili et al., 2019) and 1,6 g/m^2·day (Ceschin et al., 2020), treating sanitary effluents from ponds with the presence of *Lemna gibba* and *Lemna minor*, respectively and 8.30 g/m^2·day with *Landoltia punctata* (Mohedano et al., 2014).

Al-Nozaily et al. (2000), cultivating *Lemna minor*, had a 3.10 g/m^2·day productivity. Li et al. (2016) obtained productivity between 1.89 and 1.91 g/m^2·day for *Spirodela polyrhiza* and, for the cultivation of *Lemna aequinoctialis*, presented values of 1.32–8.90 g/m^2·day, treating sanitary effluents. The values found in this work are higher than those that treat sanitary effluents and close to those that treat swine effluents.

Table 6 shows the phosphorus recovery for steps I and II from the lemna density control produced in the MP.

Brugnago (2014) presents the pond's removal of 0.11–0.13 g/m^2·day but does not present the macrophytes biomass composition. Using the species *Lemna minor*, Monette et al. (2006) removed 0.128 g/m^2·day of phosphorus in a bench study, and Korner and Vermaat (1998), treating sanitary sewage, reached a maximum removal of 0.079 g/m^2·day with the species *Lemna gibba*.

Whereas the mean phosphorus percentage in macrophytes ranges from 0.04% to 1.00% (Chang et al., 2011; Shilton et al., 2012), thus the maximum value found in the bibliography mentioned above would be between 0.013 and 0.138 g/m^2·day, and for researches using sanitary effluents, they present values between 0.013 and 0.059 g/m^2·day. Thus, the values found in this work are within expectations when compared to other works, with higher values than those reported in the bibliography when using sanitary effluents.

The highest phosphorus recovery values are presented in the warm period for the 4.1 days HDT ($p < 0.05$), whose temperatures were also the highest obtained (27.4–35.6 °C). We register solar radiation values with higher energy intensity (>600W/m^2) in this period. The ponds are located in the south of the country, in this case in Porto Alegre - RS - Brazil, in spring/summer, the day becomes longer than at night, so there is a more extended period of exposure of photosynthetic beings to solar radiation, allowing to increase biomass production.

In the cold period, the highest phosphorus recovery also occurs for the 4.1 days HDT, being higher for the series period when compared to the parallel period ($p < 0.05$). The phosphorus recovery values to directly linked with the production of lemna biomass, and therefore, the higher the lemna production, the greater the phosphorus recovery.

The maximum values reached for phosphorus recovery were 17.2 g/day (0.12 g/m^2·day), which can reuse as a raw material used for agriculture as fertilizer. In addition, it could also be considered for use as a raw material for anaerobic digestion to generate products and by-products.

Table 5

Macrophyte biomass characterization.

Macrophyte biomass analysis report		
Parameters	Dry matter (%)	
	Step I	Step II
Organic Matter	76.62 ± 3.95	76.84 ± 2.81
Crude Protein	35.70 ± 4.10	34.20 ± 3.07
Crude Fiber	12.27 ± 2.12	12.89 ± 2.48
Ashes	23.38 ± 3.48	23.16 ± 3.12
Non-nitrogen Extract	26.33 ± 3.23	24.11 ± 2.98
Total Nitrogen	5.70 ± 0.70	5.30 ± 0.60
Total Phosphorus	0.75 ± 0.20	0.80 ± 0.10

Table 6

Phosphorus recovery from lemna excess removed at the MP.

HDT (days)	Pt Recovery - Step I		Pt Recovery - Step II	
	Pond phosphorus production (g/day)	**g/m^2. day**	**Pond phosphorus production (g/day)**	**g/m^2. day**
Warm Period			**Warm Period**	
2.2	12.7^b	0.09	9.7 ^{c}	0.07
3.3	11.2^c	0.08	14.2 ^{b}	0.10
4.1	15.4^{ab}	0.10	17.2^a	0.12
Cold Period			**Cold Period**	
2.2	11.4^c	0.08	8.4 ^{c}	0.06
3.3	10.8^c	0.07	9.2 ^{c}	0.06
4.1	11.2^c	0.08	13.1 ^{b}	0.09

This macrophyte removal method makes it possible to calculate the nutrient recovery capacity. By controlling the density correctly (always with the same density), the maximum amount of nutrients can be recovered, studying the best reproduction range of the cultivated macrophyte. However, the phosphorus and nitrogen recovered are only removed from the ponds. In this case, the biomass of macrophytes and bacteria adhered to the roots.

3.2.4. Nitrogen recovery by MP

Table 7 presents the nitrogen recovery. The warm period presents high values compared to the cold period under the same conditions. Due to higher biomass production per m^2·day, as seen in the phosphorus recovery analysis.

The percentage of nitrogen in macrophyte biomass is higher than that of phosphorus, with an average value of 5.7 ± 0.7%, and 5.3% ± 0.6%, for steps I and II respectively, these values are close to that found by Kaur et al. (2019), which presented values of 5.53%.

Nitrogen recovery had higher values for HDT of 4.1 days in the hot period for both steps, with 0.79 for step I and 0.77 g/m^2·day for step II. The bibliography reports the total removal of the pond, not specifying the nutrient recovery by the macrophytes. Brugnago (2014) found 0.63 g/m^2·day cultivating *Landoltia punctata*. Mohedano et al. (2014) obtained 1.2 g/m^2·day treating swine effluents, and Al-Nozaily et al. (2000) obtained 0.16 g/m^2·day cultivating lemna minor from swine treatment. Thus, the values found in this work are close to those seen in the bibliography.

Lemnas biomass has a high concentration of nutrients that can use as a substrate for energy production from anaerobic digestion (Gaur and Suthar, 2019; Kaur et al., 2019).

Table 7
Nitrogen Recovery from lemna excess removed at the MP.

Nitrogen Recovery - Step I		
	Pond nitrogen production (g/day)	**g/m^2·day**
Warm Period		
2.2	96.17 ^{b}	0.65
3.3	85.20 ^{c}	0.58
4.1	117.26^a	0.79
Cold Period		
2.2	86.89 ^{c}	0.59
3.3	81.83 ^{c}	0.55
4.1	85.20 ^{c}	0.58
Nitrogen Recovery - Step II		
HDT	**Pond nitrogen production (g/day)**	**g/m^2day**
Warm Period		
2.2	64.22 ^{d}	0.43
3.3	94.16 ^{b}	0.64
4.1	114.05^a	0.77
Cold Period		
2.2	55.47 ^{d}	0.37
3.3	61.26 ^{d}	0.41
4.1	86.60 ^{c}	0.59

4. Conclusions

The proposal of an innovative system that combines photosynthetic beings (microalgae and macrophytes) for the treatment of effluents presents exciting results, obtaining high removal of organic matter and nutrients, in addition to the production of energetic biomass.

For the parallel system, ammonia nitrogen removals were higher for the warm period in AP with an HDT of 4.1 days, reflecting the interaction of microbial and microalgae activities. The best configuration for the parallel system was not obtained for phosphorus, presenting similar values for the different operational conditions.

In the series period, a combination of microalgae and macrophytes treatment shows the lowest concentration for the hot period and HDT of 4.1 days, with phosphorus concentrations below 1.0 mg/L and in some samples, the ammonia nitrogen was not detected by the method, tending, due to values very close to zero.

The biomass production of macrophytes presented values of 9.7–13.9 g/m^2·day for stage I and from 7.1 to 14.5 g/m^2·day for stage II, resulting in phosphorus recovery from up to 0.12 g/m^2·day and nitrogen of 0.79 g/m^2·day for the hot period and higher HDT, allowing high recovery of nutrients from the energetic biomass removed.

This work made it possible to verify the influence of HDT and climate (solar radiation and temperature) on the removal and recovery of nutrients in microalgae and macrophyte ponds systems. Thus, the greater the HDT, the greater the possibility of developing nitrifying, denitrifying, and phosphorus accumulators.

To high removal and recovery of phosphorus from the effluent, it is necessary to evaluate the MP for higher HDT values since much of the phosphorus to absorb by macrophytes and microorganisms attached to their roots, and this posterior removal to maintain density control of macrophytes within the pond.

CRediT authorship contribution statement

José Carlos Alves Barroso Júnior: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. **Maria Cristina de Almeida Silva:** Writing – review & editing, Supervision, Funding acquisition. **Nestor Leonel Muñoz Hoyos:** Formal analysis, Investigation, Data curation, Writing – review & editing. **Luiz Olinto Monteggia:** Writing – review & editing, Supervision, Project administration, and, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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