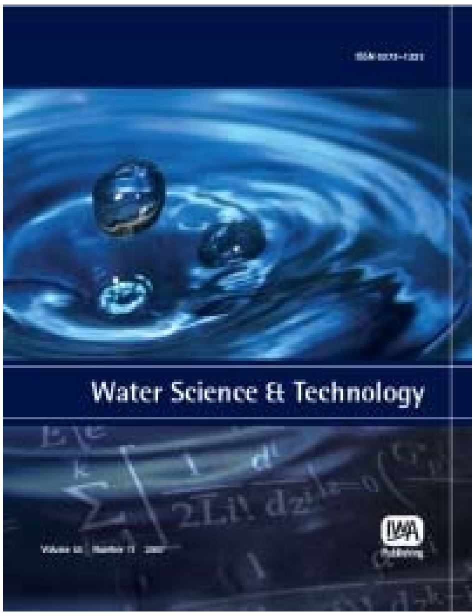
Water Science & Technology

Nutrient recovery from swine waste and protein biomass production using duckweed ponds (*Landoltia punctata*): Southern Brazil

R. A. Mohedano, V. F. Velho, R. H. R. Costa, S. M. Hofmann and P. Belli Filho

ABSTRACT

Brazil is one of the most important countries in pork production worldwide, ranking third. This activity has an important role in the national economic scenario. However, the fast growth of this activity has caused major environmental impacts, especially in developing countries. The large amount of nitrogen and phosphorus compounds found in pig manure has caused ecological imbalances, with eutrophication of major river basins in the producing regions. Moreover, much of the pig production in developing countries occurs on small farms, and therefore causes diffuse pollution. Therefore, duckweed pond have been successfully used in the swine waste polishing, generating further a biomass with high protein content. The present study evaluated the efficiency of two full scale duckweed ponds for the polishing of a small pig farm effluent, biomass yield and crude protein (CP) content. Duckweed pond series received the effluent from a biodigester-storage pond, with a flow rate of 1 m^3/day (chemical oxygen demand rate = 186 kg/ha day) produced by 300 animals. After 1 year a great improvement of effluent quality was observed, with removal of 96% of total Kjeldahl nitrogen (TKN) and 89% of total phosphorus (TP), on average. Nitrogen removal rate is one of the highest ever found (4.4 g TKN/m^2 day). Also, the dissolved oxygen rose from 0.0 to 3.0 mg/L. The two ponds produced together over 13 tons of fresh biomass (90.5% moisture), with 35% of CP content, which represents a productivity of 24 tonsCP/ha year. Due to the high rate of nutrient removal, and also the high protein biomass production, duckweed ponds revealed, under the presented conditions, a great potential for the polishing and valorization of swine waste. Nevertheless, this technology should be better exploited to improve the sustainability of small pig farms in order to minimize the impacts of this activity on the environment.

Key words | duckweed ponds, nutrient removal, protein biomass, swine waste, treatment

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INTRODUCTION

Currently, pig farming is the main source of animal protein for human nutrition and occupies a strategic position in the global scene (FAO 2009). However, the fast growth of this activity has caused major environmental impact, especially in developing countries, such as Brazil (the third largest producer of swine meat worldwide). The large amount of nitrogen and phosphorus compounds present in pig manure has caused ecological imbalances, with eutrophication of major river basins in the producing regions. Moreover, much of the pig production in developing countries occurs on small farms, which have few financial resources for the installation of waste treatment systems, and therefore causes diffuse pollution.

Because of the strictness of environmental laws in Brazil, which require environmental licensing of properties, many producers have installed anaerobic biodigesters for the treatment and valorization of pig manure to reduce the environmental impact. In addition to having low installation and operation costs, this technology produces biogas, a value-added by-product that can be used as fuel in energy generation. However, the effluent from biodigesters generally requires a polishing step before it can be released into

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the water body, mainly due to the high concentration of nutrients that must be removed. In the search for alternatives for the polishing and valorization of pig waste, duckweed ponds have arisen as an efficient and low-cost option. Duckweed is a small floating macrophyte that has a high capacity for removing dissolved nutrients from water, especially nitrogen and phosphorus compounds, as well as for reducing organic matter and suspended solids (Landolt & Kandeler 1987; Skillicorn *et al.* 1993; Alaerts *et al.* 1996). However, the great advantage of this plant group over other macrophytes used in effluent treatment is the production of a biomass with high nutritional value, reaching crude protein (CP) levels of more than 40% (Landesman *et al.* 2002). Thus, besides reducing the organic load of the effluents, the use of duckweed may generate cost savings in animal production, by minimizing the costs of animal rations.

This plant group taxonomy has undergone some changes in recent years. Duckweeds used to belong to the Lemnaceae family, but they currently are framed in the subfamily Lemnoideae within the family Araceae, with approximately 40 species in five genera (APG II 2003). Among the species of duckweeds, not all are effective in the treatment of effluents and for protein production. Bergmann *et al.* (2000) assessed 41 geographically isolated duckweeds to determine the species that have the greatest potential in the treatment of swine waste and in protein production and found that the variety *Landoltia punctata* was the best in protein production.

Several researchers worldwide have conducted studies on the potential use of duckweed in wastewater treatment, especially for the removal of nutrients. To this end, Caicedo *et al.* (2002) found that anaerobic pre-treatment improves the performance of duckweed ponds for wastewater treatment, particularly for nutrient removal. Cheng *et al.* (2002a) reported on the excellent performance of *L. punctata* in nutrient removal from swine waste (with a high ammonia concentration of 240 mg/L), with a removal rate of approximately 1.0 mg/L h for NH_4^+ and 0.13 mg/L h for PO_4^-. Additionally, Xu & Shen (2011) affirmed the great potential of *Spirodela polyrrhiza* in nutrient removal from pig manure, with approximately 84 and 89% removal of total nitrogen (TN) and total phosphorus (TP), respectively. In a survey of *Lemna minor* for the tertiary treatment of swine manure, Cheng *et al.* (2002b) reported a removal rate of 2.1 g/m^2 d for nitrogen and 0.6 g/m^2 d for phosphorus.

In addition to the environmental benefits, biomass generated during treatment may contain high nutritional value with high productivity. For over 30 years, researchers have demonstrated the potential use of duckweed in feed for farmed animals. Therefore, because of the substantial growth rate and high protein content, the protein productivity may be ten times higher than soy (Landesman *et al.* 2005). Cheng *et al.* (2002b) cite a growth rate of 29 g/m^2 d, which is equivalent to 104 t/ha year. This characteristic is positive because it can encourage low-income pig farmers to implement treatment systems because of their ability to produce value-added biomass.

Therefore, this study evaluated a swine waste treatment system, in full-scale, in a small farm in southern Brazil, using two serial duckweed ponds for nutrient recovery. In addition to effluent polishing to remove nutrients, the biomass productivity and its protein content were also assessed.

MATERIAL AND METHODS

Localization of research

This study was developed in a small pig-producing property (about 300 animals) located in the municipality of Braço do Norte, in Santa Catarina State, southern Brazil (28°13′50.1′ S and 49°06′29.2′ W under a sub-temperate climate). This region has one of the largest densities of pigs worldwide, causing serious environmental problems.

Swine waste treatment system description

The pig rearing in this property generates a volume of approximately 3 m^3 of waste daily. This residue, composed mainly of manure, urine and leftover food, passed through a treatment system that included an anaerobic digester (hydraulic retention time, HRT = 30 days), a storage pond (SP), with a variable HRT, and two duckweed ponds, termed DP1 and DP2 respectively. These ponds were constructed and covered with a high-density polyethylene (HDPE) geomembrane, with a slope of wall of 45°. DP1 and DP2 were connected in series and had dimensions of $21.0 \times 7.0 \times 0.8$ m (101 m^3) and $15.0 \times 6.0 \times 0.4$ m (33 m^3) and HRT of 101 and 33 days, respectively. After leaving the anaerobic digester, the effluent was drained to the SP, where about 2 m^3/day was used for agricultural fertilization and the rest, about 1 m^3/day, was transferred to the duckweed ponds for the polishing process (nutrient removal). Finally, the treated effluent was stored in a 5,000 L reservoir to be reused for pigsty cleaning. The entire treatment system is shown in Figure 1.

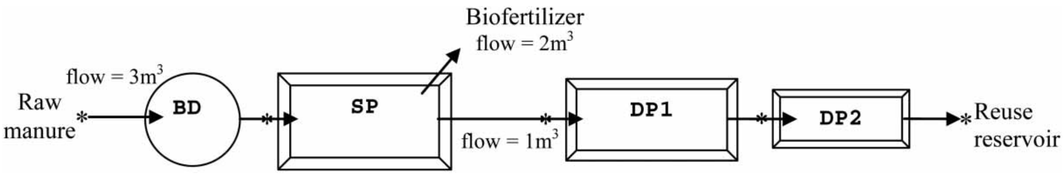

Figure 1 | Treatment System: BD – Biodigester; SP – Storage pond; DP1 – Duckweed pond 1; DP2 – Duckweed pond 2. *Points of sampling.

Duckweed ponds

The species *L. punctata* was chosen because, in addition to being a native species in southern Brazil, it has been recommended by many authors for this purpose. According to Cheng *et al*. (2002a), this type of duckweed can support high loads of ammonia and can produce high-protein biomass and is therefore adequate for swine waste treatment. Duckweeds were collected from a natural eutrophic water body located nearby and introduced into the duckweed ponds to cover the water surface at a density of approximately 220 g/m^2 and after the adaptation period (28 days), the experiment was initiated. A load was applied in batches of 15 m^3 every 15 days so that the effluent flow rate applied into the duckweed ponds was 1 m^3/d on average. Because ammonia is the primary factor that limits the growth of duckweeds in pig waste, the scaling load was set to limit the ammonia concentration to 100 mg/L (Caicedo 2005). The surface charge of ammonia applied was 36.4 kg NH_3/ha d. After the load application in DP1, the effluent went by gravity to DP2, simultaneously. Duckweed biomass was removed every 2 days at an average rate of 27 and 7.5 kg/day for DP1 and DP2.

Effluent monitoring

The effluent quality was monitored during 1 year between April 2009 and April 2010. The effluent samples were collected every 2 weeks at points of entry and exit of all stages of the system (Figure 1). After they were collected, the samples were transferred to the analytical laboratory in the Environmental Engineering Department of Federal University of Santa Catarina. The analysed parameters included total Kjeldahl nitrogen (TKN), ammonia nitrogen (N-NH_3), nitrite (N-NO_2), nitrate (N-NO_3), TP, pH, temperature and dissolved oxygen (DO), using the *Standard Methods for Examination of Water and Wastewater* (APHA 2005). To determine mean values and standard deviation, statistical inference was used to evaluate the results.

Biomass monitoring

Productivity evaluation of duckweed biomass during the experiment was evaluated based on the determination of specific growth rate (kg/kg day) and growth rate by area or relative growth rate (g/m^2 day). It was necessary to estimate ponds' total biomass through plant density. In order to carry out quantitative sampling of biomass a square float was constructed (made with PVC pipes ø32 mm), with an internal area of 1 m^2. This square was released randomly on the duckweed pond surface three times a day and the imprisoned biomass inside the square was collected and weighed (Figure 2). Therefore, the duckweed density (g/m^2) was calculated. The specific and superficial growth rates were obtained from the relation between the average

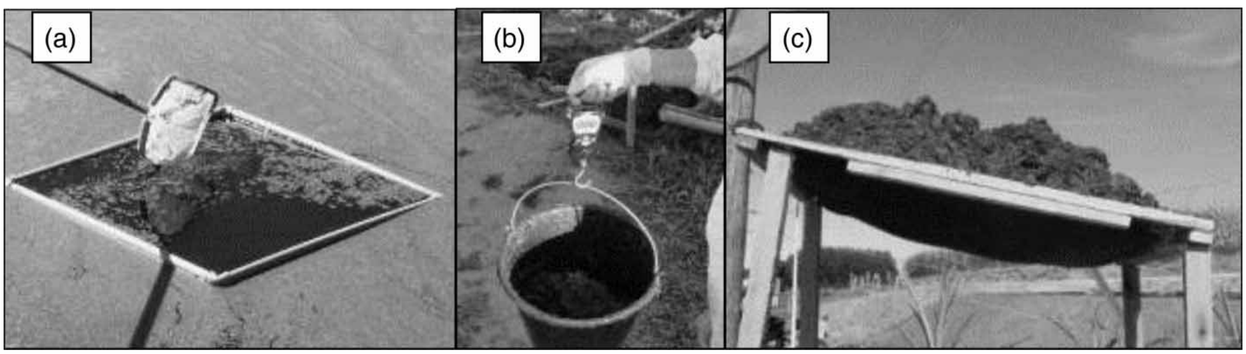

Figure 2 | Quantitative evaluation of duckweed biomass; (a) Sample collection; (b) weighing; (c) natural drying.

density (g/m^2) and the pond productivity (estimated by the total removal of biomass), as shown in Equation (1). The logarithmic ratio was not used because biomass was often removed.

$$\mathrm{RGR} = \frac{\mathrm{TB}/n}{A} \quad \mathrm{SGR} = \frac{\mathrm{TB}/n}{D \cdot A} \tag{1}$$

RGR = relative growth rates (kg/m^2 day); SGR = specific growth rate (kg/kg day); TB = total biomass harvested during the period (kg); n = number of days in the period; D = average biomass density (kg/m^2); A = surface water area (m^2).

In addition to the collected samples, fresh duckweed biomass was removed every 2 days at rates of approximately 50 kg and 22 kg from DP1 and DP2, respectively. The removal of biomass is important to the ponds' operation, which is a key factor for the success of the waste treatment.

For biomass qualitative evaluation, samples of about 1 kg were collected every 2 weeks, and were dried using laboratory oven at 55° C for 24 h. Subsequently these samples were frozen and sent for laboratory analysis for verification of CP content (CP%). The methodology is based on the determination of total nitrogen multiplied by the constant 6.25 (AOAC Method 991.20) referenced by the Association of Official Analytical Chemists (AOAC 2005). The obtained data were statistically evaluated to estimate rates of protein production.

RESULTS AND DISCUSSION

Entire treatment system efficiency

During the studied period, approximately 1,140 m^3 of swine waste was treated. The entire treatment system showed a significant efficiency of nutrient reduction (greater than 99% for TKN and TP) and DO increase (Tables 1 and 2). Most likely, this fact can be assigned to the long HRT (more than 200 days), the high concentration of raw influent, suitable temperature (24° C on average) and also to different treatment stages (with anaerobic and aerobic conditions, propitious for nitrification and denitrification process). In addition, pH values remained near neutrality, suffering a mild acidification along the system stages (7.52 to 6.68). This pH range is expected for swine wastes; however, duckweed ponds usually present low pH levels compared with maturation ponds due to the algal growth inhibition (Skillicorn et al. 1993; Costa et al. 2009). A wide variation in the raw manure composition was found throughout the studied period. This range is primarily caused by the management and hog production cycle, such as the number and age of animals, diet composition and quantity of water used, but this result was expected. This variation in raw waste composition can be seen in Table 1, as the high standard deviation from the median. Nevertheless, high treatment efficiency was observed through the stages of the system.

Table 2 | Efficiency of parameters, reduction to all stages of the treatment system

Parameters	BD (%)	SP (%)	DP1 (%)	DP2 (%)	Final efficiency (%)
N-NH$_3$	28	45	95	74	99.5
TKN	79	45	95	68	99.8
TP	85	57	89	47	99.8

BD = Biodigester; SP = Storage pond; DP1 and DP2 = Duckweed ponds.

Duckweed ponds' efficiency

The duckweed ponds showed a significant DO increase. In SP (influent) were observed DO concentrations lower than 0.2 mg/L; however, the DO at the duckweed ponds, surface reached 2.1 ± 1.4 and 3.0 ± 1.2 mg/L for DP1 and DP2 respectively (Table 1). Therefore, after passing through the duckweed ponds the effluent changed from anaerobic to aerobic conditions. Similarly, Alaerts et al. (1996) observed

Table 1 | Main values and standard deviation of parameters at all stages of the treatment system

Parameters	Raw manure	Effl. BD	Effl. SP	Effl. DP1	Effl. DP2
pH	7.52 ± 0.6	7.19 ± 0.7	7.38 ± 0.4	7.0 ± 0.6	6.68 ± 0.5
DO (mg/L)	–	0.10 ± 0.3	0.10 ± 0.19	2.02 ± 1.4	3.02 ± 1.2
N-NH$_3$ (mg/L)	1,624 ± 1,146	1,159 ± 377	636 ± 321	28 ± 14	7 ± 6
TKN (mg/L)	7,986 ± 9,573	1,622 ± 629	832 ± 435	44 ± 22	14 ± 10
TP (mg/L)	1,487 ± 898	215 ± 177	92 ± 99	10 ± 7	5 ± 6

BD = Biodigester; SP = Storage pond; DP1 and DP2 = Duckweed ponds.

DO range between 2 and 4 mg/L in duckweed ponds in domestic sewage treatment. The mean water temperatures in DP1 and DP2 were 22.8 °C ± 2.9 (max. = 26.4 °C; min. = 12.5 °C) and 23.4 °C ± 3.3 (max. = 26.7 °C; min. = 13.1 °C), respectively. Hence, the temperature was not considered a limiting factor for duckweed and microorganisms growth, being a good range for biological activity (Landolt & Kandeler 1987).

Most of the TKN load applied to the serial duckweed ponds was removed after 1 year (Table 2). The results showed that TKN load applied in duckweed ponds was 264 kg and the removed load was 259 kg, so approximately 260 kg of nitrogen was recovered from the water. Therefore, surface application rate was 46.2 kg TKN/ha day and the removal rate was 43.7 kg TKN/ha day or 4.4 g/m^2 day (Table 3). Cheng *et al.* (2002a) reported the highest removal rates in their investigation of the nitrogen removal from swine waste by *L. minor*; they found removal rates of 3.4 g TKN/m^2 day (*in vitro* experiment) and 2.1 g TKN/m^2 day (field experiment). Thus, the nitrogen removal rate presented in this research is one of the highest reported. Other reported removal rates include 0.61 g/m^2 day (Lyerly 2004), 0.95 g/m^2 day (Cheng *et al.* 2002b), 0.54 g/m^2 day (Körner & Vermaat 1998) and 1.2 g/m^2 day (Benjawan & Koottatep 2007). Based on the reports by Bergmann *et al.* (2000) and Cheng *et al.* (2002a), the species used in the present research (*L. punctata*) is one of the most efficient for this type of effluent, contributing to its success in removing nitrogen. Moreover, the warm Brazilian climate, with a gentle winter, can improve the growth rates of duckweed and microorganisms, particularly for native species such as *L. punctata.* After analysis of the biomass nitrogen content (6.6% TN dry weight, on average) it was observed that 28% of the nitrogen removal in DP1, that is 81 kg of TKN or 1.2 g TKN/m^2 day, was due to biomass absorption by the duckweed. The nitrogen remaining (72%) was removed by nitrification and denitrification processes. Strong denitrification efficiency can be justified by several factors including aerobic and anoxic zones, a large area for a biofilm to attach, optimal pH and temperature ranges, and availability of food (biological oxygen demand, BOD) for heterotrophic microorganisms. Ammonia volatilization was considered negligible because of the low pH levels. However, the N-NH_3 concentration obtained after load mixing was 97 mg/L, on average, but at high concentration periods was observed to be 182 mg/L. This value was two times higher than the maximum concentration (50 mg/L) recommended by Caicedo (2005) for *Spirodela polyrrhiza* based treatment. Hence, those results demonstrate *L. punctata*'s robustness to grow on swine waste treatment ponds, supporting high ammonia concentrations. In spite of the higher efficiency in DP1, an important contribution was observed to effluent polishing in DP2.

Table 3 | Nitrogen load rates in duckweed ponds

Duckweed ponds	Application rate (kg/ha. day) TKN	N-NH_3	Removal rate (kg/ha. day) TKN	N-NH_3
DP1	46.2	36.9	43.7	35.1
DP2	4.0	3.1	2.7	2.3

The efficiency of phosphorus recovery was also very high in DP1, around 90% (Table 2). But, unlike nitrogen, phosphorus was strongly reduced in anaerobic stage, probably due to sedimentation (Table 1). Phosphorus load applied and removed was respectively 30 and 27 kg in DP series. Thus, the TP removal rate was approximately 470 mg/m^2 day, which is in agreement with Cheng *et al.* (2002b), who described a removal rate of 590 mg P/m^2 day by *L. minor* from pig waste. Unlike nitrogen, the main route for phosphorus removal in duckweed ponds is biomass absorption. The large difference in removal rate between N and P may be due to several factors such as nutritional requirement, initial concentration of P and N and plant growth rate under temperature variations and toxic compounds (Cheng *et al.* 2002a). Nozaily *et al.* (2000), indicate TP removal rates close to 95 mg/m^2 day for duckweed pond receiving effluent produced by UASB (up-flow anaerobic sludge blanket) reactor, being a lower value.

Biomass protein production

The duckweed biomass was removed at 27 kg/day for DP1 and 7.5 kg/day for DP2, on average. Therefore, the total biomass produced in the duckweed ponds was greater than 13 tons/year (fresh weight with 90% moisture), 10.3 tons in DP1 and 2.8 tons in DP2. Thus, average yield estimate was 181 g/m^2 day (fresh weight), or 18 g/m^2 day (dry weight) for DP1. For DP2, the estimated growth rate was 83 g/m^2 day (fresh weight) or 8.3 g/m^2 day (dry weight). The maximum yield was obtained in DP1 with capacity to generate 68.8 t/ha year (dry weight). Similarly, El-Shafai *et al.* (2006) cites a production of 33 tons of *L. minor* and *L. gibba* biomass in 8 months growing in UASB reactor effluent. The average protein content in the duckweed biomass was 35% and 28% of CP in DP1 and DP2 respectively. However, CP in biomass harvested reached above 40% at beginning of experiment, only in DP1 (Figure 3). Protein

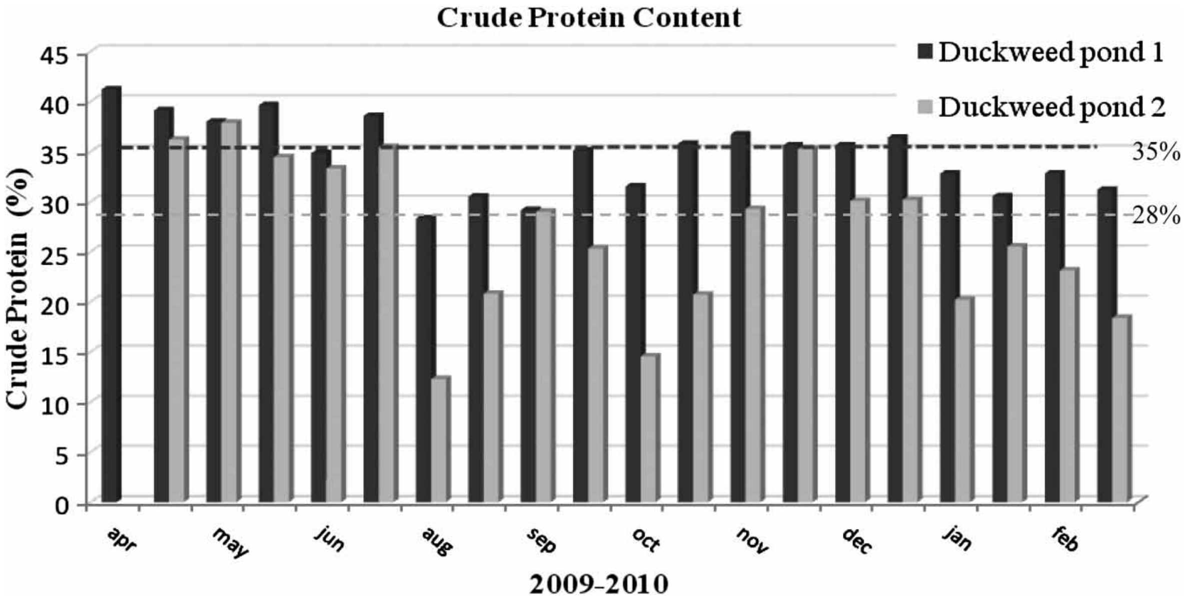

Figure 3 | Range of crude protein in duckweed biomass harvested during experimental period, in both duckweed ponds, and mean values (dashed lines).

yield was higher in DP1, probably due to high nitrogen concentration. Both duckweed ponds together produced approximately 435 kg CP, with a productivity of 24 t/ha year. This production represents approximately 20 times the mean soybean protein productivity in Brazil and two times the production reported by Landesman *et al.* (2005). CP content range during the period can be seen in Figure 3.

CONCLUSIONS

Due to the high rate of nutrient removal, and also the high protein biomass production, duckweed ponds revealed, under the presented conditions, a great potential for the polishing and valorization of piggery waste. The rate of nitrogen removal presented here was one of the highest reported in specific literature. Maybe the warm Brazilian climate, with a soft winter, can improve duckweed growth rates, mainly for native species like *L. punctata*. Moreover, the biomass produced during the waste treatment may be used for animal feed (for example fish farming), generating economic gains and encouraging farmers to apply this technology with resulting environmental benefits. However, other tests should be done to ensure the sanitary security for animal feed and human health, such as analysis to determine heavy metal concentrations and presence of pathogenic organisms. Thus, this technology should be better exploited to improve the sustainability of small piggery farms in order to minimize the impacts of this activity on the environment.

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