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High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds

Rodrigo A. Mohedano [a,*], Rejane H.R. Costa [a], Flávia A. Tavares [b], Paulo Belli Filho [a]

[a] *Department of Sanitary and Environmental Engineering, Federal University of Santa Catarina – UFSC/CTC/ENS, CEP 88010 970 Florianópolis, SC, Brazil*

[b] *Brazilian Company of Agricultural and Livestock Research (EMBRAPA), Fishery and Aquaculture, Conjunto 01, Lote 17 Térreo, CEP 77015-012, Palmas, Tocantins, Brazil*

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ABSTRACT

Duckweed ponds have been successfully used in swine waste polishing, generating a biomass with high protein content. Therefore, the present study evaluated the efficiency of two full-scale duckweed ponds considering nutrient recovery from a piggery farm effluent (produced by 300 animals), as well as the biomass yield and crude protein (CP) content. A significant improvement in the effluent quality was observed, with the removal of 98.0% of the TKN (Total Kjeldahl Nitrogen) and 98.8% of the TP (Total Phosphorous), on average. The observed nitrogen removal rate is one of the highest reported (4.4 g/m^2 day of TKN). Additionally, the dissolved oxygen level rose from 0.0 to 3.0 mg/L, on average. The two ponds together produced over 13 tons of biomass (68 t/ha year of dry biomass), with 35% crude protein content. Because of the excellent nutrient removal and protein biomass production, the duckweed ponds revealed a great potential for the polishing and valorisation of swine waste, under the presented conditions.

1. Introduction

Currently, pig farming is the main source of animal protein for human nutrition and occupies a strategic position in the global food production (FAO, 2010). However, the fast growth of this activity has caused major environmental impacts, especially in developing countries, such as Brazil (the third largest producer of pork meat worldwide). The large amount of nitrogen and phosphorous 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, which have few financial resources for the installation of waste treatment systems and therefore causes diffuse pollution.

Because of the strictness of environmental laws, which require environmental licensing of properties, many producers have installed anaerobic biodigesters for the treatment and valorisation of pig manure to reduce the environmental impact. In addition to having low installation and operation costs, this technology produces biogas, a value-added byproduct 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 a body of water because of the high concentration of nutrients that must be removed. In the search for alternatives for the polishing and valorisation of pig waste, duckweed ponds have arisen as an efficient and low-cost option (Bergmann et al., 2000a,b; Cheng et al., 2002a,b; Xu and Shen, 2011). Duckweed is a small floating macrophyte that has a high capacity for removing dissolved nutrients from water, especially nitrogen and phosphorous compounds, as well as for reducing organic matter and suspended solids (Landolt and 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 minimising 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 5 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., (2000a) 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

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* Corresponding author. Tel.: +55 48 3721 7743.
E-mail address: rodrigomohedano@gmail.com (R.A. Mohedano).

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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 and Shen (2011) affirmed the great potential of *Spirodela polyrrhiza* in nutrient removal from pig manure, with approximately 84% and 89% removal of TN and TP, respectively. In a survey (scale) 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 phosphorous.

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 (dry weight – dw), 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. In addition to the nutritional value, Cheng and Stomp (2009) describe the production of significant quantities of starch that can easily be converted to bioethanol, which can serve as a potential biofuel source produced in wastewater.

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.

2. Methods

2.1. Swine waste treatment system description

This study was developed in a pig-farming property located in Santa Catarina State in 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 in the world, which causes serious environmental problems. The property is a small farm with approximately 300 animals that generates 3 m^3 of waste daily. This residue, composed mainly of manure, urine and leftover food, passed through a treatment system that includes an anaerobic digester (hydraulic retention time (HRT) = 30 days), a storage pond (SP) with a variable HRT and two duckweed ponds, called DP1 and DP2, connected in series. After leaving the digester, the effluent was drained to the SP where approximately 70% was used for agricultural fertilisation and the rest (30% – approximately 1 m^3/day) was transferred to the duckweed ponds for the polishing process (nutrient removal). Finally, the treated effluent was stored in a 5000-L reservoir to be reused for pigsty cleaning. The entire treatment system is shown in Fig. 1.

2.2. Duckweed ponds

Two ponds were constructed for the duckweed-based treatment (named DP1 and DP2) and covered with a high-density polyethylene (HDPE) geomembrane (0.8 mm). The dimensions of the ponds were based on parameters such as the available area, effluent flow and HRT. The dimensions are shown in Table 1.

Initially, the duckweed ponds were filled with rain water and river water and then received low-concentration loads of swine waste that had been pre-treated by the biodigester (approximately 1% of waste). For the adaptation period, duckweeds (*L. punctata*) were collected from a natural eutrophic water body located nearby and introduced in the duckweed ponds to cover the water surface at a density of approximately 220 g/m^2 (fresh weight – fw). The specie *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. Additionally, bamboo dividers were floated across the pond to minimise the wind drag.

After the adaptation period (28 days), the experiment was initiated, and 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). After the load was applied to DP1, the effluent travelled by gravity to DP2. The duckweed biomass was removed every two days at an average rate of 27 kg/d (fw) from DP1 and 7.5 kg/d (fw) from DP2, or one-fifth of the pond surface per day. To calculate the biomass removal, the specific growth rates of duckweeds in stabilisation ponds that were reported in the manuscripts of Cheng et al. (2002a), Caicedo (2005), Driever et al. (2005), Landesman et al. (2005), and others were used. The growth rates ranged from 16 to 58 g/m^2 d (dw). The biomass management is described later.

Table 1
Engineering parameters of the duckweed ponds (DP1 and DP2).

Dimensions	DP1	DP2
Length (m)	20.5	15
Width (m)	7.5	6.0
Area (m^2)	153	90
Depth (m)	0.8	0.4
Volume (m^3)[a]	102	34
HRT (days)	102	34

[a] Ponds slope of 45°.

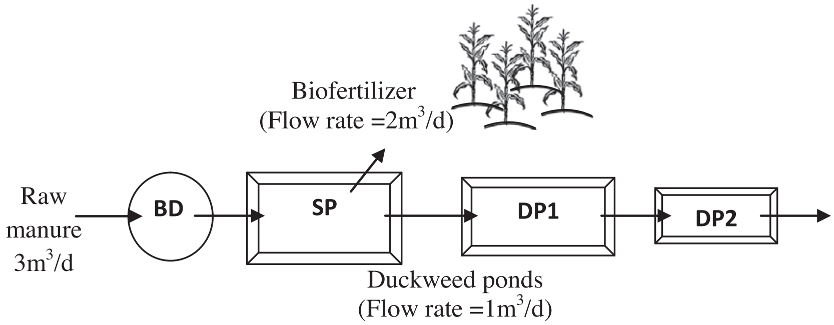

Fig. 1. Treatment system outline: BD = Biodigester; SP = Storage pond; DP1 and DP2 = Duckweed ponds 1 and 2.

2.3. Effluent quality monitoring

The effluent quality was monitored for one year between April 2009 and April 2010. The effluent samples were collected every two weeks at the points of entry and exit of each system stage (Fig. 1). After they were collected, the samples were transferred to the analytical laboratory in the Environmental Engineering Department of the Federal University of Santa Catarina. The analysed parameters included total Kjeldahl nitrogen (TKN), ammonia nitrogen ($N{-}NH_3$), nitrite ($N{-}NO_2$), nitrate ($N{-}NO_3$), total phosphorus (TP), pH 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. The nitrogen mass balance was calculated according to Eq. (1), where total nitrogen removed (TNR) was obtained by the sum of biomass removal (BMR), ammonia volatilization ($NH_3\uparrow$), nitrogen sedimentation (NS) and nitrification–denitrification process ($N_2\uparrow$).

$$\mathrm{TNR} = \mathrm{BMR} + \mathrm{NH_3} \uparrow + \mathrm{NS} + \mathrm{N_2} \uparrow \tag{1}$$

TNR = total nitrogen removed
BMR = removed by biomass
$NH_3\uparrow$ = ammonia volatilization
NS = nitrogen sedimentation
$N_2\uparrow$ = nitrification–denitrification process.

The nitrogen removed by duckweed biomass was obtained by nitrogen content analysis which is a step for protein determination. Ammonia volatilization was calculated based on $N{-}NH_3$ concentration, pH end temperature (at pH of 7 and 20 °C, only 0.4% of ammonia is in the volatile form). For NS measurement, a metallic box ($25 \times 25 \times 10$ cm) was placed on the bottom of the duckweed ponds in order to collect sediment samples and proceed the weighing and TKN analysis. The denitrification contribution was obtained by subtracting the total nitrogen removal in another ways.

2.4. Biomass monitoring

The production of duckweed biomass during the experiment was evaluated based on the determination of the specific growth rate (kg/kg d) and growth rate per area or relative growth rate (g/m^2 d). The growth rate was based on fresh biomass weight; however dry weight was also measured. After collection, the biomass was placed on a plastic screen (for 15 min approximately) for the water drain until no drops were observed and weighed in a digital balance. Therefore, a biomass sample (1 kg) was oven dried at 55 °C for 24 h. Thus, it was necessary to estimate the average percentage of dry weight to determinetotal fresh biomass harvested. It was necessary to estimate the total biomass of the ponds by measuring the plant density. To carry out quantitative sampling of the biomass, a square floating was constructed (from PVC pipes, ø32 mm), with an internal area of 1 m^2. This square was released randomly on the surface of the duckweed ponds three times per day, and the biomass inside the square was collected, and weighed. Therefore, the duckweed density (g/m^2) was calculated. The specific and superficial growth rates were obtained from the relation between the average density (g/m^2) and the pond productivity (estimated by the total removal of biomass), as shown in Eq. (2). (The logarithmic ratio was not used because biomass was often removed.)

$$\mathrm{RGR} = \frac{\mathrm{TB/N}}{\mathrm{A}} \cdot \mathrm{SGR} = \frac{\mathrm{TB/N}}{\mathrm{D \cdot A}} \tag{2}$$

RGR = Relative growth rate (g/m^2 d)
SGR = Specific growth rate (g/g d)
TB = Total biomass harvested during the period (kg)
N = Number of days in the period
D = Average biomass density (g/m^2)
A = Surface area of the water (m^2).

In addition to the collected samples, fresh duckweed biomass was removed every two days at rates of approximately 50 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 the qualitative evaluation of the biomass, samples of approximately 1 kg were collected every two weeks (total of 25 samples) and oven dried at 55 °C for 24 h. The duckweeds were weighed before and after drying to determine the moisture content. Subsequently, these samples were frozen and sent for laboratorial analysis and verification of crude protein content (CP%) in accordance with the Association of Official Analytical Chemists (Method 991.20) (AOAC, 2005). The data were statistically evaluated to estimate the protein production rates.

3. Results and discussion

3.1. Entire treatment system efficiency

During the studied period, approximately 1140 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 2 and 3). Most likely, this is because of the long HRT (more than 200 days), the high concentration of raw influent and the use of different treatment stages (with aerobic and anaerobic conditions) (Bortone, 2009). In addition, the pH values remained nearly neutral, suffering a mild acidification along the system stages (from 7.52 to 6.68). This pH range is expected for swine wastes; however, duckweed ponds usually have low pH levels compared to maturation ponds because of the algae growth inhibition (Skillicorn et al., 1993; Costa et al., 2009). Phosphorus retention in anaerobic units occurs mainly because of the settling of inorganic phosphate compounds under anaerobic conditions. Additionally, a high concentration of iron ions that are present in this region could contribute to the formation of these compounds. However, the greatest nutrient removal efficiency was observed in the duckweed ponds, which is discussed in the following sections.

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 was expected. This variation in raw waste composition can be seen in Table 3, as the high standard deviation from the median. Nevertheless, high treatment efficiency was observed through the stages of the system.

3.2. Duckweed ponds' efficiency

Nutrient removal was significant in the duckweed ponds in series (after both ponds), particularly for nitrogen, with efficiencies of approximately 98.3% and 98.8% for TKN and $NH_3{-}N$ removal,

Table 2
Nutrient removal efficiency, in all stages of the treatment system.

Nutrients	BD (%)	SP (%)	DP1 (%)	DP2 (%)	Total effic. (%)
$N{-}NH_3$	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.

Table 3
Mean values (median) and standard deviation of the concentration of variables in the effluent to all stages of the treatment system (N = 25 samples).

Parameters	Raw manure	BD	SP	DP1	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
TKN (mg/L)	7986 ± 9573	1622 ± 629	832 ± 435	44.7 ± 22.6	14.1 ± 10.6
$N–NH_3$ (mg/L)	1624 ± 1146	1159 ± 377	636 ± 321	28.2 ± 14.5	7.2 ± 6.1
$N–NO_2$ (mg/L)	–	0.03 ± 0.01	–	0.9 ± 1.2	–
$N–NO_3$ (mg/L)	–	–	0.1 ± 0.2	11.3 ± 21.8	1.4 ± 3.3
TP (mg/L)	1487 ± 898	215 ± 177	92 ± 99	10.0 ± 7.2	5.2 ± 6.1

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

respectively, and TP (efficiency of 94.5%). Although the concentration of nutrients varied greatly, primarily because of the raw manure variations and rain fluctuations that occurred during the experimental period, the efficiency remained high. The median values and standard deviation obtained during the experimental period can be observed in Table 3 and Fig. 2.

Most of the TKN load applied to the serial duckweed ponds was removed after one year. Moreover, approximately 260 kg of nitrogen was recovered from the water (Table 4). However, it is necessary to express the applied load in terms of the application rate to compare the results with other authors. In this way, the TKN surface application rate was approximately 46 kg/ha day, and the removal rate was 43.7 kg/ha day or 4.4 g/m^2 day (Table 5). Cheng et al. (2002a) reported the highest removal rates in their investigation of the nitrogen removal from swine waste by *Lemna 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 and Vermaat, 1998) and 1.2 g/m^2 day (Benjawan and Koottatep, 2007). One factor that influences the nitrogen removal efficiency is that most nitrogen is present as ammonium, released after organic matter degradation because of the anaerobic pre-treatment (TKN/$N–NH_3$ ratio = 3/2). Ammonia nitrogen is easily absorbed by duckweed, especially when the pH is near to neutral or slightly acidic, as in this system (Skillicorn et al., 1993; Caicedo, 2005).

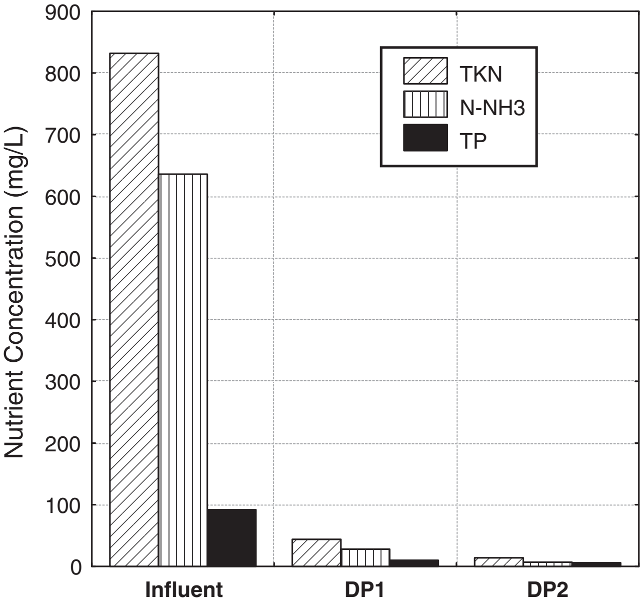

Fig. 2. Nutrient removal by the duckweed ponds. Mean values (median) for nutrient concentrations. n = 25 samples. (DP1 and DP2 = Duckweed ponds 1 and 2, respectively).

In full-scale treatment systems, duckweed ponds form an ecosystem with diverse organisms, in which the nutrient dynamics are complex compared to *in vitro* or pilot experiments. According to Zimmo et al. (2004), nitrogen elimination can occur in one of four ways: incorporation by duckweed biomass, nitrification and denitrification processes, sedimentation and volatilisation. In the present work, volatilisation and sedimentation were considered negligible because of the low pH levels and the lack of sludge formation. Also, minimal ammonia volatilisation in duckweed ponds has been confirmed by several authors including Van Der Steen et al. (1988), Zimmo et al. (2004), Caicedo (2005) and El-Shafai et al. (2007).

Analysis of the biomass nitrogen content demonstrated a percentage of 6.6% ± 0.8 of total nitrogen (dw), in average. Therefore, knowing total biomass produced was possible to calculate 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 duckweeds. Additionally, (72%) was removed by nitrification and denitrification processes (Fig. 3). Strong denitrification efficiency can be justified by several factors including aerobic and anoxic zones (2.1 mg DO/L on the surface and 0.5 mg DO/L on the bottom) always being present, a large area for a biofilm to attach, optimal pH and temperature ranges, and availability of food (BOD) for heterotrophic microorganisms (denitrificants). Also, nitrate (NO_3) was detected sometimes, usually during the summer, reaching 32 mg/L. However, because nitrate can be used by both duckweeds and denitrificant bacteria, the nitrate concentration ranged widely (Table 3).

However, in DP2, a different proportion was found; 96% of the total removed nitrogen was due to biomass absorption, and only 4% was caused by denitrification. The applied nitrogen load was larger in DP1 than DP2, so it is possible that almost all of the nitrogen applied to DP2 was required for duckweed growth. Hence, it can be concluded that at low nitrogen rates, the main removal

Table 4
Total nutrient loads applied to and removed from the duckweed ponds.

Ponds	Applied load (kg)			Removed load (kg)			Efficiency (%)		
	TKN	$N–NH_3$	TP	TKN	$N–NH_3$	TP	TKN	$N–NH_3$	TP
DP1	264.5	202.1	30.1	250.3	192.2	27.1	95.1	95.6	89.5
DP2	13.2	10.2	3.0	8.9	7.4	1.4	68.8	74.2	47.6
DP1 + DP2	264.5	202.1	30.1	259.2	199.6	28.5	98.3	98.8	94.5

DP1 and DP2 = Duckweed ponds 1 and 2, respectively.

Table 5
Nutrient rates (application and removal) in the duckweed ponds.

Duckweed ponds	Application rate (kg/ha day)			Removal rate (kg/ha day)		
	TKN	$N–NH_3$	TP	TKN	$N–NH_3$	TP
DP1	46.2	36.9	5.3	43.7	35.1	4.7
DP2	4.0	3.1	0.93	2.7	2.3	0.45
DP1 + DP2	29.3	22.4	3.9	28.8	22.2	3.6

DP1 and DP2 = Duckweed ponds 1 and 2, respectively.

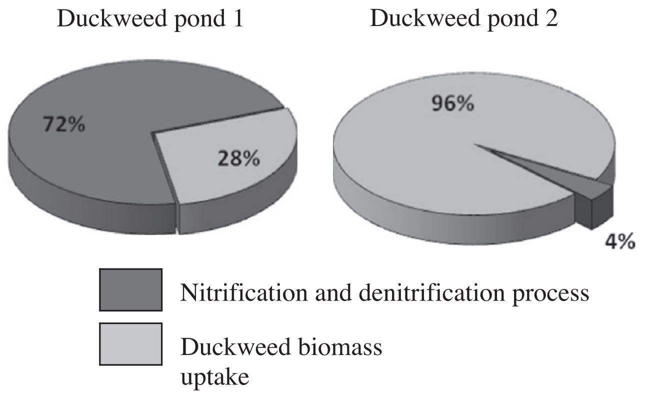

Fig. 3. Nitrogen balance. Percentage of nitrogen removal processes in the duckweeds ponds (DP1 and DP2 = Duckweed ponds 1 and 2, respectively).

process is biomass absorption, and at high loads, nitrification and denitrification become more important. In addition, the nitrogen removal efficiency was higher in DP1 than in the other stages (Table 2).

The ammonium concentrations obtained after load mixing (in DP1) was 97 mg $N–NH_3$/L on average, but during some periods with a high NH_3 concentration, it was observed to be 182 mg $N–NH_3$/L. This value was two times higher than the maximum concentration (50 mg/L) recommended by Caicedo (2005) for a *S. polyrrhiza*-based treatment. On the other hand, Cheng et al. (2002a) reported that duckweed (*L. punctata*) grows well with an $N–NH_4^+$ concentration of 240 mg/L. These results demonstrate the robustness of *L. punctata* to grow on swine waste treatment ponds, as well as its ability to support high ammonia concentrations and to take up nitrogen.

Based on the reports by Bergmann et al. (2000a) 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. Additionally, 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*. However, under full-scale conditions, raw manure variances and the biomass harvest may more strongly affect the performance of duckweed ponds than do the seasons. In the present work, statistical relationships between the treatment efficiency and the seasons were not found.

The efficiency of phosphorus recovery was also higher in DP1 (approximately 90%) than in DP2 (Table 2). However, unlike nitrogen, phosphorus was strongly reduced in the anaerobic stage, probably because of sedimentation (Table 3). The phosphorus loads applied and removed were 30 and 28.5 kg, respectively, in the 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 mgP/m^2 day by *Lemna 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 requirements, initial concentrations of P and N and the plant growth rate with varying temperatures and in the presence of toxic compounds (Cheng et al., 2002a). However, the present data showed that nitrifications/denitrification processes were improved by duckweed mat, may strongly affect the difference in removal rate between N and P, mainly in DP1. Al-Nozaily et al. (2000), reported TP removal rates close to 95 mg/m^2 day for duckweed ponds receiving effluent produced by a UASB reactor, five times less than what is reported here.

It was concluded that during the studied period, approximately 260 kg of nitrogen and 28.5 kg of phosphorus were recovered by the duckweed ponds, preventing nutrient overflow to the environment.

3.3. Biomass production and protein content

After one year, the duckweed biomass was removed at 27 kg/day (fw) from DP1 and 7.5 kg/day (fw) from DP2, on average. However, the harvest frequency fluctuated according to the biomass production, which is affected by many factors, such as temperature, biomass density, photoperiod, toxic compounds, and nutrient availability. Therefore, the total biomass production in the duckweed ponds was greater than 13 tons (fresh weight), of which 10.3 tons was removed from DP1 and 2.8 tons from DP2. The moisture average percentage was 90.1% ± 2.2 with slight variation. Thus, the average yield was 181 g/m^2 day (fresh weight) or 18 g/m^2 day (dry weight) from DP1. For DP2, the estimated growth rate was 83 g/m^2 day (fresh weight) or 8.3 g/m^2 day (dry weight). This difference between DP1 and DP2 production was mainly due to the different nutrient loads, which was higher in DP1. Other authors reported large variations in growth rates (dry weight) for several species, for example, 5.5 g/m^2 day (Körner et al., 1998), 13.8 g/m^2 day (El-Shafai et al., 2007), 15.1 g/m^2 day (Alaerts et al., 1996), 29 g/m^2 day (Cheng et al., 2002b) and 32 g/m^2 day (Cheng et al., 2002a). The maximum yield was obtained in DP1, which had the capacity to generate 68.8 ton/ha year (dry weight), on average. This production is a good value for full-scale experiments. For example, El-Shafai et al. (2007) reported a production of 33 tons (dw) of *L. minor* and *L. gibba* biomass after 8 months growing in a UASB reactor effluent. Tavares et al. (2010), working with domestic wastewater polishing, found a biomass productivity of approximately 50 t/ha (dw), for *Lemna valdiviana*. However, the specific growth rate (SGR) was high in DP1 (0.24 g/g on average), indicating that each gram of duckweed biomass produces nearly 0.24 g per day. In DP2, the SGR was lower (approximately 0.15 g/g). The SGR in DP1 was similar to those cited by Driever et al. (2005), Landsman et al. (2005) and Caicedo (2005), which were 0.3, 0.2 and 0.28 g/g, respectively.

Duckweed biomass density is an important parameter for protein productivity, as is the nutrient uptake efficiency (Landesman et al., 2005; Driever et al., 2005). However, during the entire period, a large range of biomass density was observed. On average, the duckweed biomass density was 752 g/m^2 in DP1 and 560 g/m^2 in DP2 (fresh weight); however a great variation can be seen in Fig. 4. In full-scale systems, a range of densities is normal and

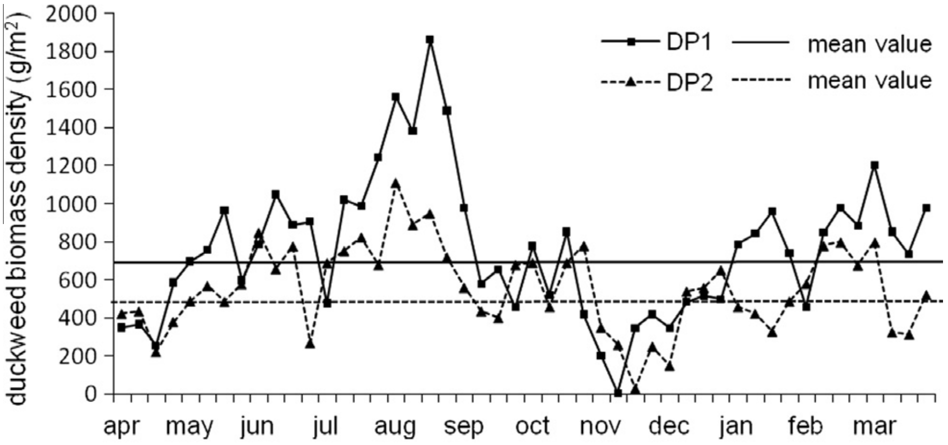

Fig. 4. Duckweed biomass density and mean values during the assessed period (DP1 and DP2 = Duckweed ponds 1 and 2, respectively).

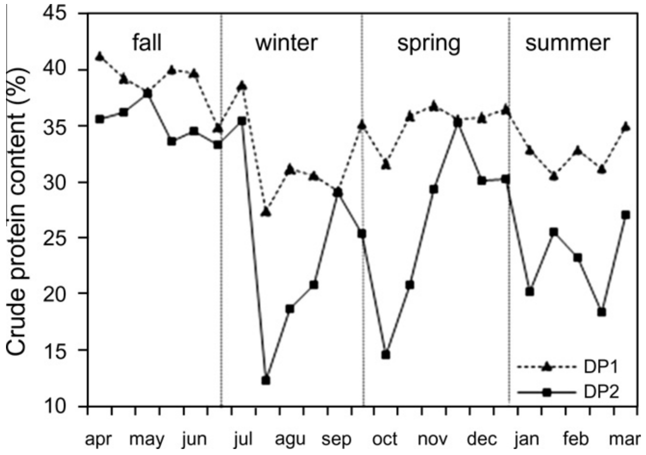

Fig. 5. Seasonal duckweed crude protein content range in the ponds (DP1 and DP2 = Duckweed ponds 1 and 2, respectively).

expected, predominately because of the climate conditions, nutrient availability, harvest frequency and endogenous factors such as flowering. It was observed that at both low and high biomass densities, the effluent quality and protein content became worse. Driever et al. (2005) reported a similar result observing that at high and low densities (greater than 180 g/m^2 or lower than 9.0 g/m^2 dry weight), the net growth rate became negative. For example, during high-density periods (August – early spring) grey spots were seen scattered in the floating duckweed coverage, indicating mortality (Fig. 4). Contrarily, the biomass density became low in November (late spring), with high water temperatures (reaching 30 °C), which sparked flowering. Is possible that during flowering, duckweeds plants deviate their metabolic energy, and vegetative growth is reduced. Therefore, in full-scale open experiments, plants are subject to environmental variations, which affect their reproductive cycle as in wild environments.

The average protein contents of the duckweed biomasses in DP1 and DP2 were 35% and 28% crude protein (CP), respectively. However, the CP in the harvested biomass reached greater than 40% at beginning of the experiment in DP1 (Fig. 5). The protein yield was greater in DP1, probably because of a 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). The range of CP contents during the period can be seen in Fig. 5. El-Shafai et al. (2007) found a protein yield of approximately 11.1 t/ha year (*Lemna gibba*) and estimated a biomass value of US $6600.00/year, based on other feed sources. In addition, the protein production appears to have been negatively affected by the high biomass density. After statistical analysis between the protein content and biomass density, it was possible to verify a decay tendency; however a weak correlation was found ($r^2 = 0.73$).

4. Conclusions

The duckweed ponds revealed, under the presented conditions, a great potential for polishing and valorisation of piggery waste. The observed nitrogen removal rates were one of the highest reported in the literature. The biomass produced during the treatment showed a high protein content and a fast growth rate. Possibly, the warm Brazilian climate can improve the duckweed growth rate, but under full-scale conditions, raw manure variances and harvests may strongly affect duckweed performance. Thus, this technology should be better exploited to improve the sustainability of pig farms to minimise the impact of this pig farming on the environment.

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