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Effects of the Hydraulic Retention Time on Pig Slurry Purification by Constructed Wetlands and Stabilization Ponds

M. A. Muñoz · R. M. Rosales · M. Gabarrón · A. Faz · J. A. Acosta

Abstract The overuse of pig slurry for fertilization purposes could involve an environmental risk. Pig slurry has been scarcely treated using constructed wetlands and stabilization ponds. Further information on hydraulic retention time comparison at full-scale in farms is desired. This survey aims to optimize a low-cost system comparing two hydraulic retention times (3 and 7 days) to purify pig slurry. Physical, chemical and microbial parameters were tested. A mechanical separator provided homogenous influent to feed the constructed wetland. Seven days of retention presented higher COD and N removal while 3 days of retention was more effective to remove TP and SO_4^{2-} in the constructed wetland. However, higher removal efficiencies were registered performing 7 days of retention for Mn (148.1 %), TP (113.4 %), KN (102.6 %), COD (102.5 %), NH_4^+-N (94.0 %), TC (87.9 %), Cu (64.2 %), FS (47.4 %), NO_3^- (36.6 %), Ca^{2+} (32.1 %), and Br^- (26.0 %) in the whole system, pointing out the positive effect of the storage pond. Though the main potential pollutants were effectively reduced, parameters such as Fe, SO_4^{2-}, SS, Zn and NO_2^- increased after purification.

1 Introduction

The agricultural sector has become more intensified in the last decade worldwide. The sustainable management of wastewater from livestock is an important concern and the implementation of friendly environmental techniques to treat them are highly demanded (Science for the Environment Policy 2013). The international normative regulates the spreading of pig slurry in agricultural soils based on the annual N contribution (EEC 1991). Spain is the second pig producer in the European Union with 25,494,715 of heads (MAGRAMA 2015). Bayo et al. (2012) estimated 6.5 Hm^3 of slurries produced in Murcia Region (SE Spain). In these regions with high farm concentration, the spreading of the pig slurry previously needs treatment for removing N to respect the international normative on nitrates (Tambone et al. 2015).

The high cost of the fertilizers and the progressive degradation of soils and their natural fertility have triggered the application of the pig slurry in agricultural soils (Delgado et al. 1999; Antolín et al. 2005; Sánchez and González 2005). However, its overuse could involve an environmental risk due to water pollution caused by nitrate leaching, eutrophication and air and

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soil pollution, becoming a potential pollutant (Gómez-Garrido et al. 2014; Tambone et al. 2015).

On the other hand, the majority of the studies focused on the wastewater purification have been conducted on municipal or industrial wastewaters (Larse et al. 2004; Wallace and Kadlec 2005; Domingos et al. 2007; Rai et al. 2015) while wastewaters from livestock are being left to one site. The use of plants to purify water (phytoremediation) in systems such as constructed wetlands (CWs) has emerged as a low-cost, non-invasive and publicly acceptable way to remove environmental pollutants (Pastor et al. 2003; Vymazal 2005; Sehar et al. 2015). Moreover, CWs and stabilization ponds are low-cost technology for wastewater treatment. Photosynthetic reactions of plants and algae and biological, physical and chemical mechanisms occur in them. On the other hand, the hydraulic retention time (HRT) is considered one of the main operation parameters to achieve the best performance of the CWs (Sehar et al. 2015). The literature refers to 7 days as an HRT frequently used in full-scale CWs (Fernandes et al. 2015), whereas Borin et al. (2013) established 4–5 days in hybrid (vertical + horizontal) CW. However, longer retention requires more available land and facilities to treat the same volume of wastewater. In order to optimize the operational conditions of the purification system, the recommended HRT was reduced to 3 days and it was compared with 7 days of retention. No further studies on the comparison of HRT treating pig slurry have been conducted at full-scale in farms.

Previous surveys have pointed out that the purification process continued when industrial wastewaters or landfill leaches are disposed in stabilization ponds (Polprasert and Kittipongvises 2011; Fernandes et al. 2013). However, scarce studies have been carried out using wastewaters from livestock, particularly pig slurries in storage ponds (Caballero-Lajarín et al. 2015). With the aim to gain knowledge about the suitability of the final purified pig slurry to be reused as organic fertilizer, physical, chemical and microbial parameters were studied. This survey aims to optimize a low-cost system comparing two HRT (3 and 7 days) in order to use the pig slurry as organic fertilizer. It is hypothesized that higher HRT will trigger higher removal of potential pollutants. The information generated from this study will be essential to optimize the operational conditions of a low cost purification system to treat livestock wastewaters in a real farm. It will be a noticeable contribution to obtain an environmentally friendly organic fertilizer from livestock wastewater.

2 Materials and Methods

2.1 Study Zone and Purification System

The study zone was located in a farm at the Lorca municipality (Murcia Region, SE Spain) under the Mediterranean climate. The coordinates were 37° 39′ 47.8″ N and 1° 33′ 08.1″ W. The mean temperature and rainfall were 17.5 °C and 119.8 mm, respectively, for the period of the experiment (SIAM 2016). The mean evapotranspiration was 1110 mm with a very high hydric deficit. The farm had 300 heads of mothers with piglets from Duroc-Jersey breed with an average weight of 300 kg. The experiment was conducted from September to December 2014.

The purification system consisted of an open concrete tank (200 m^3) to collect the raw pig slurry directly from the livestock housing in a first step. The pig slurry was maintained in this tank over 48 h. After that, the raw influent was driven by a pump through a fan press screw (mechanical separator) of 5 m^3 h^{-1} (Westfalia, Germany) to remove solids, and then, the liquid phase was transferred into a second concrete tank (200 m^3) where it was maintained over 48 h to stabilize it (Fig. 1). Table 1 shows the characterization of the raw pig slurry and after the mechanical separator performance as well as the efficiency on this step.

Then, the influent was conducted to a waterproofed pond (100 m^3) where it was stored for 24 h before feeding the CW. The constructed wetland consisted of three cells of $27 \times 2.5 \times 1$ m deep with horizontal subsurface flow (HSFCW) and a bottom slope of 1 %. Each cell contained 80 cm of gravel from limestone rocks (diameter 23–40 cm) consisting of hydrated carbonates and alkali and alkaline earth metals and 20 cm of washed sand in the surface layer. *Phragmites australis* (Cav.) was planted with a density of ten plants m^{-2} based on results from recent studies with similar HSFCW used to purify pig slurry (Caballero-Lajarín et al. 2015). All the plants were certified to avoid physiopathies and exhibited the same height in order to homogenize the behaviour of the three cells as
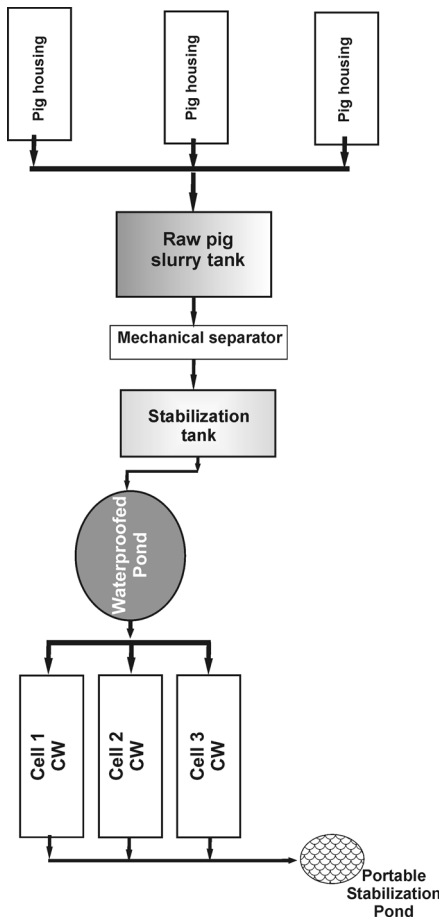
Fig. 1 Purification system layout (plan view)

Table 1 Efficiency of purification (E) using the mechanical separation (Mechan. Separ.)

Parameters	Raw	Mechan. Separ.	E (%)
T (°C)	15.3 ± 0.2	16.8 ± 0.1	−9.8
pH	7.4 ± 0.0	7.7 ± 0.0	−4.1
EC (dS m^{-1})	27.8 ± 0.0	19.6 ± 0.0	29.5
SS (mg L^{-1})	1.0 ± 0.3	2.3 ± 0.4	−130.0
TSS (mg L^{-1})	3052 ± 668	2903 ± 675	4.9
COD (mg L^{-1})	32033 ± 2196	9907 ± 851	69.1
KN (g L^{-1})	2.8 ± 0.2	1.6 ± 0.1	42.9
NH$_4$−N (g L^{-1})	2.5 ± 0.1	1.4 ± 0.0	44.0
NO$_3^-$ (mg L^{-1})	110.7 ± 15.3	94.9 ± 11.7	14.3
TP (mg L^{-1})	129.6 ± 10.6	84.5 ± 4.4	34.8
Na$^+$ (mg L^{-1})	1148.8 ± 37.3	998.2 ± 43.0	13.1
K$^+$ (mg L^{-1})	1930 ± 62.2	1292 ± 25.7	33.1
Ca^{+2} (mg L^{-1})	279.7 ± 33.6	149.8 ± 13.1	46.4
Mg^{+2} (mg L^{-1})	254.7 ± 8.4	246.0 ± 4.4	3.4
Cl$^-$ (mg L^{-1})	4926.7 ± 512.4	3822.9 ± 695.4	22.4
Br$^-$ (mg L^{-1})	97.2 ± 7.8	79.8 ± 11.2	17.9
SO$_4^{-2}$ (mg L^{-1})	97.3 ± 7.2	55.2 ± 3.8	43.3
F$^-$ (mg L^{-1})	852.5 ± 49.8	304.4 ± 33.6	64.3
NO$_2^-$ (mg L^{-1})	1.7 ± 0.3	1.9 ± 0.6	−11.8
Cu (mg L^{-1})	2.4 ± 0.2	1.4 ± 0.0	41.7
Fe (mg L^{-1})	8.2 ± 0.2	3.5 ± 0.1	57.3
Mn (mg L^{-1})	1.0 ± 0.3	0.3 ± 0.0	70.0
Zn (mg L^{-1})	6.2 ± 0.1	4.4 ± 0.1	29.0
MA (Log (UFC mL^{-1})	7.3 ± 0.0	6.9 ± 0.0	5.3
TC (Log (NMP mL^{-1})	3.3 ± 0.0	3.9 ± 0.2	−17.8
FC (NMP mL^{-1})	<3	<3	0
FS (Log NMP mL^{-1})	5.7 ± 0.0	4.2 ± 0.1	26.2
E. coli	Positive	Positive	0
Salmonella sp.	Positive	Positive	0
Shighella sp.	Positive	Positive	0

EC electrical conductivity, *SS* settleable solids, *TSS* total suspended solids, *COD* chemical oxygen demand, *KN* Kjedahl nitrogen, *TP* Total phosphorous, *MA* mesophilic aerobic, *TC* total coliforms, *FC* faecal coliforms, *FS* faecal streptococcus, *E. coli* Escherichia coli

purification units. Before starting the experiment, *P. australis* was kept planted over two months in the HSFCW using irrigation water to promote its adaptation. Finally, the purified effluent was in part conducted to two different portable ponds, one of each HRT, where it was stored over 10 days (Fig. 1). The capacity of each portable stabilization pond was 12 m^3. The rest of the purified effluent was kept in a general storage pond to be applied on agricultural soils in the farm. The times of residence in the concrete tanks and ponds were selected considering the mean times that the farmers usually manage in the Murcia Region when the pig slurry is used as fertilizer. Moreover, the HSFCW was used over 4 years to purify pig slurry from the same farm before starting this survey, thereby biofilm development was expected. Conversely, since decaying plant was observed in some zones, a new plantation of *P. australis* was carried out in the HSFCW.

2.2 Experimental Design

Two different HRTs were considered (7 and 3 days) to perform the HSFCW under field conditions. Both HRTs were tested three times following 3 cycles of filling and empting in the three cells of the HSFCW which were continuously maintained under saturated

conditions. The influent hydraulic load was 25.3×10^{-3} m^3 m^{-2} day^{-1} for HRT = 7 days and 59.3×10^{-3} m^3 m^{-2} day^{-1} for HRT = 3 days. One sample was collected at the entrance of each cell when they were filled. Another one sample was collected at the exit of each cell when they were emptied. Three samples from each cell were pseudo-replicates. Samples were collected 10 min after opening the keys to fill or empty the cells to ensure a homogeneous flow. Taking into account the three cycles of treatment per HRT and the three replicates, the total number of samples per treatment was nine at the entrance and other nine samples at the exit.

The purified influent from the HSFCW was directly transferred from the exit of each cell to the portable pond until 4 m^3 for each cycle of treatment. The three cycles of treatments completed 12 m^3 in each pond for each HRT. Three samples were collected in each stabilization pond 10 days after the whole filling. This design ensured representative samples from the HSFCW and the ponds. Samples were collected using 500-mL sterilized vials and maintained at 4 °C and then analysed in a short time.

2.3 Physical, Chemical and Microbial Parameters

The following parameters were determined on site: temperature (T) and pH, using a handle probe (Hanna model, HI 9025, Spain), electrical conductivity (EC) using (Hanna, HI 9033, Spain) and settleable solids (SS) were determined in an Inhoff vessel, after 60 min (2540-F method, APHA-AWWA-WEF 2012). The next parameters were determined in the laboratory following APHA-AWWA-WEF (2012) methodology with the modifications suggested by Peters et al. (2003) for pig slurry. Total suspended solids (TSS) were measured using a vacuum pump and filters of 0.45 μm. Chemical oxygen demand (COD) was determined by photometric determination of chromium (III) concentration after 2 h of oxidation with potassium dichromate/sulphuric acid and silver sulphate at 148 °C (Macherey-Nagel GmbH & Co. KG. Nanocolor Test, Ref 985 028/29), (DIN 38 409 - H41 - 1, DIN ISO 15 705 - H45).

Total phosphorus (TP) was photometrically determined as molybdenum blue after acidic hydrolysis and oxidation at 120 °C (Macherey-Nagel GmbH & Co. KG. Nanocolor Test; ref 985 055). Copper (Cu) and zinc (Zn) were measured after an acid digestion by atomic absorption spectrophotometry (A-Analyst 800 Perkin Elmer).

Total nitrogen (TN) was determined by the Kjeldahl method (Duchaufour 1970), modified using 5 mL of pig slurry in the digestion. Ammonium nitrogen (NH$_4^+$-N) was determined by steam distillation and titration with HCl 0.1 N. Nitrate (NO$_3^-$) was measured by photometric determination with 2–6 imetilphenol in sulphuric acid/phosphoric acid mixture (Macherey-Nagel GmbH & Co. KG. Nanocolor Test; ref 985 064). Ions (Cl$^-$, Br$^-$, SO$_4^{-2}$, F$^-$ and NO$_2^-$) were analysed by high-performance ion chromatography (IC) (Methorm, model 861). Cations (Na$^+$, K$^+$, Ca^{2+} and Mg^{2+}) were determined using atomic absorption spectrometer (Perkin Elmer AA-Analyst, 800).

Microorganisms were determined by preparing serial dilutions of samples with autoclaved peptone water. Recount of mesophilic aerobic (MA) bacteria was determined by culturing each sample with trypticase soy agar (31 °C over 72 h). Total coliforms (TC) were determined by culturing each sample in triplicate in Brilliant Green Bile Lactose Broth (BGBL) 2 % tubes (37 °C over 24/48 h). Faecal coliforms (FC) were determined by culturing the positive total coliform tubes in BGBL 2 % tubes (44.5 °C over 24–48 h). Faecal streptococcus (FS) was determined by culturing each sample in triplicate in Kanamicina-aesculina-azida (37 °C over 24–48 h). *Escherichia coli* (*E. coli*) was determined by culturing FC with eosin methylene blue (37 °C over 24–48 h). *Salmonella sp.* and *Shigella sp.* were determined by a selective enrichment in selenite cystine broth. These three parameters were confirmed by API 20 E gallery.

2.4 Statistical Analysis

The normality of the data distribution was studied by Shapiro-Wilk's Test (Shapiro and Wilk 1965). Transformations into Ln were carried out to achieve normality. Bartlett's Test confirmed the homogeneity of the variance. Principal components analysis (PCA) was carried out to identify the causes of variability. After this, one-way ANOVA followed by post hoc Tukey's Test at $P < 0.05$ were completed to identify significant differences through the comparisons of all possible pairs of means considering the results at the entrance and at the exit of the cells for 7 and 3 days. To assess possible differences between the exit for 7 and 3 days and the purified pig slurry in the stabilization pond as well as the sum of efficiencies for HRT = 7 and 3 days, data were analysed by two sample t test for paired samples

(Statistix 9.0). Principal component analysis (PCA) was applied to study the variation of the properties determined in the pig slurry at the entrance and at the exit for 7 and 3 days of retention in the HSFCW, extracting the first two principal components (PCs) corresponding to the entrance and exit for a HRT of 7 and 3 days (SPSS 21.0).

3 Results

3.1 Purification at the HSFCW at 7 Days of Retention

Based on the standard deviation values (Table 2), all three cells provided stable treatment performances for both studied HRT. According to the Tukey test results (Table 2), significant variations after the HSFCW with HRT = 7 days was observed for COD, KN, NH_4^+-N, Na^+, Ca^{2+}, SO_4^{2-}, Fe, Mn and Zn ($P < 0.05$). Efficiencies for COD, KN, NH_4^+-N removal were above 30 %. Accordingly, Table 1 shows removal efficiencies in the mechanical separator for COD (69.1 %), KN (42.9 %) and NH_4^+-N (44.0 %).

In the case of Na^+, Ca^{2+}, SO_4^{2-}, Fe and Zn significant differences were found between the entrance and the exit and efficiencies were negative; thereby, the contents of these elements were higher at the exit of the HSFCW. Conversely, positive removal efficiencies were observed in the mechanical separator for Fe (57.3 %) and Ca^{2+} (46.4 %).

The PCA exhibited differences at the entrance and at the exit for HRT = 7 days (Fig. 2 a, b). PC 1 and 2 explained 67.2 % of the variation at the entrance and 54.6 % at the exit. The PC 1 grouped SS, TSS, KN, NH_4^+-N, NO_2^-, Br^-, F, Fe, K^+, SO_4^{2-}, Cu and Zn at the entrance. It grouped EC, K^+, Br^-, NH_4^+-N and KN at the exit with positive correlations, whereas T, SO_4^{2-}, Mg and Fe presented negative ones at the exit.

The PC 2 grouped T, COD, Na^+, Ca^{2+} and Mg^{2+} at the entrance with positive correlations while for pH the correlation was negative (Fig. 2 a, b). PC 2 grouped TSS, Na^+, Ca^{2+}, Cl^-, NO_2^- and Mn with positive correlations while correlations for T, SS, and NO_3^- were negative at the exit. All these properties were significant in the explanation ($P < 0.05$) of the data variation.

3.2 Purification at the HSFCW at 3 Days of Retention

Significant variations ($P < 0.05$) between the entrance and the exit in the HSFCW were registered for T, KN, Total P, Ca^{2+}, Fe, Mn and Zn for HRT = 3 days (Table 2). For these parameters, positive efficiencies over 30 % were merely observed in the case of TP in accordance with the mechanical separator results (Table 1), while the negative ones were assessed for Ca^{2+}, Fe and Mn (Table 2).

Figure 1c, d) shows PC1 and PC2 results, explaining 59.4 % of the variation at the entrance and 51.9 % at the exit. PC1 grouped the EC, COD, KN, TP, F^-, NO_2^- and Mn with positive correlations. Negative correlations were registered for TSS, Fe, SO_4^{2+} and KN at the entrance. This component grouped TP, Mn, Ca^{2+}, Na^+, K^+, Fe and Mn with positive correlations, whereas the negative ones were observed for KN, NH_4^+-N, EC and pH ($P < 0.05$).

PC 2 grouped pH, SS, NO_3^-, K^+, Cu and Zn at the entrance with positive correlations and T and Ca^{2+} with negative ones. In the exit, the positive correlation was observed for COD, SS, F^- and Mg, while negative ones were registered for NO_3^-, NO_2^- Cu and Zn (Fig. 2c, d). All these properties were significant in the explanation ($P < 0.05$) of the data variation.

3.3 Comparison Between 7 and 3 Days of Retention at the HSFCW

Comparison among entrances for 7 and 3 days pointed out similarities for most of the studied parameters in the influent with the exception of T, EC, NO_3^- and F^- (Table 2). Significant differences were registered for KN, NO_3^-, Ca^{2+}, Mg^{2+}, Br^-, SO_4^{2-}, F^- and Mn ($P < 0.05$) in the effluents after 7 and 3 days of retention.

Based on the differences between the effluent from 7 to 3 days, we could infer higher removals for 7 days, mainly for N (KN, N-NH_4^+ and NO_3^-) and COD. However, the HRT = 3 days presented significantly higher SO_4^{2-} removal. Conversely, significantly increases of Na^+, Ca^{2+}, SO_4^{2-}, Fe and Zn were registered for 7 days of retention. Increases were significant for T, Ca^{2+}, Fe and Mn at 3 days of retention.

3.4 Purification at the Stabilization Ponds

Table 3 shows the physico-chemical properties on the pig slurry from the exit of the HSFCW after 7 days of

Table 2 Efficiency (*E*) of purification atn the HSFCW for TRH = 7 days and TRH = 3 days

Parameters	Entrance 7 days	Exit 7 days	Entrance 3 days	Exit 3 days	*E* 7 days (%)	*E* 3 days (%)
T (°C)	20.8 ± 5.1 a	18.2 ± 1.3 ab	14.7 ± 1.3 b	18.9 ± 2.0 a	12.5	−28.7
pH	7.7 ± 0.1 ab	7.6 ± 0.1 b	7.9 ± 0.1 a	7.8 ± 0.1 ab	2.2	2.4
EC (dS m^{-1})	15.9 ± 1.6 a	14.7 ± 1.4 ab	14.3 ± 1.4 b	13.5 ± 0.3 b	7.8	5.6
SS (mg L^{-1})	0.5 ± 0.4 n.s.	0.9 ± 0.5 n.s.	1.1 ± 0.5 n.s.	0.6 ± 0.3 n.s.	−71.9	44.9
TSS (mg L^{-1})	1497 ± 344 n.s.	1076 ± 571 n.s.	1479 ± 571 n.s.	1056 ± 630 n.s.	28.1	28.6
COD (mg L^{-1})	5123 ± 1048 a	3338 ± 340 c	4372 ± 481 ab	3810 ± 255 bc	34.8	12.9
KN (g L^{-1})	1.0 ± 0.1 ab	0.6 ± 0.2 c	1.0 ± 0.1 a	0.8 ± 0.1 b	35.5	17.5
NH$_4$–N (g L^{-1})	0.8 ± 0.1 a	0.5 ± 0.0 b	0.9 ± 0.0 a	0.7 ± 0.1 a	38.2	13.8
NO$_3^-$ (mg L^{-1})	104.8 ± 14.5 a	96.0 ± 25.9 ab	61.0 ± 25.9 bc	69.4 ± 38.7 c	8.5	−13.7
TP (mg L^{-1})	67.4 ± 18.8 a	57.9 ± 13.7 ab	73.9 ± 13.7 a	44.0 ± 13.3 b	14.0	40.5
Na$^+$ (mg L^{-1})	794.0 ± 39.1 b	857.7 ± 41.2 a	809.9 ± 41.2 b	832.9 ± 28.0 ab	−8.0	−2.8
K$^+$ (mg L^{-1})	779.2 ± 52.6 n.s.	927.6 ± 135.8 NS	922.31 ± 135.8 NS	913.5 ± 44.2 NS	−19.0	0.9
Ca^{+2} (mg L^{-1})	108.2 ± 24.3 bc	157.2 ± 12.7 a	78.3 ± 12.7 c	118.0 ± 38.7 b	−45.3	−50.7
Mg^{+2} (mg L^{-1})	227.2 ± 16.1 ab	239.8 ± 11.0 a	213.4 ± 11.0 bc	203.9 ± 8.7 c	−5.6	4.5
Cl$^-$ (mg L^{-1})	1338 ± 3 b	1392 ± 84 ab	1404 ± 58 ab	1425 ± 60 a	−4.0	−1.5
Br$^-$ (mg L^{-1})	26.0 ± 3.0 ab	23.8 ± 2.2 b	28.5 ± 2.2 a	27.5 ± 2.0 a	8.7	3.6
SO$_4^{-2}$ (mg L^{-1})	14.9 ± 3.4 b	112.0 ± 25.9 a	37.9 ± 25.9 ab	13.8 ± 3.4 b	−652.9	63.5
F$^-$ (mg L^{-1})	66.8 ± 11.6 a	43.7 ± 13.0 a	16.7 ± 13.0 b	11.8 ± 8.8 b	34.6	29.3
NO$_2^-$ (mg L^{-1})	0.6 ± 0.2 a	0.4 ± 0.1 ab	0.4 ± 0.1 ab	0.3 ± 0.1 b	31.5	25.2
Cu (mg L^{-1})	0.6 ± 0.2 NS	0.6 ± 0.0 n.s	0.7 ± 0.0 NS	0.6 ± 0.1 NS	4.3	14.5
Fe (mg L^{-1})	1.7 ± 0.2 b	4.2 ± 0.1 a	1.8 ± 0.1 b	4.2 ± 0.4 a	−581.7	−131.9
Mn (mg L^{-1})	0.6 ± 0.2 c	0.6 ± 0.0 a	0.1 ± 0.0 c	0.4 ± 0.2 b	3.5	−203.4
Zn (mg L^{-1})	1.5 ± 0.2 ab	1.2 ± 0.1 c	1.7 ± 0.1 a	1.3 ± 0.2 bc	−93.0	21.9

Different letters indicate significant differences among zones (*P* < 0.05) according to Tukey's Test

EC electrical conductivity, *SS* settleable solids, *TSS* total suspended solids, *COD* chemical oxygen demand, *KN* Kjedahl nitrogen, *TP* total phosphorous, *NS* not significant

retention and on the pig slurry storage at the pond after 10 days of stabilization. The comparison exhibited statistical significant differences (*P* < 0.05) in all the parameters with the exception of T, pH, EC and Fe. Positive efficiencies of removal over 30 % were identified for SS, Ca^{2+} and Mn. In contrast, negative efficiency over 30 % was registered for NO$_2^-$.

In the case of HRT = 3 days, the comparison between the exit of the HSFCW and the pond showed significant differences in the studied parameters with the exception of T, pH, EC, TP, Br$^-$ and SO$_4^{2-}$. Positive efficiencies over 30 % for SS, TSS, Ca^{2+} and Mn were identified. No negative efficiencies over 30 % were observed in those parameters with statistical significant differences. The comparison between the purified pig slurries from 7 to 3 days kept in the pond pointed out significant differences in the parameters with the exception of TP, Br$^-$ and F$^-$ (Table 3).

Related to the microbial determinations (data not shown) MA for 7 and 3 days of retention were 6.7 ± 0.1 and 6.3 ± 1.1 Log CFU mL^{-1}, respectively. Total coliforms were 0.4 ± 0.0 and 1.4 ± 0.0 Log MPN mL^{-1}, respectively. Faecal coliforms were <3 MPN mL^{-1} in both cases. Faecal streptococci were 3.0 ± 0.6 and 3.5 ± 0.2 Log CFU mL^{-1}, respectively. The bacteria *Salmonella sp.* and *Shighella sp.* were positive while *E. coli* was negative for 7 days and positive for 3 days.

3.5 Total Efficiencies at the Purification System

The efficiencies found in the different stages of the treatment (mechanical separator + tanks + HSFCW + open pond) for 7 days of retention showed efficiencies over 30 % in the whole of the parameters with the exception of T, pH, TSS, Na$^+$, K$^+$, Mg^{2+} and Br$^-$ (Table 3). In contrast, negative efficiencies over 30 %
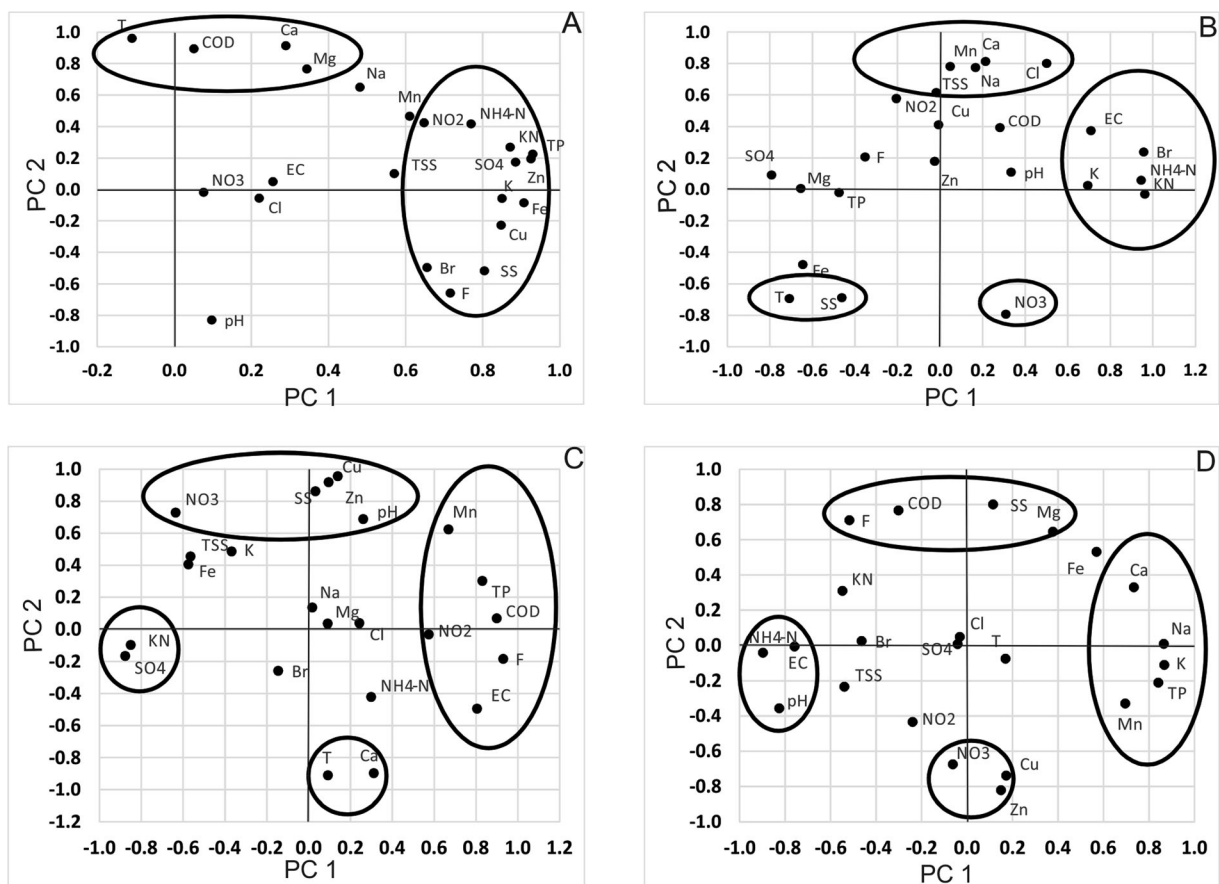
Fig. 2 Principal component analysis (PCA) performed with results at the entrance of 7 days of retention (**a**), at the exit of 7 days of retention (**b**), at the entrance of 3 days of retention (**c**) and at the exit of 3 days of retention (**d**) for each parameter. *PC1* principal component 1, *PC2* principal component 2

were observed for SS, SO_4^{2-}, NO_2^-, Fe and Zn. Considering 3 days of retention, the addition of efficiencies exhibited percentages over 30 % for all the parameters except for T, pH, SS, Na^+, Mg^{2+}, Cl^- and Br^-. Conversely, negative efficiencies over 30 % were identified for NO_2^-, Fe and Mn.

4 Discussion

4.1 Purification at the HSFCW at 7 Days of Retention

The parameters COD, KN and NH_4^+-N, showed the highest positive efficiencies with significant differences between the entrance and exit in the HSFCW. Knight et al. (2000) also registered a decrease in COD treating livestock wastewaters in CWs because of the biodegradation reactions of bacteria, as well as Caballero-Lajarín (2013) who reported 5371 and 1744 mg L^{-1} of COD at

the entrance and exit, respectively, after 1 month of retention of the pig slurry in a HSFCW. The aerobic and facultative bacteria decompose the organic matter using the oxygen produced by algae while this one uses the CO_2 released from the bacterial respiration to produce energy and fix carbon for growth via photosynthesis (Polprasert and Kittipongvises 2011). According to Polprasert et al. (1998), the biofilm bacteria growing on the substrate surface and the root zone are the major organisms responsible for the organic matter removal.

Regarding the legislation, special attention should to be paid to the N contents. Similar N removal percentages were reported by other authors. Zhang et al. (2015) assessed 40–50 % of KN removal, whereas Polprasert and Kittipongvises (2011) reported a range between 10 and 50 %. Nitrification and denitrification processes influence the KN and NH_4-N variation in CWs (Zimno et al. 2003). The oxygen from the atmosphere coupled with the oxygen leakage from the rhizosphere is

Table 3 Efficiency (E) of purification at the storage ponds for HRT = 7 and 3 days of retention in the HSFCW

Parameters	Pond HRT =7 days	E Pond 7 days (%)	Σ E7 days (%)	Pond HRT = 3 days	E Pond 3 days (%)	Σ E3 days (%)	t Student
T (°C)	15.0±0.3	17.7	20.4	15.7±0.3	16.6	−21.9	T7=−1.6 NS; T3=0.4 NS; T7−3=3.6*
pH	8.0±0.0	−5.4	−7.3	7.9±0.0	−1.5	−3.2	T7=−0.1 NS; T3=2.2 NS; T7−3=4.7**
EC (dS m⁻¹)	12.2±0.0	17.1	54.4	13.3±0.0	1.5	36.6	T7=−1.2 NS; T3=0.9 NS; T7−3=4.7**
SS (mg L⁻¹)	0.2±0.1	74.5	−127.4	0.2±0.0	66.7	−18.4	T7=4.1**; T3=6.2**; T7−3=19.3***
TSS (mg L⁻¹)	1340.0±764.6	−24.5	8.5	646.7±97.8	38.8	72.3	T7=−11.3***; T3=−6.9**; T7−3=−7.9**
COD (mg L⁻¹)	3386.7±123.4	−1.4	102.5	3576.7±265.6	6.1	88.1	T7=−13.9***; T3=−11.2***; T7−3=−21.4***
KN (g L⁻¹)	0.5±0.0	24.2	102.6	0.8±0.1	10.0	70.4	T7=5.2**; T3=6.9**; T7−3=35.3***
NH₄-N (g L⁻¹)	0.5±0.0	11.8	94.0	0.7±0.0	5.2	63.0	T7=5.8**; T3=7.2***; T7−3=32.0***
NO₃⁻ (mg L⁻¹)	82.7±16.8	13.8	36.6	90.0±9.2	−29.8	−29.2	T7=−5.1**; T3=−3.3*; T7−3=−4.6**
TP (mg L⁻¹)	20.5±5.3	64.6	113.4	37.2±2.2	15.4	90.7	T7=−2.2*; T3=−1.5 NS; T7−3=1.6 NS
Na⁺ (mg L⁻¹)	873.2±9.7	−1.8	3.3	826.4±27.8	0.8	11.1	T7=−10.9***; T3=−8.5***; T7−3=−13.7***
K⁺ (mg L⁻¹)	898.1±10.8	3.2	17.3	893.4±22.7	2.2	36.2	T7=−10.6***; T3=−8.5***; T7−3=−14.8***
Ca⁺² (mg L⁻¹)	108.6±2.6	31.0	32.1	76.9±4.3	34.8	30.5	T7=−5.5**; T3=−2.8*; T7−3=−3.3*
Mg⁺² (mg L⁻¹)	232.0±4.1	3.3	1.1	188.7±5.7	7.5	15.4	T7=−7.6***; T3=−4.9**; T7−3=−6.8**
Cl⁻ (mg L⁻¹)	1528.3±34.7	−9.8	8.6	1505.1±48.9	−5.6	15.3	T7=−12-3***; T3=−9.7***; T7−3=−16.5***
Br⁻ (mg L⁻¹)	23.9±0.7	−0.6	26.0	27.9±1.6	−1.4	20.1	T7=−2.6*; T3=−0.7 NS; T7−3=1.3 NS
SO₄⁻² (mg L⁻¹)	8.3±1.5	92.6	−517.0	20.5±1.6	−48.1	58.7	T7=−1.3*; T3=0.6 NS; T7−3=16.9***
F⁻ (mg L⁻¹)	22.7±5.1	48.0	146.9	3.7±0.6	68.2	161.8	T7=−2.3*; T3=2.2*; T7−3=2.1 NS
NO₂⁻ (mg L⁻¹)	0.6±0.0	−51.0	−31.3	0.6±0.1	−106.5	−93.1	T7=7.8***; T3=−3.3*; T7−3=16.1***
Cu (mg L⁻¹)	0.5±0.0	18.2	64.2	0.5±0.0	3.3	59.5	T7=5.4**; T3=7.8***; T7−3=19.9***
Fe (mg L⁻¹)	4.1±0.1	1.1	−523.3	4.6±0.1	−10.2	−84.8	T7=1.3 NS; T3=3.6*; T7−3=10.0***
Mn (mg L⁻¹)	0.2±0.1	74.6	148.1	0.1±0.0	79.1	−54.3	T7=4.2**; T3=6.1**; T7−3=15.1***
Zn (mg L⁻¹)	0.9±0.0	24.4	−39.6	1.2±0.1	10.3	61.2	T7=4.0*; T3=5.6**; T7−3=20.6***

EC electrical conductivity, *SS* settleable solids, *TSS* total suspended solids, *COD* chemical oxygen demand, *KN* Kjedahl nitrogen, *TP* Total phosphorous, respectively;

*, **, ***: significant differences among surface and surface at *P* < 0.05, 0.01 and 0.001, respectively; *NS* not significant

presented on the surface of the CWs which favours nitrification processes while anaerobic conditions are presented in the CW bed favouring denitrification (Yadav 2010; Doherty et al. 2015). However, no significant differences between the entrance and the exit were observed for NO_3^- and NO_2^- at HRT = 7 days.

Different surveys have showed that nitrate and nitrite removal also depend on nitrifying bacteria such as *Nitrosomonas sp*. and *Nitrobacter sp*. (Bock et al. 1989; Scholz 2006) which likely were not very active in our HSFCW explaining similar NO_3^- and NO_2^- contents before and after the HSFCW. For that reason, we can infer N plant uptake (Polprasert and Kittipongvises 2011) and adsorption in the substrate (Reddy and Patrick 1984) as the main removal mechanisms. Moreover, precipitation of NH_4^+-N favoured by the alkaline pH of the pig slurry (Phillips et al. 2000) and volatilization as Kadlec and Wallace (2008) suggested could be taking place. However, further studies should be carried out to gain understanding about these figuring out.

Conversely, Ca^{2+}, SO_4^{2-}, Fe, and Zn showed the highest negative efficiencies (higher than −30 %). Some authors described increases in the content of ions and metals in wastewaters treated using CW due to the interaction between the biofilm and the substrate which blocks the adsorption in the substrate (Kadlec and Knight 1996; Fynlayson and Chick 1983). On the other hand, Polprasert and Kittipongvises (2011) indicated precipitation and adsorption as predominant removal mechanisms of heavy metals. Precipitation enhances by increasing pH. However, no significant differences in the pH before and after the HSFCW treatment were observed indicating no precipitation. Regarding the negative efficiencies, it can be inferred that the substrate provided these elements to the effluent based on the limestone nature, which is composed by calcite crystals where metals are linked. Caballero-Lajarín et al. (2015) registered similar values of Ca^{2+} and Zn for pig slurry purified in a HSFCW and higher SO_4^{2-} contents.

PCA showed that PC1 and PC2 presented changes at the entrance and exit for HRT = 7 days. PC1 was associated to solids, TP, anions, metals and nitrogen. These properties could be correlated in the entrance while merely nitrogen (NH_4^+-N and KN), Br^-, SO_4^{2-} and Fe were grouped by this component at the exit. The high correlation between NH_4^+-N and KN has been widely described in the literature on pig slurry (Bonmati and Flotats 2003; Sánchez and González 2005). PC1

supported the efficiency results as elements such as SO_4^{2-} and Fe which increased significantly after the HSFCW.

PC2 seemed to refer those properties more correlated with alkalinity such as T, COD, Ca^{2+} and Mg^{2+} with a positive correlation, conversely to pH which exhibited a negative one. However, this pattern was partially observed merely for T, Ca^{2+} and Na^+ at the exit. Therefore, we could infer that the HSFCW treatment triggered the organic matter removal according to the COD efficiency and the statistical analyses. Conversely, cations responsible for alkalinity such as Ca^{2+} and Na^+ remained in the purified effluent. Another variable grouped by PC2 is T which influences highly the removal of some elements such as cations as it regulates the microbial function and the nutrient uptake (Molina-Grima 1999). However, T of the influent did not exhibit significant differences before and after the HSFCW. Authors such as Hiley (2003) highlighted that CWs cushion the variation of the environmental and influent T supporting our findings.

4.2 Purification at the HSFCW at 3 Days of Retention

The highest positive efficiencies were identified for TP. In connection with our results, percentages of removal from 40 to 90 % were found in some surveys (Anderson et al. 2005; Vymazal 2007; Polprasert and Kittipongvises 2011; Zhang et al. 2015). Our findings were consistent with Sehar et al. (2015) who described the presence of polyphosphorous accumulating bacteria (PAO) in the biofilm which use soluble phosphorous as a substrate. Moreover, P is removed by plant uptake and sedimentation (Polprasert and Kittipongvises 2011).

Sehar et al. (2015) assessed that further increase in HRT triggered no significant reduction in P contents as the maximum saturation of phosphate adsorption takes place in the substrate. In our case, although a decrease in P contents registered at 7 days of retention, no statistical significant differences were found between 7 and 3 days. In contrast, negative efficiencies were found for Ca^{2+}, Fe and Mn as were observed for HRT = 7 days, thereby the reduction of the HRT did not imply these element decreases. Conversely, they could be supplied by the substrate (Fynlayson and Chick 1983; Caballero-Lajarín et al. 2015).

PCA showed that PC1 grouped EC, TSS, COD, N, TP, F^-, Mn and NO_2^- at the entrance and EC, TSS, N, TP, F^- and Mn at the exit after 3 days of retention.

Therefore, this component correlated almost the same variables at the entrance and at the exit with the exception of COD and NO_2^-. This parameter did not show significant differences before and after the HSFCW and the efficiency of removal was lower than 30 % (Table 2). For that reason, we could not assess their significant removal after 3 days of retention in spite of PCA results. PC2 grouped T, pH, SS, Ca^{2+}, Cu and Zn at the entrance and, from these, SS, NO_3^- Cu and Zn at the exit. In this case, only Ca^{2+} and Zn presented significant differences before and after the HSFCW and negative efficiency for Ca^{2+}.

4.3 Comparison Between 7 and 3 Days of Retention at the HSFCW

Considering that significant differences were merely observed for T, EC, NO_3^- and F^- in the influent used to perform 7 and 3 days of retention, we assessed the homogeneity of the influent and its suitability to compare both HRTs.

The parameters with the highest removal and statistical significant differences for 7 days of retention were COD, KN and NH_4^+-N. Although these parameter efficiencies decreased at 3 days of retention in comparison with 7 days, merely KN showed significant differences between 7 and 3 days. Therefore, just for KN lower plant uptaking, adsorption, precipitation, biodegradation and volatilization when the HRT was 3 days could be inferred (Reddy and Patrick 1984; Phillips et al. 2000; Kadlec and Wallace 2008). Moreover, the decrease of the T in the influent during the experiment (from 20.8 ± 5.1 °C in the entrance of 7 days to 14.7 ± 1.3 °C in the entrance of 3 days) could also affect the inhibition of N removal at 3 days of retention as Zhang et al. (2015) suggested.

Therefore, higher removals were observed for 7 days of retention mainly for COD and N while HRT = 3 days was effective to remove TP and SO_4^{2-}. In contrast, significant increases of Ca^{2+}, Fe and Mn were observed for both HRT and additionally Na^+ and SO_4^{2-} also increased for 7 days.

4.4 Purification at the Stabilization Ponds

The storage of the pig slurry from HRT = 7 days in the pond was identified as a continuity of the purification treatment for most of the studied parameters with the exception of T, pH, EC and Fe with no significantly differences. The highest efficiencies were identified for SS, Ca^{2+} and Mn. Caballero-Lajarín et al. (2015) observed similar Ca^{2+} removal, higher SS and lower Fe removals.

Polprasert and Kittipongvises (2011) indicated ultraviolet (UV) light inactivation, high pH during day time because CO_2 is uptaken by algal cells, grazing by protozoa and sedimentation as the main removal mechanisms of stabilization in ponds. The sedimentation could explain the high SS content decrease coupled with the Ca^{2+} and Mn^{2+} reduction which could be absorbed in the solid precipitated at the bottom of the pond. Gerritse (1993) explained the P and Ca^{2+} decrease because of the precipitation of these elements as calcium phosphate when pH >6. Furthermore, Mashauri et al. (2000) suggested the calcium immobilization by microalgae. Other surveys point out that some Mn oxides could be retained in the sediment (Tam and Wong 1996). However, further studies focused on the analyses of the sediment and microalgae performance in the pond should be carried out to clarify this suggestion. In accordance with Polprasert and Kittipongvises (2011), pH increased in the pond although no statistical significant differences were reported. This increase could favour NH_4^+-N precipitation (Phillips et al. 2000). NO_3^- decrease and NO_2^- increase could be explained based on simultaneous nitrification-denitrification processes as well as absorption of NO_3^- by microalgae (Vymazal 2002; De Godos et al. 2009). This observation is supported by the anoxic conditions in the pond which favours denitrification processes.

In the case of HRT = 3 days, the purification in the pond continued except for T, pH, EC, TP, Br^- and SO_4^{2-}. The same parameters were identified for both HRT with the exception of TP, Fe and Br^-. However, negative efficiencies with significant differences were also observed not only for NO_2^- but also for NO_3^- (pointing out nitrification processes), SO_4^- and Fe for HRT = 3 days. Statistical differences between 7 and 3 days were found in all the studied parameters except for TP, Br^- and F^-. This finding pinpointed the better performance of 7 days in comparison with 3 days of retention.

4.5 Total Efficiencies at the Purification System

Considering the results of the total efficiencies (mechanical separator, tanks, HSFCW and stabilization ponds), the highest efficiencies of removal were identified for 7 days of retention for TP (113.4 %), KN (102.6 %),

COD (102.5 %), NH_4^+-N (94.0 %), NO_3^- (36.6 %), and other elements as Mn (148.1 %), Cu (64.2 %), Ca^{2+} (32.1 %) and Br^- (26.0 %). Other studies carried out merely in CWs with different types of wastewaters generally pointed out lower efficiencies than our findings (Walker et al. 2010; Puigagut et al. 2007) which evidenced the complementation of HSFCW and the pond. Likewise, Caballero-Lajarín et al. (2015) found lower efficiencies in a combined system of HSFCW and open pond except for Ca^{2+}, Br^- and Cu after 1 month of retention. In general, 3 days of retention showed higher removal for TSS (72.3 %) and ions including Na^+ (11.1 %) and Cl^- (15.3 %) in comparison with HRT = 7 days.

However, noticeable total negative efficiencies were observed for 7 days of retention in the case of Fe (−523.3 %), SO_4^{2-} (−517.0 %), SS (−127.4 %), Zn (−39.6 %), N, and O_2^- (−31.3 %). These parameters should be monitored when purified pig slurry is used as fertilizer in order to prevent crop physiopathies. Negative efficiencies were identified for NO_2^- (−93.1 %), Fe (−84.8 %) and Mn (−54.3 %), NO_3^- (−29.2 %), SS (−18.4 %) at 3 days of retention. This observation is extremely relevant for NO_3^- taking into account the risk of nitrate leaching. Nitrates increased 29.2 % in total when the HRT was 3 days.

Regarding the microbial parameters, 7 days of retention (data not showed) presented higher efficiencies for TC (87.9 %) and FS (47.4 %). The contents were noticeably lower than those reported by Massé et al. (2011) and Ros et al. (2006) in raw pig slurry. Neither *E. coli* nor FC was registered. In this sense, Steer et al. (2002) reported 99 % of removal for FC using CWs. In contrast, HRT = 3 days exhibited higher efficiencies for MA (13.7 %) with similar values to those observed by Tofant et al. (2006) in raw pig slurry. Consequently, 7 days of retention appeared as the most efficient in the removal of the faecal pollutants.

5 Conclusions

The influent from the mechanical separator was homogeneous and the three cells provided stable treatment performances. Seven days of retention under field conditions showed the higher removal in comparison with 3 days. The PCA supported the efficiency results. The stabilization ponds were identified as the continuity of the purification process. Higher removals were observed for 7 days of retention mainly for COD and N while HRT = 3 days was effective to remove TP and SO_4^{2-} in the HSFCW. However, higher efficiencies for Mn (148.1 %), TP (113.4 %), KN (102.6 %), COD (102.5 %), NH_4^+-N (94.0 %), TC (87.9 %), Cu (64.2 %), FS (47.4 %), NO_3^- (36.6 %), Ca^{2+} (32.1 %), and Br^- (26.0 %) were identified for 7 days of retention in the whole purification system, pointing out the positive effect of the storage pond. Conversely, total negative efficiencies were observed for Fe (−523.3 %), SO_4^{2-} (−517.0 %), SS (−127.4 %), Zn (−39.6 %), NO_2^- (−31.3 %) at 7 days of retention coupled with NO_3^- (−29.2 %) at 3 days of retention. Though these parameters should be monitored, the main potential pollutants were effectively reduced. Therefore, 7 days of retention in a low-cost purification system at full scale seems to perform as optimized treatment of pig slurry providing a suitable organic fertilizer.

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