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Abstract Pig manure is a complex mixture with excessive nutrients such as ammonium, microbial pathogens and may contain contaminants such as antibiotics. Conventional pig manure management practices caused water contamination. Sludge treatment wetland has been evaluated to determine its potential use under Mediterranean climate aiming at a parsimonious use of water and preventing water contamination, two major steps to preserve water resources in the Mediterranean Basin. Preliminary NH_4-N degradation was tested using aeration process and/or addition of commercial bacterial products. Aeration alone appeared to be sufficient to ensure nitrogen transformation of the pig manure at lab small-scale (10 L) and medium-scale (300 L). Selected plant species e.g., *Carex hispida* for use in the integrated constructed wetland tolerated the nitrogen content after aeration enabling their use in a treatment vertical bed.

Keywords Pig manure · Water preservation · Sustainable solution · Mediterranean climate · Constructed wetland · Ammonium · Aeration

Introduction

In 2012, the production of pig meat was globally estimated ca. 112.4 million tons (FAO 2014), making pork production one of the largest world meat productions. With the recent increase in worldwide demand for pig meat, the main environmental issues associated with pig production are water and air pollution due to slurry management. Traditionally used, land disposal of pig manure may, in many ways, cause several environmental damages such as eutrophication due to excess of nitrogen and phosphorus (Basset-Mens and Van der Werf 2005), and soil and surface water contaminations by heavy metals (L'Herroux et al. 1997), pharmaceutical and antibacterial treatments (Domínguez et al. 2014; Hou et al. 2015) extending to river and groundwater (Hooda et al. 2000; Jiang et al. 2015). To comply with the European Nitrates Directive (91/676/EEC), improvements in pig manure treatment are currently evaluated (Martinez et al. 2009). Wide ranges of equipments and systems have proven their efficiency in Europe and North America (Burton and Turner 2003). However, a research effort is still needed in a perspective of sustainable solutions such as water reuse (Cronk 1996; Meers et al. 2008; Harrington and Scholz 2010; Dong and Reddy 2010).

Constructed wetlands (CWs) are designed to efficiently remove suspended solids, biodegradable organic matter, pathogen microorganisms, heavy metals, persistent organic substances, and emergent pollutants (Vymazal 2014) that are parts of pig slurry composition. Depending on effluent characteristics to treat, different types of CWs may be combined and adapted (Vymazal 2005, 2008). Given its high total solids

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(TS) and ammonia concentrations that can reach ca. 4.25 % and 3 g.l^{-1}, respectively (Zhang et al. 2004; Seydoux et al. 2008), pig manure cannot be directly treated by CWs. Both characteristics can cause subsurface CW clogging thus limiting bed lifespan (Tanner and Sukias 1995; Cronk 1996; Cadelles-Osorio et al. 2007) and phytotoxicity for most of the plant species leading to growth inhibition or death (Finlayson et al. 1987; Hill et al. 1997; Clarke and Baldwin 2002). To avoid clogging and phytotoxicity, reduction of the TS influent concentration and of NH_4-N concentration below 200 mg.l^{-1} are generally recommended (Clarke and Baldwin 2002; Harrington 2005). Moreover, excess of ammonia is also responsible of bacterial nitrogen transformation inhibitions (Anthonisen et al. 1976; Suthersan and Ganczarczyk 1986).

After costly phase separations (e.g., centrifugation), anaerobic (e.g., biogas digester) and aerobic treatments of pig manure, CWs may be used as a secondary or tertiary treatment for pig manure (Poach et al. 2003; Lee et al. 2004; Hunt et al. 2009; Harrington and Scholz 2010; Chen et al. 2010; McCarthy et al. 2011; Borin et al. 2013). However, these systems are expensive and/or require a large ground area (Martinez et al. 2009). Ammonia is mostly concentrated in the liquid pig manure phase (Coillard 1997), and authors often recommend reducing it by dilution or by prior nitrification (Hunt and Poach 2001; Poach et al. 2003). Under Mediterranean climate, dilution is however not a sustainable solution in a parsimonious water-use objective.

Another perspective is to design CWs integrating as a first step a special type of vertical flow bed (VF) called sludge treatment wetland (STW). It consists of a planted drying bed often used as a less expensive alternative for drying sludge from conventional wastewater treatment plants (Uggetti et al. 2010). Therefore, to completely treat aerated pig manure without any phase separation it is necessary to design a complementary planted bed to supply the treatment of leachates from the drying bed. Moreover, plant species selection has to integrate their tolerance to ammonium and also to Mediterranean climate limiting factors (e.g., drying–rewetting effects on summer, osmotic stress and high sunlight).

The aim of this study was to design CWs adapted to sustainable pig farms under Mediterranean climate corresponding to an increasing demand in environmental solutions in areas where deposal of pig slurry is still legal. A global treatment system consisting in an aeration tank followed by a CW composed of a sludge treatment wetland and a horizontal bed was evaluated. An experiment in two steps was conducted in order to test the feasibility and efficiency of this pig manure processing system. A first small-scale experiment of pig manure aeration was performed including additions of commercial bacterial products to enhance ammonia transformation. On the basis of the results of this first experiment, a medium-scale experiment was then conducted including pig manure aeration and its treatment in a hybrid constructed wetland designed and planted with ammonia tolerant species.

Materials and methods

Manure origin

Manure used for these experiments was obtained from a finishing pig farm in southeastern France (Miramas; 43° 32' 53" N, 4° 58' 12" E). Slurry was collected in the main pit receiving manure of two barns (ca. 300 pigs each), in November 2013 and May 2014 for the small- and the medium-scale experiments, respectively. Samples were done down the discharge pits under the livestock buildings in the main pit permitting the homogenization of the manure.

Small-scale experiment

Experimental design

The experimental system was composed of 18 buckets (propylene 20 l) each filled with 10 l of raw pig manure. In half of them (nine buckets), a circular ventilation rail (PVC perforated hose 30 holes of 2 mm) was installed in their bottom and connected to an air pump (AIRTECH 130–110 l.min-1) providing a flow rate of 20 l.s^{-1}.m^3 to reach oxygen saturation a few hours after starting the aeration process (Zhang and Zhu 2005). Aeration lasted the whole experiment time (21 days). To enhance ammonia transformation, two commercial bacterial products were added separately in three aerated buckets and in three non-aerated ones, each, left buckets (three aerated and three non-aerated) were without any bacterial addition.

Commercial bacterial products, preparation, and amendment modality

The two commercial bacterial products tested were "Bacta-Pur® XLG" produced by IET-Aquaresearch Ltd. (Quebec) abbreviated as "P1A" and a combination of "Sanifosse" and "Bactiboost" commercialized by Bioxem (France), respectively, abbreviated as "P2A" and "P2B". P1A is sold as a liquid suspension of a balanced community of natural beneficial microorganisms, which have been selected for their capacity to biodegrade organic waste digesting sludge and reducing ammonia. P2A is a concentrated solution of non-pathogenic bacteria and P2B is an unspecified biological activator, both are usually sold for the treatment of septic tanks. P1A was used to produce a bacterial enhanced solution (abbreviated "P1"), following the recommended incubation instructions: 5 ml of P1a with 5 ml Bacta-Pur®Activator (carbon source) in a 1000 ml beaker filled with deionized water, aerated (Otto, Air Pump SA-2500 Single Outlet 150 l.h^{-1}) and warmed during 24 h (VisiTherm, VT0 25 W). P2A and P2B were used without preliminary dilution. During the 4 weeks of the small-scale experiment, in the P2-treated bucket, 2 ml of P2A ware added both on Tuesday and Thursday and 2 ml of P2B were added both on Wednesday and Friday. One the other hand 30 ml of P1A was added to the P1-treated buckets on Tuesday, Wednesday, Thursday and Friday. Each bucket (treated or not) was homogenized after the addition of the bacterial products avoiding strict anaerobic conditions in the non-aerated buckets. Buckets that did not receive any bacterial inoculum were called P0-treated.

Medium-scale experiment

Experimental design

Pig manure (300 l) was aerated in a 500 l polyethylene tank (1.095 × 1 m, height x diameter) during 28 days with an air pump (SP0045-1MA550-1, 45 $m^3.h^{-1}$) providing a flow rate of 41.6 $l.s^{-1}.m^{-3}$ (Fig. 1). After aeration, tank was allowed to stand and pig manure was regularly amended in an arrangement of nine constructed wetlands (mesocosms). Each of them consisted of a vertical sub-surface flow CW made with a rectangular plastic (polypropylene) tanks (430 × 325 × 350 mm, length x width x depth) followed by a horizontal sub-surface flow CW made with EPDM tarp in a wood tank (1100 × 200 × 300 mm, length × width × depth). Both vertical and horizontal tanks were filled of pozzolan (7–12 mm diameter) to 250 and 200 mm depth, respectively. Pozzolan was used as substrate because its mechanical characteristics are favorable for water filtration processes (Dumont et al. 2008). Two individuals of *Carex hispida* and *Typha latifolia* were planted in each vertical CW. Three individuals of *Alisma lanceolatum*, *Carex cuprina*, *Iris pseudacorus* and *Juncus inflexus* were planted in each horizontal CW. Apart *A. lanceolatum*, all species were selected based on their tolerance to ammonium (Cronk 1996; Finlayson et al. 1987; Clarke and Baldwin 2002; Harrington 2005) and their natural occurrence in Mediterranean climate. Plantlets were collected from a natural wetland situated at approximately 30 km from the pig farm (Les Paluns wetland, Marignane, France) and were replanted in the mesocosms 6 months before the experiment beginning to ensure their acclimation. In order to distinguish the impact of the aerated swine manure on vegetation, three mesocosms received tap water amended with a nutritive solution and the six others only the aerated-manure. To reproduce alternately feeding conditions conventionally found in the constructed wetland currently operating (Uggetti et al. 2010), vertical filters of each mesocosms were amended 4 days per week by 2 l of effluent. Given mesocosms dimensions, hydraulic retention time was ca. 14 days. To perform plant species ammonia acclimation (Cronk 1996), aerated swine manure was half-diluted with tap water during the first 15 days of experiment and then amended without dilution during the next 28 days. In order to assess plant species tolerance to aerated manure (Clarke and Baldwin 2002), a mortality rate (MR) monitoring was conducted following the formula:

$MR = \sum \frac{n}{N} \times 100$ where n is the number of dead individuals for each species and N is the total number of individuals for each species.

Physicochemical analyses

During small-scale aeration experiment, pH and temperature within the buckets were measured every 3 days using a portable multiparameter probe (Hanna Instrument – HI 991301). To perform chemical analyses, 200 ml of manure was collected in each bucket every 7 days (respectively noted T0, T_{+7}, T_{+14}, T_{+21}) to analyze chemical oxygen demand (COD), total solids (TS), and ammonia nitrogen (NH_4–N) concentrations.

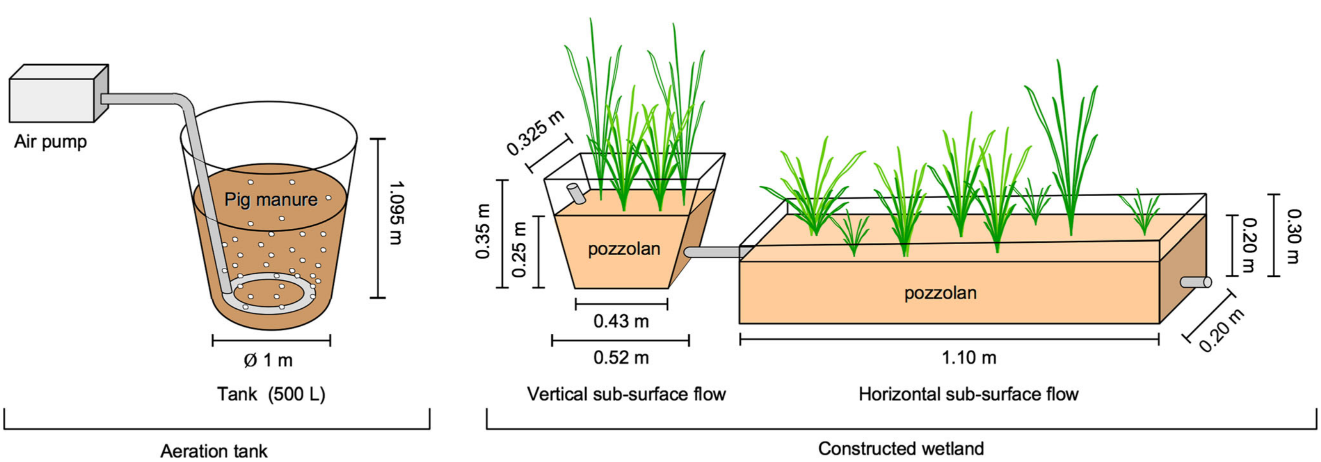

Fig. 1 Experimental design of the medium-scale experimentation including first pig manure aeration in tank (500 l) and secondly a hybrid constructed wetland (vertical and horizontal sub-surface flow)

During medium-scale experiment, 200 ml of manure was collected in the aeration tank before aeration starting, after 14, 24, 28 days of aeration and 6 days after aeration stopped (respectively noted T_0, T_{+14}, T_{+24}, T_{+28}, T_{+34}). After 20 days of CWs feeding, water samples were collected at the outlet of each microcosm for determining residual pollutant concentrations. These samples were used to analyze COD, biochemical oxygen demand (BOD_5), TS, Total Organic Carbon (TOC), total Kjeldahl nitrogen (TKN), nitrites (NO_2-N), nitrates (NO_3-N), ammonia (NH_4-N), and total phosphorus (Ptot).

COD was determined by potassium dichromate oxidation of samples according to ISO 6060:1989 method. TKN was measured according to international standard method ISO 5663:1984. TS were analyzed according the American Public and Health Association Standard methods (APHA et al. 1998). BOD_5 was assessed by measurement of microbial catabolic activity using a fluorescent redox indicator in 96-well microplates (Envolure, France) (Muller et al. 2014). TOC measurements were made using high temperature catalytic oxidation techniques (EN 1484:1997). The combustion of 250 µL of sample was realized at 800 °C in a NC2100S C/N analyzer (Analytik Jena, Germany) and the combustion products were carried by high purity oxygen (Linde Gas, France) through a nondispersive infrared sensor that performed CO_2 analysis. Each sample was analyzed in triplicate. Anions (NO_3^-, NO_2^-) analysis was carried out by ion chromatography (EN ISO 10304–2:1996) on a ICS-3000 HPLC system (Dionex, Sunnyvale, CA, USA), driven by Chromeleon® (6.80 version) equipped with a guard column (Dionex AG11-HC), an analytic Dionex AS11-HC (4 × 250 mm) column and using a 25 µL loop injection valve. Analysis were performed in an isocratic mode (22.5 mM NaOH in helium sparged demineralised water) at 30 °C, with a flow rate set at 1.5 mL/min. Ammonium was determined by modified Roth's fluorimetric method using derivatization with o-Phthaldialdehyde/N-acetyl-cysteine (OPA/NAC) and fluorescence measurement of the formed isoindols (Meseguer-Lloret et al. 2005).

To evaluate treatment effectiveness, removal rates (RR) were calculated as follows:

$RR = \frac{(C_{in} - C_{out})}{(C_{in} * 100)}$ where C_{in} is the influent concentration and C_{out}, the effluent concentration. For aeration experiments, removal rate between beginning of experiment and the measurement time was called total removal rate (TRR).

To understand phenomena leading to NH_4-N reduction during pig manure aeration, different nitrogen form concentrations ($g.l^{-1}$) have been reported as nitrogen concentrations ($g.l^{-1}$ N) and converted into mass (g N) by multiplying it by pig manure volume in the tank (300 l). The amount of total nitrogen (TN) was estimated from the sum of NTK, nitrites, and nitrates; the organic nitrogen was estimated by subtracting NH_4-N to NTK. Inorganic nitrogen was estimated as the sum of NH_4-N, NO_2-N, and NO_3-N.

Statistical analysis

All statistical analyses were performed using R software (version 3.0.2.) and p value ≤0.05 was considered as significant. Significance of differences was tested by the Student test when samples followed a normal distribution (Shapiro test). Chi^2 test was used to test significance of differences between mortality rate percentages.

Results

Microbial treatment efficiency

After 7 days of aeration during small-scale experiment, significantly higher values of temperature and pH averages were observed in the aerated-manure than in the non-aerated one with an average delta of 0.4 °C and one unit, respectively (Table 1). After 14 and 21 days, these differences were maintained for pH but faded for temperature. Temperature augmentations in both aerated and non aerated-manure between T_0 et T_{+7} followed the ambient temperature variations.

As expected, aeration led to a faster and higher reduction of ammonia concentrations than without aeration. Starting with a NH_4-N concentration above 2 $g.l^{-1}$, aeration allowed reaching the objective of below 250 $mg.l^{-1}$ NH_4-N concentrations (phytotoxicity threshold) in 21 days that represented a total removal rate ca. of 89.98 %. Without aeration, total removal rate was only ca. 56.77 % and brought NH_4-N concentrations ca. 880 $mg.l^{-1}$. During experiment time, no significant bacterial treatment effect was observed in both aerated and not-aerated pig manure.

Moreover, no effect of bacterial inoculums on COD and TS concentrations were found in both aerated and non-aerated manure (supplementary data Table 1).

Evaluation of effects of aeration on physicochemical characteristics of pig manure

Total solids, chemical oxygen demand, 5-day biochemical oxygen demand, and total organic carbon changes

In small- and medium-scale experiments, initial TS percentages were, respectively, 1.84 and 5.38 %. In both experiments, no significant TS variations were recorded between the beginning and the end (Table 2).

During small-scale experiment, 21 days of aeration permitted to reach a COD concentration of 7369.44 $mg.l^{-1}$ and led to a COD significant reduction of 34 % after 7 days. However, after this first reduction, COD concentrations

Table 1 Evolution of pH, temperature (T°C), ammonium (NH_4-N) average concentrations (±SE), and NH_4-N total removal rate (%) over time (total removal rate) during small-scale experiment (0, 7, 14 and 21 days of aeration)

Parameters	Time	Aeration							
		Without				With			
		Bacterial treatment			Mean	Bacterial treatment			Mean
		P0 ($n=3$)	P1 ($n=3$)	P2 ($n=3$)	All (n = 9)	P0 ($n=3$)	P1 ($n=3$)	P2 ($n=3$)	All ($n=9$)
pH	T_0	7.97 ± 0.03 a C	7.94 ± 0.03 a B	7.96 ± 0.03 a B	7.96 ± 0.02 a' C	7.98 ± 0.01 a B	7.91 ± 0.01 a B	7.93 ± 0.11 a B	7.94 ± 0.04 a' B
	T_{+7}	8.04 ± 0.03 b B	8.08 ± 0.02 b B	8.05 ± 0.04 b B	8.06 ± 0.02 b' B	9.10 ± 0.10 a A	9.08 ± 0.06 a A	9.03 ± 0.03 a A	9.07 ± 0.04 a' A
	T_{+14}	8.29 ± 0.07 b B	8.32 ± 0.06 b A	8.33 ± 0.06 b A	8.31 ± 0.03 b' A	9.21 ± 0.09 a A	9.21 ± 0.01 a A	9.10 ± 0.04 a A	9.17 ± 0.03 a'A
T (°C)	T_0	18.4 ± 0.06 a C	18.43 ± 0.03 a B	18.43 ± 0.09 a B	18.42 ± 0.03 a' C	18.57 ± 0.12 a C	18.70 ± 0.03 a C	18.63 ± 0.06 a C	18.63 ± 0.04 a' C
	T_{+7}	23.17 ± 0.03 a A	23.37 ± 0.15 a A	23.47 ± 0.07 a A	23.33 ± 0.06 b' A	23.87 ± 0.18 a A	23.83 ± 0.15 a A	23.90 ± 0.07 a B	23.87 ± 0.07 a' A
	T_{+14}	21.87 ± 0.42 a B	22.10 ± 0.50 a A	22.33 ± 0.45 a A	22.10 ± 0.24 a' B	21.3 ± 0.12 a B	21.90 ± 0.42 a B	22.07 ± 0.06 a A	21.97 ± 0.13 a' B
NH_4^+ (mg.l^{-1})	T_0	2028.27 ± 114.19 a A	1953.9 ± 28.75 a A	2178.8 ± 57.72 a A	2053.66 ± 50.27 a'	2110.63 ± 74.14 a A	2196.17 ± 21.81a A	2098.57 ± 37.11 a A	2135.12 ± 29.13a'
	T_{+7}	1991.93 ± 5.49 a A	1946.23 ± 100.85 a A	1887.07 ± 51.96 a A	1941.74 ± 36.13a'	677.13 ± 247.93 b B	739.60 ± 199.08 ab AB	874.07 ± 617.36 b B	763.60 ± 202.56 b'
	T_{+14}	1168.18 ± 92.96 a B	1091.01 ± 57.27 a B	1120.36 ± 69.14 a B	1126.51 ± 38.96 a'	142.97 ± 65.48 b B	497.27 ± 214.84 ab AB	391.67 ± 235.47 ab B	343.97 ± 150.88 b'
	T_{+21}	913.17 ± 38.85a B	836.47 ± 137.63 a B	892.77 ± 78.60 a B	880.80 ± 48.48 a'	87.37 ± 38.91 b B	288.40 ± 191.11 ab B	275.27 ± 271.53 ab B	217.01 ± 101.82 b'
NH_4^+ total removal rate (%)	T_{+7}	1.11 ± 6.05 b B	0.36 ± 4.73 b C	13.37 ± 1.36 b B	4.95 ± 3.08 b' C	67.11 ± 12.53 a A	67.18 ± 9.42 ab A	58.37 ± 27.15 a A	64.22 ± 9.17a' A
	T_{+14}	41.69 ± 7.40 c A	43.85 ± 3.25 c A	48.49 ± 5.33 bc A	44.68 ± 2.97 b' B	93.17 ± 3.09 a A	77.97 ± 10.20 abc A	81.38 ± 19.17 ab A	84.17 ± 6.74 a' AB
	T_{+21}	54.61 ± 3.80 b A	56.87 ± 6.77 b A	58.85 ± 5.43 b A	56.77 ± 2.80 b' A	95.78 ± 1.87 a A	87.25 ± 9.08 ab A	86.92 ± 11.97 ab A	89.98 ± 4.60 a' B

In a same line, values of bacterial treatments followed by a same lower-script letter (a, b, c) and means followed by a same lower-script letter (a',b',c') are not significantly different $p < 0.05$ (*t* test)

In a same column and for a given parameter, values followed by a same upper-script letter (A, B, C) are not significantly different $p < 0.05$ (*t* test)

P0: without bacteria inoculum; P1: bacterial mix 1; P2: bacterial mix

Table 2 Total solids (TS %), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) and total organic carbon (TOC) average concentrations (±SE) changes during small (S) and medium (M) scale experiments. For each parameter, total removal rates (%) between T_0 and measurement times are indicated in *grey rows*

		Scale	Experiment time					
			T_0	T_{+7}	T_{+14}	T_{+21}	T_{+28}	T_{+34}
COD	Concentration ($mg.l^{-1}$)	S	10583.33 ± 225.46 a	6938.89 ± 175.55 b	7675.00 ± 466.37 b	7369.44 ± 624.20 b	–	–
		M	29041.67 ± 324.79 a	–	–	–	21841.67 ± 814.75 b	21766.67 ± 758.47 b
	Total removal rate (%)	S	–	34.41 %	26.94 %	29.38 %	–	–
		M	–	–	–	–	24.79 %	25.05 %
TS	Percentage (%)	S	1.84 ± 0.06 a	1.57 ± 0.07 b	1.99 ± 0.16 a	1.75 ± 0.20 ab	–	–
		M	5.38 ± 0.03 a	–	–	–	5.38 ± 0.21 a	5.36 ± 0.02 a
	Total removal rate (%)	S	–	13.61 %	– 8.90 %	3.86 %	–	–
		M	–	–	–	–	11.53 %	0.37 %
BOD	Concentration ($mg.l^{-1}$)	M	4269.67 ± 241.08 a	–	–	–	2886.00 ± 535.57 a	3573.33 ± 555.57 a
	Total removal rate (%)	M	–	–	–	–	32.41 %	16.31 %
TOC	Concentration ($mg.l^{-1}$)	M	3000.00 ± 174.90 a	–	–	–	2118.47 ± 28.48 b	2276.42 ± 157.77 b
	Total removal rate (%)	M	–	–	–	–	29.38 %	24.12 %

Means followed by a same letter (a, b, c) in the same raw are not significantly different $p < 0.05$ (*t* test, S: small scale $n = 9$, M: medium scale $n = 3$)

remained stable until the end of the experiment. During medium-scale experiment, pig manure aeration during 28 days led to COD, BOD, and TOC reduction, respectively, of 24.79, 32.41, and 29.38 %. Unlike the significant decrease in COD and TOC, no significant evolution in BOD concentrations could be highlighted. Between T_{+28} and T_{+34} (aeration stop to feed CWs), no significant variation in COD, BOD, TOC, and TS was recorded.

Nitrogen transformation

In aeration tank at T_{+28}, total losses in TN mass were estimated as 569.49 g N (Table 3); TN mass diminution was kept after aeration stopped (ca. 125.18 g N). Such as for TN, inorganic nitrogen mass decreased steadily during aeration time leading to a total loss of 633.97 g N and still decreased after aeration stopped with a loss of ca. 108.09 g N. Organic nitrogen mass increased during the first 28 days of aeration (gain of ca. 65.48 g N) and then slightly decreased when aeration was stopped, a gain of ca. 65.48 g N and a loss of ca.17.09 g N were recorded, respectively. During the first 24 days of aeration, a loss of more than 450 g N of NH_4-N was observed without any equivalent NO_2-N and NO_3-N mass formations. Their mass variations resulted in a gain of 2.46 g N and a loss of 6 g N, respectively. Between T_{+24} and T_{+28} days of aeration, NH_4-N mass decreased by 253.89 g N, NO_2-N mass increased by 85.94 mg but no NO_3-N formation has been recorded. Between T_{+28} and T_{+34}, losses of 33.05 g N and 75.04 g N, respectively, of NH_4-N and NO_2-N were recorded without any NO_3-N formation.

Constructed wetland early performances

Sludge layer production

As expected, sludge layer formations were observed on vertical bed surfaces. Their heights and fresh weights were measured after 25 days of experiment. Average sludge layer height was 3.21 cm for a 5.01 kg fresh weight and it represented an average volume of 6.01 l. Ca. 25 % of the pig manure applied remained in vertical surfaces during this period.

Constructed wetland efficiency

Analyses on CW outlet effluent were conducted after 1 month of experiment consisting of a total of 22 l of aerated pig manure applied per mesocosms (16 l of diluted aerated pig manure and 6 l of raw aerated pig manure). In order to not overestimate CW removal rates and given the fact that the horizontal filters were kept in water prior to feeding them with aerated pig manure, it has been considered for these inlet-outlet concentrations that inlet concentrations were half-diluted (Table 4). COD, BOD, TOC, TKN, NH_4-N, and Ptot effluent concentrations were significantly lower than those measured in the influent and their associated removal rates were, respectively, 96.95, 99.79, 91.41, 98.23, and 98.21 %.

Plant tolerance

In the vertical flow filter, phytotoxicity effects on *Typha latifolia* appeared after 45 days and were mainly characterized in early stages by wilting. *T. latifolia* mortality rate reached 58 and 75 % after 45 and 52 days, respectively. These mortality

Table 3 Mass balance of the various nitrogenous forms, during pig manure aeration (medium-scale experiment). In *grey columns*, values are concentrations (g $N.l^{-1}$) measured at T_0 and T_{+34}. In *white columns*, mass variations between two-measurement times are noted (concentrations have been transformed into mass through multiplication by the slurry volume in the tank). Mass gains and losses are indicated by (+) and (−), respectively

Nitrogen forms	Initial mass (g N)	Mass variation between two measurement times (Δ g N)					Final mass (g N)
		T_0 / T_{+14}	T_{+14} / T_{+24}	T_{+24} / T_{+28}	T_{+28} / T_{+34}	T_0 / T_{+34}	
Inorganic nitrogen	814.03	−367.34	−98.68	−167.95	−108.09	−742.06	71.97
Ammonium (NH_4–N)	807.92	−365.27	−97.11	−253.89	−33.054	−749.22	58.60
Nitrites (NO_2–N)	0.01	+4.03	−1.57	+85.94	−75.039	+13.36	13.37
Nitrates (NO_3–N)	6.10	−6	0	0	0	−6	<0.34
Mass variation between two measurement times (Δ g *N*)							
			T_{+28} – T_0		T_{+28} / T_{+34}	T_0 / T_{+34}	
Total nitrogen	1281.04		−569.49		−125.18	−693.67	587.37
Organic nitrogen	467.01		+65.48		−17.09	+48.39	515.40
Total Kjeldahl nitrogen (TKN)	1274.93		−650.79		−50.14	−700.93	574.00

rates were significantly different to those measured in bed filled with water (chi^2 test, $p \leq 0.05$). Nevertheless no significant differences were found between these two-measurement times and no increase of *T. latifolia* mortality rates over time could be highlighted (chi^2 test, $p = 0.3865$). No significant mortality rate was recorded for *Carex hispida* between the two conditions (manure vs. water). In horizontal flow beds, in those filled with water as well as those filled with aerated pig manure, no plant individuals died during the experiment.

Discussion

Bacterial inoculum effect on ammonia transformation during pig manure aeration

Bacterial inoculation of pig manure to favor and guide its biological degradation has been tested in composting processes and has proven its efficiency in some cases (Tam and Vrijmoed 1990; Tiquia et al. 1997; Jiang et al. 2015). Considering these results, commercial bacterial products were tested because of their expected properties to enhance and accelerate NH_4-N transformation. During pig manure transformation under aeration in our experiment, addition of bacterial inocula did not seem to change enough microbial functionalities and/or activities in order to accelerate organic matter degradations. Even if bacteria inocula have proven their efficiency in pig manure composting, in liquid aerated environment, inoculated bacteria had to be in too strong competition with indigenous ones and aeration appeared to be sufficient to ensure nitrogen transformation. Aeration might force a biological selection between the high amount of microorganisms initially present in pig manure (Snell-Castro et al. 2005) and enable to transform NH_4-N. But, aeration might also promote physiochemical ammonia release into atmosphere (Burton 1992).

However, pig manure inoculation with commercial bacterial products may be necessary after a barn washing operation with bactericide (e.g., bleach) or a pig antibiotic treatment campaign that both would destroy the microbial populations initially present in the manure and required for NH_4-N transformation under aeration. Considering in this case the lack of

Table 4 Chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), ammonium (NH_4-N), total phosphorus (P_{tot}) average concentrations (±SE) at inlet (aerated pig manure diluted by half) and outlet (drained water) of the constructed wetlands and removal rates associated during the starting phase

Measured parameters						
	COD ($mg.l^{-1}$)	BOD ($mg.l^{-1}$)	TOC ($mg.l^{-1}$)	TKN ($mg.l^{-1}$ N)	NH_4^+ ($mg.l^{-1}$)	P_{tot} ($mg.l^{-1}$)
Inlet	10883.33 ± 379.24 a	1786.67 ± 277.79 a	10883.33 ± 78.92 a	956.67 ± 23.33 a	125.57 ± 7.96 a	416.67 ± 6.67 a
Outlet	332.17 ± 43.14 b	3.83 ± 1.33 b	97.80 ± 6.08 b	16.93 ± 2.29 b	0.82 ± 0.19 b	7.06 ± 2.33 b
Removal rate (%)	96.95	99.79	91.41	98.23	99.35	98.31

Means followed by a same letter in the same column are not significantly different $p < 0.05$ (*t* test, inlet $n = 3$; outlet $n = 6$)

competition phenomenon by indigenous microbial communities, inoculation might allow changes in necessary microbial functionalities and activities to NH_4-N transformation.

pH and foaming as quality indicators of aeration

During small-scale experiment, pH variations were consistent with the results of Zhang and Zhu (2005) who showed that aeration can sharply increase liquid manure pH by more than 1.0 unit within the first 6 h. This increase can be due to an ammonia production generated by the enhanced hydrolysis of urea, the breakdown of organic matter due to manure aeration (Luo et al. 2001) and by the potentially H^+ consumption by the aerobic growth of heterotrophs with supply of nitrate and ammonia (Grady et al. 1999). Even if dissolved oxygen concentration could not be monitored during our experiments, pH variations indicated sufficient manure aeration. Concerning temperature evolution, aerobic digestion of waste releases energy and it results in a production of heat (Burton 1992). Temperature differences after 7 days and it disappearance after 21 days can be explained by the rapid rise in microbial activity with peaks and then falls back as the level of available nutrients in the slurry diminishes as generally observed in manure aeration (Burton 1992).

Moreover, in both small- and medium-scale experiments, foaming was observed quickly after aeration starting. Foaming normally results when airflow rates exceed 2.8 $l.s^{-1}.m^{-3}$ for manure aeration treatment (Burton 1992) and is provided by an overgrowth of filamentous bacteria and an insufficient microbial floc (Cumby 1987; Grady et al. 1999).

Thought, foaming phenomenon underscored a potential excess of aeration that can be responsible of ammonia amount releases into atmosphere. Moreover, pH increase could neutralize a part of volatile fatty acids generation but can also lead to a high ammonia emission (Zhang and Zhu 2005). Therefore, monitoring of different nitrogen forms is necessary to know and understand the phenomena involved in its transformation and the attendant environmental impacts.

Monitoring transformation of organic matter in pig manure under continuous aeration for small- and medium-scale experiments

Even if pig manure were collected in same pit for small- and medium-scale experiment, major fluctuations in its composition can be noted. Globally, pig manure used in the medium-scale experiment was more loaded than in the small-scale. TS and COD concentrations almost tripled and doubled in the small- and the medium-scale, respectively (Table 2). Variations (volume, composition and production rate) are regular in livestock wastewater (Boursier et al. 2005; Sage et al. 2006) and are conditioned by livestock species, their type and age, the nature of their feed and how they are fed, whether or not the livestock are housed, weather, and climate (Knight et al. 2000). These variations are part of the issues making biological treatment of pig manure a complex issue. Scaling up in aeration design can also lead to treatment efficiency variations. Indeed, pig slurry is a viscid material in which the establishment of a homogeneous aeration may lead to some adjustments when it comes to large volumes and high height of effluent above ventilation rail. So aeration time and airflow were increased in medium-scale experiment after prior tests that revealed that pig manure height above ventilation rail was a limiting factor to aeration diffusion and homogenization and a pump pressure increase were necessary to counteract it.

According to the obtained results, microbial potential of organic matter transformation was conserved between both experiments despite scaling up and pig manure composition fluctuations. COD reduction observed in the medium-scale experiment is consistent with the results at small-scale even though the removal rate was quite lower. In agreement with the literature, no significant TS reduction was recorded when aeration flow rate reach 20 $l.s^{-1}.m^{-3}$ and even at 41.6 $l.s^{-1}.m^{-3}$ (small- and medium-scale, respectively). Indeed, Ginnivan (1983) found significant TS reduction in pig manure under aeration (greater than 35 %) with an airflow of 138 $l.m^{-3}.s^{-1}$ applied during 12.5 days. But no significant reduction between non-aeration and aeration has been found when airflow rate was 29.7 $l.m^{-3}.s^{-1}$ that corresponded to the order of magnitude applied during our experiments. At last, organic carbon losses might be due to the formation of volatile organic compounds and their release into atmosphere (Westerman and Zhang 1997; Zhu 2000) and CO_2 production due to microbial respiration and organic matter transformation.

Nitrogen transformation under continuous aeration

During pig manure aeration, total nitrogen losses are explained by degassing that occurs under the influence of two classical nitrogen transformation pathways (Burton et al. 1993; Münch et al. 1996; Bernet et al. 2000; Ni et al. 2000): (1) the biological nitrification-denitrification and (2) the physicochemical ammonia stripping. Nitrification implies a first oxidation of NH_4^+ into NO_2^- (nitration) followed by its oxidation into NO_3^- (nitratation). Denitrification implies a first reduction of NO_3^- into NO_2^-, followed by its reduction into nitric oxide (NO) and then into nitrous oxide (N_2O) to forms molecular nitrogen (N_2) that can be releasing into atmosphere (Fig. 2). Ammonia stripping consists in a desorption process: in pig manure, depending on pH and T°C, ammonium ions are in equilibrium with dissolved ammonia (NH_4^+/NH_3 redox couple). Given the NH_4^+/NH_3 acid-base couple dissociation constant (pKa = 9.25), dissolved ammonia is the predominant form at high pH and can be transferred from the liquid phase

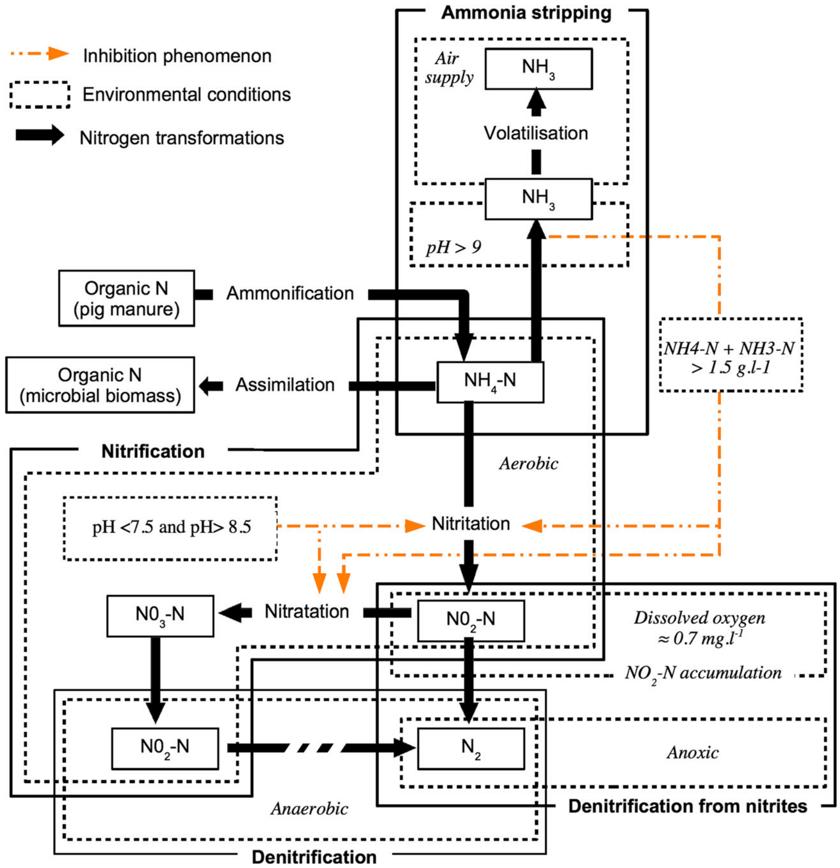

Fig. 2 NH_4-N potential transformations in pig manure under aeration depending on environmental conditions and inhibition phenomena (adapted from Anthonisen et al. 1976; Josserand 1983; Bock et al. 1989 and Ruiz et al. 2006)

into the atmosphere with an air supply (Bonmatí and Flotats 2003; DelaRubia et al. 2010).

During this first 24 days of aeration characterized by NH_4-N diminution without any NO_2-N and NO_3-N formations, NH_4-N could have been partly assimilated into microbial biomass (Azam et al. 1993) leading to the augmentation of organic nitrogen during this period. Moreover ammonia stripping seems to replace nitrification-denitrification mechanisms. It can be explained by a nitrification inhibition and favorable physicochemical conditions for stripping. Indeed, nitrification can be inhibited by many parameters such as: (1) a NO_2-N concentration greater than 300 mg.l^{-1} (Bock et al. 1989), (2) an unsuitable pH (pH <7.5 and pH > 8.5) (Josserands 1983; Bock et al. 1989) and (3) NH_3 and HNO_2 excessive concentrations (Anthonisen et al. 1976). In the medium-scale experiment, NO_2-N concentrations did not reach 300 mg.l^{-1} in the first 24 days (supplementary data table 2). Nevertheless in the small-scale experiment pH average was about 9.2 units (Table 1) promoting NH_3-N form and thus facilitating ammonia stripping (Fig. 2). Furthermore, nitrifying bacteria can be inhibited by free ammonia forms (Anthonisen et al. 1976). The high pH of pig aerated-manure therefore favored the formation of NH_3, potentially responsible for the inhibition of nitrification.

Between T_{+24} and T_{+28}, nitrification seemed to take place in the aeration tank (NO_2-N production) but it appeared to be limited to the nitration step of nitrification (no NO_3-N formation). Nitrite accumulation (partial nitrification) may be due to inhibition of bacteria growth and their activities (such as *Nitrobacter)* by free ammonia concentrations higher than 150 mg.l^{-1} (Anthonisen et al. 1976). At the beginning of this period, NH_4-N concentrations were higher than 300 mg.l^{-1} (c.a. 1480 mg.l^{-1}) and can be a potential explanation. Though, others parameters such as pH, temperature and dissolved oxygen concentration can be the cause of nitrite accumulation (Suthersan and Ganczarczyk 1986; Yoo et al. 1999; Bae et al. 2002). Between T_{+28} and T_{+34} whereas aeration has been stopped to feed constructed wetland, NO_2-N and NH_4-N losses without any NO_3-N formation could traduce a denitrification directly from NO_2-N forms (Ruiz et al. 2006).

In order to develop a low environmental impact pig manure treatment, degas NH_3 under aeration could be a limitation. Indeed this compound can directly injures ecosystem (soil acidification and eutrophication) and human health (Butterbach-Bahl et al. 2011; Dise et al. 2011; Brunekreef and Holgate 2002) and contributes through secondary conversions to particles, to climate change (Renard et al. 2004). Generally, in swine manure production 50 % of the ammonia emissions originate from the shelter and the slurry storage, other 50 % are emitted following land application (Martinez et al. 2009). However, Loyon et al. (2007) shown that aerobic biological treatment without separation of pig slurry can greatly reduce 68 % emissions of the polluting gases NH_3, N_2O, CH_4 and CO_2 when compared to traditional manure management based on 6 months storage before land spreading. Moreover, treatment systems that exclude long storage in animal houses avoid health risks to animal and man induced by long-term exposure to NH_3 as severe lung diseases (Seedorf and Hartung 1999) and can increased animal performance (Martinez et al. 2009).

Constructed wetland early evaluation

In order to evaluate early performances of the constructed wetlands, three major aspects were studied: (1) the sludged layer formation above the vertical bed, (2) the drained water quality (Stefanakis and Tsihrintzis 2012), and (3) plant tolerances to the influent.

The solid loading rate (SLR) defined as the total solid (TS) weight of influent applied to the system per unit surface area and time need to be adapted to climate location (Uggetti et al. 2010). In this experiment, SLR was about 122.32 kg TS $m^{-2}.year^{-1}$. Stefanakis and Tsihrintzis (2012) showed that under Mediterranean climate it could be probably up to 85–90 kg TS $m^{-2}.year^{-1}$ and higher range of load has been tested and validated by Koottatep et al. (2005) and Kengne et al. (2008) in tropical regions. However, sludge layer composition was not analyzed during this first experiment step and was left for further study steps when sludge layer mineralization will begin. Indeed, transformation of the sludge layer involved evapotranspiration occurring in period of active plant growth, percolation just after the bed filling, and mineralization that is a variable phenomenon in time and space (e.g., mineralization gradient between the surface and the bottom of the sludge layer) (Nielsen 1990; Uggetti et al. 2009). Mineralization quality can be monitored by different sludge layer analyses as TS, COD, BOD, nutrients, and organic matter content (Uggetti et al. 2009).

Drained waters or leachates are mainly composed by the pore water fraction (66.7 % of the total sludge water content) of the total sludged water content that percolates after each sludge-feeding event into the vertical bed (Uggetti et al. 2010). Capillary water (25 %) and adsorbed and structurally bound water (8.3 %) remain into the sludge layer and will be eliminated by evaporation, evapotranspiration, and microbial development (Uggetti et al. 2010). In sludge treatment wetland without horizontal bed, the drained water shows low organic matter content around 100 $mg.l^{-1}$ of COD, a total phosphorus between 7 and 25 mg $P–PO_4$ l^{-1}, TKN below 10 mg $NH_3–N$ l^{-1}, nitrite below 0.3 mg NO_2-N l^{-1}, but a high nitrate content that can reach 280 $mg.l^{-1}$ (Uggetti et al. 2010). Given these high nitrogen contents, drained water is usually recycled to headwork constructed wetland (Uggetti et al. 2010). Adding a horizontal bed to treat these leachates permitted to reduce COD, total phosphorus, and TKN, respectively, below are previous findings to reach removal rates up to 90 %. However, these first results considered only diluted aerated pig manure at the inlet though 6 l of raw aerated pig manure have been added in CW. Furthermore, except for nitrogen that is mainly converted by microbial pathways, it is noteworthy that the short operation period of the CW did not enable to lead to conclusions on real efficiencies of microbial degradation (Ragusa et al. 2004) and plant uptake but rather on filtration and adsorption capacities of the substrate. Adsorption is closely linked to redox potential that is quite stable in horizontal beds. Implementation of this type of filter at the end of the treatment system offers the advantage to avoid the issue of release of pollutants during redox condition variations du to the alternate aerobic and anaerobic conditions that occurs in vertical beds (Vymazal 2007). Finally, adsorption is a saturable phenomenon and raises the question of this treatment system lifetime.

Concerning plant tolerance, all species excepted *A. lanceolatum* were chosen because of their ammonium tolerance (Clarke and Baldwin 2002; Harrington 2005) and their natural occurrence in the surrounding aquatic environments (Cronk 1996) ensuring better climate condition adaptations and maintain of populations. *T. latifolia* was the only species showing early toxicity symptoms as wilting and mortality. Even if it is one of the most commonly plant used in treatment wetland for livestock wastewater (Cronk and Fennessy 2001; Uggetti et al. 2010) due to its high initial growth rate (De Maeseneer 1997) and its high ammonia tolerance (Baldwin and Clarke 2002), Koottatep et al. (2005) found that *Typha augustifolia* could show signs of wilting. It was at the initial stage of operation (first 6 month) of a sludge treatment CWs (once-a-week loading) and *T. augustifolia* was heavily impacted at a SLR higher than 250 kg TS $m^{-2}.year^{-1}$. Moreover, Stefanakis and Tsihrintzis (2012) advise to lightly load them during the initial growth to avoid plant death. Mixing *T. latifolia* with other species as *C. hispida* that did not show toxicity symptoms in the early stage appears to be a favorable option to ensure plant treatment contribution as sludge dewatering, sludge mineralization, and drainage efficiency preservation (Uggetti et al. 2010) at the CWs initial stage operation. To go further, complementary studies of biometrics parameters monitored during this experiment (width, length, and number of leaves, maximum aerial length etc.) and physiological parameters (chlorophyll, anthocyan, and flavonol concentrations and stress proteins) will permit to underscore other phytotoxicity symptoms as growth inhibition or loss of the original sheet (Finlayson et al. 1987; Baldwin and Clarke 2002; Guittonny-Philippe et al. 2015). These further studies will permit to select between the tolerant species highlighted in this studies, even if they do not present significant mortality rate, those which present less symptoms in order to optimize plants growth and maximize their role in pig wastewater treatment (Baldwin and Clarke 2002).

In situ large-scale pilot prospects

Despite the wide range of equipment and system potentially available to treat pig manure in Europe and North America (Burton and Turner 2003), few were adapted at a large scale because of their heavy investment and operating cost without an equivalent return, their complexity and impracticality for the livestock operator and their poor adaption for the livestock farm or their need to transport to another region (Martinez et al. 2009). Indeed, Martinez et al. (2009) highlight the point that whatever the options considered either being so-called "technological options" (based on energy, concrete, steel, chemicals) or "natural options" (based on sun, wind, land) there is clearly no best solution, but rather a range of options which needs to be adapted and implemented according to the local situation and context (social, economical, regulatory). Therefore, some situations may require designing a complete on-site treatment practicable by farmers, in order to utilize treated effluent for water reuse (Cronk 1996; Meers et al. 2008; Harrington and Scholz 2010; Dong and Reddy 2010).

Aeration of manure is an expensive process requiring a considerable amount of energy (Martinez et al. 2009). Nevertheless in Mediterranean climate energy requirements can be provided by solar panels or wind trubine. Further, in pig farms, pig manures are usually stored first in pig house and then in a main pit. This latter can be cheaply transformed in two compartments connect with a pump, one for continuous aeration and another to stock aerated pig manure to feed constructed wetlands. In some case an odor control system by aeration can be also installed (Van Der Hoek 1977; Zhu 2000) and could be used in this purpose after some operating parameters adjustments. To proportion correctly and adapt aeration during time, it is thus interesting to monitor cheap indicators as pH, T°C or visual indications (foaming) rather than set up costly probes. For instance pH and temperature increases could be early indicators to a successful aeration rate.

In southern France unlike in Brittany, livestock buildings are mostly built on farms with varied activities including agricultural areas and thus areas between the fields and building are left without any use. Necessary surface to integrated constructed wetland can be found. Further, if adapted monitoring tools are developed as plant biometric indicators, constructed wetland operation could be done by farmers. Depending on the plant species selected, valorisation of plant biomass into farms activities can be considered as mulching, compost, or wood fuel.

Finally, even if ecological and sustainable treatment systems could more or less be adapted to pig farm effluent, swine manure remains a fluctuant and highly loaded effluent that requires being treated on a case by case basis. Upstream improvement of farming practices could achieve better results in reducing the impact of pig manure impacts on environment.

Conclusions

- Aeration appeared to be sufficient for the selection of microorganisms initially present in pig manure and which can ensure NH_4-N degradation.
- Airflow rate might need to be continuously monitored during pig manure aeration in order to avoid foaming and to reduce ammonia stripping.
- Purifying functionalities were conserved between both experiments despite scaling up (pig manure volume, airflow rate and aeration time) and pig manure composition fluctuations.
- During aeration, nitrogen losses were mainly caused by ammonia stripping the first weeks then nitrification occurred but seemed to partially be inhibited by free ammonia concentrations and pH value.
- Considering the early CWs purification efficiency, adding a horizontal bed after the sludge treatment vertical bed permitted to improve drained water quality and to reach COD concentrations permitting water reuse in irrigation.
- Mixing vegetal species planted in the sludge treatment vertical bed permitted to ensure plant functionalities despite phytotoxicity symptoms shown by other authors.

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