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Ecological engineering: from concepts to applications

Biomass production and water purification from fresh liquid manure – Use of vermiculture, macrophytes ponds and constructed wetlands to recover nutrients and recycle water for flushing in pig housing

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Abstract

A wastewater treatment system including a screen, a vermifilter, macrophytes ponds, and constructed wetlands has been built after a pig housing on slatted-floor. The aims were, all at once, to recycle water for excretion washing and to produce, from the nutrients contained in the effluent, organic matter and plants that can be either sold or reused on the farm to reduce inputs. Analyses, made on the effluent at different steps of the treatment plant, show that the concentrations of the nitrogen, micro-organisms and endocrine disruptors are drastically reduced, while the phosphorus and potassium removal go through the by-products harvesting.
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Keywords: lagooning; macrophytes ponds; pig manure; recycling; vermifiltration

1. Introduction

Water, soil and air pollution, related to the carbon and nitrogen cycles, affect climate, health, biodiversity and the safeguarding of resources [1]. Now, the increase in crop and animal production following population increase on a global scale should increase the use of fertilizers in the regions with the highest potential yields [2]. Furthermore water consumption by animal farms is high. In many regions their wastewater pollutes the ecosystem, whereas water

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treatment is expensive compared to the income of animal production. The eutrophication of coastal waters and the resultant algal blooms are a concrete example of regional water contamination [3]. Finally the storage of liquid manure below the pigs in animal houses is not conducive to animal health, and results in gaseous losses of around 25% of the excreted nitrogen in the form of reactive nitrogenous molecules (ammonia, nitrous oxide).

Agro-environmental ecosystems should therefore together minimize pollution transfer, improve their efficiency of resource use, especially for water and nutrients, and attempt to improve health and meat quality. As a significant part of water is used to wash the dejection, the efficiency of nutrient and water inputs of animal farms could be increased by systems that combine nutrient recovering and water recycling. This can be achieved by means of complementary animal and crop production, designed to maximize short distance recycling of by-products. Moreover, system designs should integrate the specific know-how and technologies, sufficiently well understood to be controlled, available in the different regions of the world.

Just for the example of a national swine production, 21 million m^3 of liquid manure are produced each year in France. In Brittany, where the majority of animal production is concentrated on 5 % of the national area (e.g. 50% of the French pig production), nitrogen leakages due to agricultural practices lead to the development of *Ulva sp*. in coastal waters, of which about 100,000 m^3, stranded on the beaches, are harvested each year. Nevertheless, with 24 million pigs raised per year, France is far behind China, the main world producer, with nearly 500 million animals.

It thus became necessary to 1find alternative solutions for the elimination of the liquid manure to storage beneath the pigs and spreading. The liquid manure from the piggery can be removed as soon as possible by scraping or flushing. The first technique leads to fresh liquid manure often treated by intensive processes. The second leads to a diluted fresh liquid manure which requires extensive treatment. The flushing system is much used in Australia, but without effective treatment of the diluted fresh liquid manure, so resulting in malodorous gas emissions from anaerobic lagoons [4].

Pig production and effluent management (flushing system), might be associated with extensive water treatment systems in agro-environmental ecosystems, which allow maximum vegetative growth per hectare by reducing limiting factors and minimizing uncontrolled release to the surrounding natural ecosystem.

Various systems combining water treatment and production of biomass have been described in the literature; vermiculture [5], macrophyte ponds [6,7] and helophyte filters [8]. It thus seemed worthwhile to devise a way of treating the diluted fresh liquid manure, using these processes with a dual aim: to obtain purified water, and to maximize various forms of production, starting from the elements contained in the effluent.

Tests were performed from 2003 to 2007, at the experimental station of Guernévez (Finistère, France), on the piggeries effluent nutrients abatement and the biomass production by passing through separate vermifilter or macrophytes lagooning pilots. Afterwards, a demonstration treatment plant including both systems, together with a screen and a storage lagoon, was settled behind a 30 pregnant sow's piggery. This paper presents the first results obtained, and discusses the concept of the association between nutrient recovering and water recycling.

2. Materials and methods

2.1. Pilot scale plants

The experimental vermifilter was composed of four elementary sub-units 5 m long × 2.5 m wide × 1 m high, placed in a room with natural ventilation. They were filled with a mixture of wood chips (one-third of total mass) and earthworm (*Eisenia andrei*) litter (two-thirds of total mass), the latter made of bark, wood chips, peat, straw, and vermicompost resulting from previous experiments and containing the earthworms. The material was about 0.7 m high [9]. After sieving, fresh liquid manure was sprinkled onto the vermifilter, at different loads and frequencies, according to the experiments (Fig. 1).

The pilot of pollution abatement by macrophytes was composed of three lines (a, b and c) of 4 basins (1, 2, 3 and 4) in series, alternatively lagoons and constructed wetlands with horizontal subsurface flow, except for basin 2c subdivided into two and which could be managed with horizontal or vertical flow. The depth of the basins varied from 1.1 m for the first lagoons to 0.35 m (planted beds for completion) and the time of retention was 5 d for the first lagoons and 4 d for the other basins (except basin 2c, when it functioned with vertical flow, one week in water, one week in air). The support of the constructed wetlands was made up mainly of gravel, particle size being 6 to 10 mm. Areas of the basins were of 0.65 m^2 at level 1, 2.5 m^2 at level 2 (2 m^2 for 2c), 1.1 m^2 at level 3 and 5 m^2 at level 4 [10].

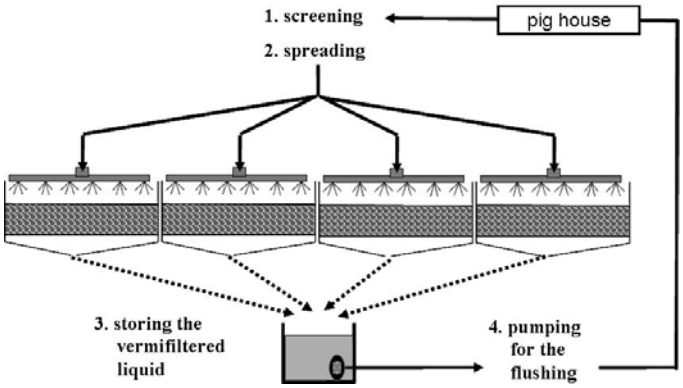

Fig. 1. Experimental vermifilter and principle of the effluent liquid circulation at pilot scale

2.2. Demonstration plant

The whole prototype comprised a livestock building and a fresh manure treatment plant, itself composed from a screen, a vermifilter, a settling tank and a macrophyte combined system. All equipment was controlled automatically with float level switches and a clock.

The livestock building was of about 12 m x 5 m for 30 pregnant sows on a slatted floor. Flushing was performed 5 to 6 times per day by the swinging of 2 stainless troughs of 400 L each (i.e. between 130 and 160 L d^{-1} sow^{-1}). The effluent flowed under the slatted floor and was collected in a concrete tank and then pumped to the screen.

The screen was a vibrating screen with a mesh of 600 µm, made out of stainless steel (Guillerm, Finistère, France). The liquid effluent fell into a concrete tank containing a pump to transfer it to the vermifilter.

The vermifilter consisted of a layer of coarse wood chips, about 0.5 m thick, resting on a stainless slatted floor with 5 mm slits, between two concrete walls on either side of a sloping floor (96 m²). A slow-moving crane was used both to sprinkle the wood chips and to stir them weekly by means of a rotating fork (Lombrimat®, Cadiou, Finistère, France). The part receiving the fresh manure occupied approximately half of the area. The second half received the old vermifilter material moved by the fork, and the sludge collected in the settling tank. The liquids ran out by gravity towards the settling tank through a grid which retained the wood chips.

The settling tank consisted of a concrete tank 2.5m x 1m x 2m. The water flowed out from the top of the tank, on the opposite side from the supply pipe, into a PVC tank, from which it was pumped to the first pond.

Macrophyte treatment was carried out in five basins terraced at successive levels. Macrophyte ponds (P1 and P3 corresponding to levels 1 and 3) alternated with constructed wetlands (CW) with horizontal subsurface flow (P2 and P4 corresponding to levels 2 and 4). The pilot plant for macrophytes described above was connected to the demonstration plant to have a similar input for both macrophyte plants. The demonstration plant was built with the same hydraulic retention time (HRT) of 5 days for the first basin and 4 for the others, as for the pilot plant. Basins P1 to P4 had areas of 50, 95, 45 and 160 m^2 and maximum depths of 1.5, 0.6, 0.6 and 0.45 m respectively, i.e. water volumes of about 25 or 20 m^3. The basins were lined to ensure that they were waterproof. The support for the CW was made of 6-10 mm grade gravel. Water flowed by gravity between the basins. Filters P2 and P4 were planted with reeds (*Phragmites australis*) and a mixture of *Glyceria aquatica*, *Iris pseudoacarus* and *Carex* spp. respectively. In 2008, P1 and P5 were covered with water hyacinths (*Eicchornia crassipes*) and P3 with water ferns (*Azolla caroliniana*). The water ferns were used to feed some pigs as described in [10]. In 2009, all lagoons were covered with water lettuces (*Pistia stratiotes*).

The storage lagoon (P5) covered a maximum of 180 m^2. Its volume of 250 m^3 was chosen to compensate for the evaporative needs during the dry months. It received both full scale plant and pilot outflows. A submersible pump raised the water to the level of the piggery. Its HRT of approximately 50 d when full after rainfall, decreased with evaporation. It was covered with water ferns or water hyacinth depending on the season. Microphytes settled or disappeared depending on the covering with macrophytes. 100 young goldfish were used as bioindicators of the water quality and to improve the stability of the aquatic ecosystem.

2.3. Sampling and analyses

The effluent was sampled at the successive levels, about twice a month, and chemical analyses, following the Standard Methods, were made. The analytical parameters assessed in this study were total solids (TS), suspended solids (SS), chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), total phosphorus (TP) and potassium (K) according to the Standard Methods. pH and temperature were measured *in situ*. Plants were also sampled and sent to COOPAGRI-Bretagne laboratory to be analyzed by Standard Methods in order to determine dry matter (DM), volatile solids (VS) and elemental composition. For the demonstration plant, on September 19th 2008, the sampling was completed in order to perform microbial and endocrine disruptors' analyses.

For microbial analysis, 10 g of each sample taken was transferred into 90 mL of peptone water and then 10-fold diluted. *E. coli* were counted using 3MTM Petrifilm E. coli (incubated 24 h at 44°C). Enterococci were counted on selective Slanetz–Bartley agar (Biokar, France), incubated 48 h at 37°C, with subsequent confirmation on Bile Esculin Agar (Biokar, France) incubated 4 h at 44°C. Spores of *Clostridium perfringens* were counted after a thermal shock at 80°C for 20 min, according to the protocol described by Sartory et al. [11]. Tests were performed in triplicate. All results were expressed as wet weight of sample.

The endocrine disruptors were searched by the estrogenic activity, because of the presence of pregnant sows in the piggery. Sampling of 1 L has been done after each step of treatment. Samples were split into pre-cleaned 500 mL glass bottles and then freezed at -20°C. Samples were thawed 24 h before extraction. Water (1 L) was filtered with a Whatman GF/C filter. The filtrate was concentrated by solid phase extraction (SPE) on mini-columns. After rinsing, drying under vacuum, and storing at –20°C, the SPE columns were thawed and extraction was made by 10 ml of ethyl acetate:methanol (5:1 v/v) mixture. The eluates were dried, then solved again with 1 mL of ethanol for bioassay. Water extracts were applied to cells lines at concentrations varying from 0.001% to 0.3% (vol/vol) of the test culture medium.

Cell cultures and bioassay procedures were based on previously described methods [12]. Summarily described, the medium used for MELN cell line culture was Dulbeco's Modified Eagle Medium (DMEM) with red phenol and supplemented with 5% foetal calf serum (FCS). For the bioassay, 5 days prior to the experiments the medium was replaced by DMEM, without red phenol, supplemented with 5% stripped serum (DCC-FCS medium). Water extracts to be tested were prepared 4× concentrated in the same medium and were tested at different dilutions in order to achieve a dose–response curve in the bioassay. MELN cells were plated in 96-well plates and water extracts were added 1 day after seeding. Cells were incubated with the samples for 16 h at 37°C. Then the medium was replaced by a culture medium containing 3×10^4 M of luciferin. Luminescence was measured in intact cells using a Microbeta Wallac Luminometer for 2 s and expressed as relative luminescence units (RLU). RLU of MELN cells with samples was expressed relative to the value obtained with E_2 (10 nM). MELN cell basal activity was around 20% of the maximum activity.

Biological activities were expressed in estrogenic equivalents (E_2-EQ) for 1 L of water. Equivalents were calculated as the concentration of E_2 resulting in the same activation of luciferase expression in MELN cells as tested sample. Calculation was based on the 50% effective concentration (EC_{50}) from the estrogen-responsive gene transactivation dose-response curve, with E_2 as positive control (EC_{50} of estradiol: 17.6 pM).

The bioassays were performed in quadruplicate and repeated three times.

3. Results and discussion

First experiments concerning the vermifilter and the macrophytes lagooning plant at the pilot scale, performed from 2003 to 2007, permitted to define the design of a demonstration plant. On this basis, this larger unit was designed and built in 2007. It was modified in July 2008 following a first semester of observations, by the addition of the settling tank after the vermifilter. The prototype is continuously managed since this date. Nevertheless, the experiments at the pilot scale have continued, with the aim to improve the demonstration plant. Some results, already published, are summarized, and completed here with the data necessary for discussing the changes to be brought to it.

3.1. Pilot vermifilter

Trials on the volumetric load of the raw effluent (5600 mg C L^{-1}, 820 mg N L^{-1}, 210 mg P L^{-1}, and 790 mg K L^{-1}) permitted to reach a removal efficiency around 60% for ammoniacal nitrogen, 40% for dry matter, COD and total nitrogen, and 20% for phosphorus and potassium [9].

3.2. Macrophyte lagooning pilot

For the macrophyte lagooning system combining 2 lagoons and 2 constructed wetlands, the efficiency was 70% in yearly average for COD, N and P, with a hydraulic retention time between 4 and 5 days (N load of 1.2 g m^{-2} d^{-1}). The plant productivity, the capacity of the plants to withstand climatic variations and various effluent loads, the complementarity of the treatment stages were also observed. Four species of floating plants (*Azolla caroliniana*, *Eichhornia crassipes*, *Hydrocotyle vulgaris*, *Pistia stratiotes*) and two of rooted plants (*Phragmites australis* and *Glyceria aquatica*) were found to be best suited, either in series or in alternation with time, to maximise nitrogen abatement and exportation of potassium and phosphorus [5].

The productivity of the basins in biomass was found to be around 20 t MS ha^{-1} y^{-1}, a little less for some floating plants, like *Hydrocotyle*, or when put too belatedly in the lagoons [10]. The nutrients' content has also been determined (Table 1).

Floating plants are more efficient to remove nutrients and heavy metals than rooted plants (factors of 2 for N and K, 4 for P, Cu and Zn, with, however, individual features, like the few quantity of copper removed by *Azolla*). This can be due to the fact that the whole plants are harvested for the first ones and only the aerial part for the second ones.

Table 1. Concentrations of N, P, K, Cu, Zn in plants of the macrophyte pilot. Concentrations are given as means obtained from 3 harvests for the floating plants (Nov. 2006, July and Dec. 2007) except *Lemna* spp., and from one harvest for the rooted plants and *Lemna* spp. (July 2007)

Plants	**dry weight (DW) (% wet weight)**	**N (% DW)**	**P (% DW)**	**K (% DW)**	**Cu (mg/kg DW)**	**Zn (mg/kg DW)**
Eicchornia crassipes	4,15	5,62	1,40	4,56	26,1	170
Pistia stratiotes	4,88	4,34	0,74	4,67	83,7	327
Azolla caroliniana	4,35	5,04	1,16	3,72	8,63	116
Hydrocotyle vulgaris	4,80	4,76	0,96	5,01	14,2	114
Lemna spp.	5,29	6,39	1,75	2,64	54,3	286
Phragmites australis	25,0	2,88	0,30	2,49	9,80	73,2
Typha latifolia	14,3	2,37	0,39	2,25	11,9	37,2
Glyceria aquatica	23,8	1,56	0,27	1,58	3,08	24,1
Juncus inflexus	30,8	1,65	0,15	1,54	4,11	30,3

3.3. Demonstration plant first steps efficiency

Nutrients removal of the different steps of the demonstration plant is given in Fig. 1. Only 20% of the phosphorus was retained by the screen while 80% was expected. The nitrogen and potassium removal during screening was negligible. Only the COD and SS removal were acceptable (about 50%) but not optimal. The vermifilter gave lower removal efficiency of COD and nitrogen, compared to pilot scale results [9]: around 20 and 40% respectively, against 85 and 65% previously achieved. The phosphorus removal was similar, around 22%. This difference can be explained by a higher porosity of the second vermifilter, which had to be maintained for continuous water recycling. Porosity maintenance was achieved by particulate organic matter removal (worm casts carried away by the water) and weekly declogging and vermifilter moving with the rotating fork. The high release of particulate organic matter from the vermifilter made it necessary to install the settling tank between the vermifilter and the first macrophyte pond, with regular sludge removal, to avoid the saturation of the pond by sludge. Therefore, a second vermifilter for sludge retention was added to the design. It was not necessary to place it on a slatted floor because the water input during one sludge removal was limited to the free air space of the vermifilter.

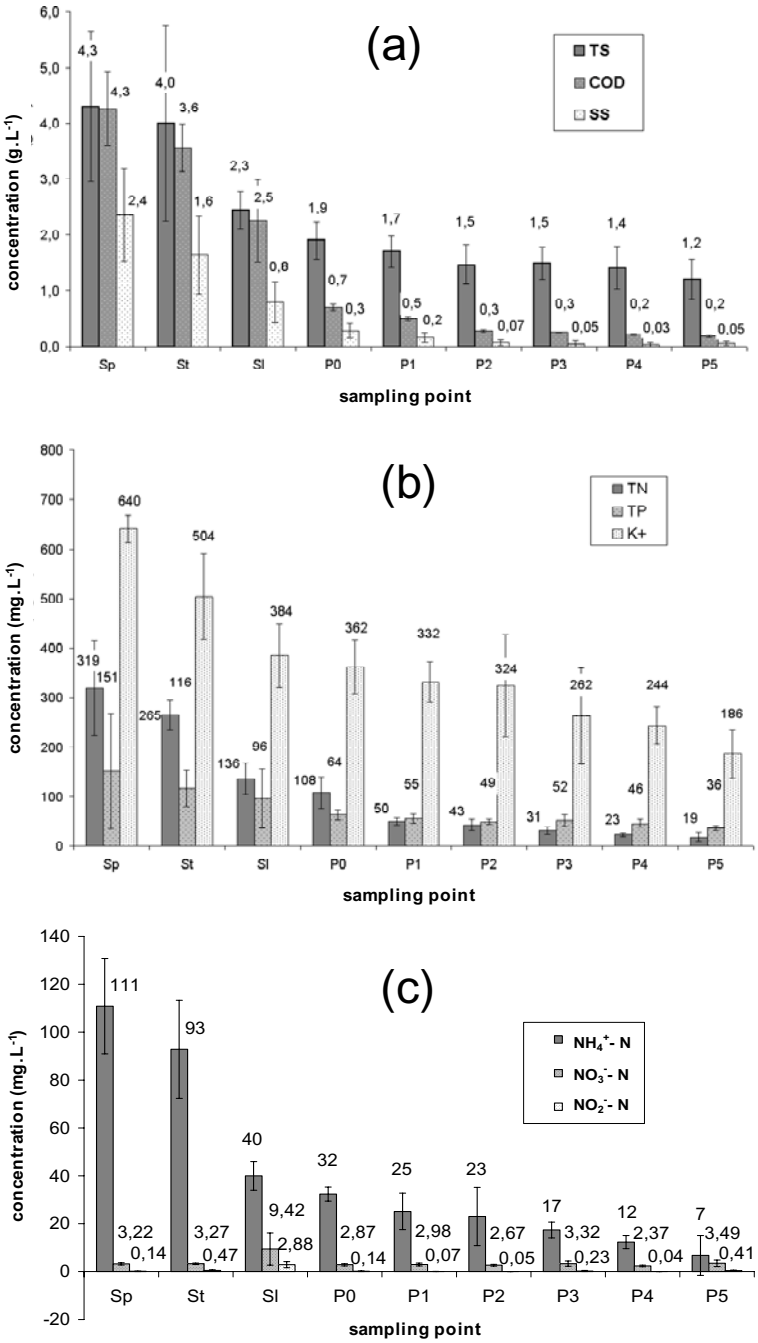

Fig. 2. Concentration abatement along the circulation of the effluent for recycling. Sampling points refer to outputs of piggery (Sp), screen (St), vermifilter (Sl), settling tank (P0), lagoon 1 (P1), basin 2 (P2), lagoon 3 (P3), basin 4 (P4), storage lagoon (P5). The values are the averages of samplings taken between July and Oct. 2008, given with their standard deviations. (a) evolution of total solids (TS), chemical oxygen demand (COD), suspended solids (SS); (b) evolution of total N, P, K; (c) evolution of N as ammonium ($N\text{-}NH_4^+$), nitrates ($N\text{-}NO_3^-$) and nitrites ($N\text{-}NO_2^-$)

3.4. Nutrient abatement in the effluent

TS, SS and COD removal efficiencies of the overall experimental plant were 72, 98 and 96% respectively (Fig. 2a). The settling tank was efficient (SS removal (64%) and COD (69%) calculated from its input to its output). The nature of the COD, initially mostly composed of reactive organic matter, is modified by the vermifilter into more stable compounds produced by the metabolism of macrofauna, bacteria and fungi, and is rich in worm casts, which facilitate aggregation and settling. The removals for the whole vermifilter/settling tank were thus 82 and 80%. The first CW (P2) also had a noticeable contribution to the COD removal (43%).

The treatment plant had a high performance on total N, P, and K: 95% overall removal for N, 76% for P, 71% for K (Fig. 2b). They were mostly removed by the organic compartments (St to P0). The residual concentration of N (19 mg l^{-1}) was low enough for flushing and is acceptable in the case of overflow after very high rainfall. The vermifilter removed a further 20%, as previously observed by Li et al. [9]. Adsorption on reactive organic particles can explain this removal. K was not retained in the sludge of the settling tank. It was further removed by absorption by plants: removal by the macrophyte ponds ranged between 10% (P1) and 20% (P3 and P5). The constructed wetlands were less efficient for all these parameters. The difference is due to the high biomass production of the floating macrophytes in summer and their high nutrient concentration.

Ammoniacal nitrogen was reduced by 95 % (Fig. 2c). The concentrations of nitrites and nitrates throughout the process are characteristic of the system's operation. The vermifilter caused nitrification, as shown by the maximum nitrate concentrations. In the settling tank, there was rapid denitrification as shown by the big decrease in nitrate concentration despite the smallest HRT of the system. The sludge can bring it about because it consists of worm casts, known to be rich in denitrifying bacteria (Alain Brauman, pers. comm.), and because the water is rich in dissolved carbon. The effect of the vermifilter on nitrogen transformation (abatement of ammoniacal nitrogen and nitrification) is similar to that of a vertical filter [see e.g. 13].

The concentration evolutions through the system were similar one year later, i.e. end 2009 [14].

3.5. Micro-organisms removal

Fig. 3 summarizes the behavior of three types of micro-organism used to indicate pathogen removal throughout the system. Regardless of the type of bacteria, a progressive decline of bacterial numbers was observed, confirming observations in the literature [15]. At the end, the microbiological quality was satisfactory for water to be reused (less than 10 bacteria / mL) within the piggery. The *E. coli* counts were reduced in each of the five basins, with the greatest contribution at the CW stages. The succession of basins resulted in a large decrease in the bacterial counts (over 4 log. units, i.e. 10,000-fold), compared to other biological manure treatments which reduce the numbers of *E. coli* by two logarithmic units (100-fold) [16,17].

3.6. Endocrine disruptors abatement in the effluent

Estrogenic activity was assessed in samples by measuring luciferase activation in MELN cells. All samples showed estrogenic activity when present at 0.3% concentration in test culture medium (Fig. 4). The first tested level effluent, after screen (St), was the most active and for the other samples, there was a linear decrease of the activities depending of treatment. The last sample, taken off after lagoon 5 (P5), exhibited only very little activity. Taking into account the EC_{50} of the reference ligand E2, we evaluated the concentration of estrogenic compounds in the samples. Concentrations ranged between 1011 to 2 ng/L. At the effluent **"P0"** the estrogenicity removal efficiency was 89% and the treatment P1 increased the removal to 96%. Estrogenic activity removal from "P1" to **"P5"** weakly increased from 96% to 99.8%, but at each step, except in P2 where no decrease in endocrine disruptors is observed, the individual abatement is very similar. The experimental treatment system removal efficiency results in underlining the important role of the vermifilter (Sl) and of the settling tank (P0) for estrogenic compounds removal in pig housing. It is interesting to note that the macrophyte lagoons (P1 and P3) and the water storage pond (P5) improve sensibly estrogenic activity removal.

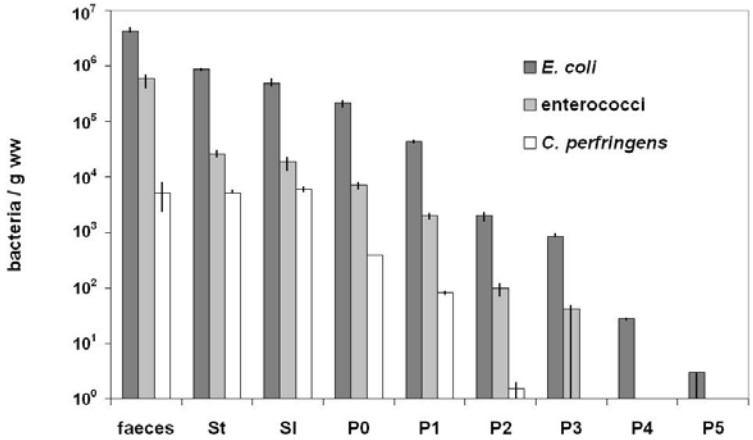

Fig. 3. Concentrations of *E. coli*, enterococci and *C. perfringens* along the treatment plant. Sampling points refer to Fig. 2; the feces replace the output of the piggery (Sp). Bars indicated minimum and maximum values

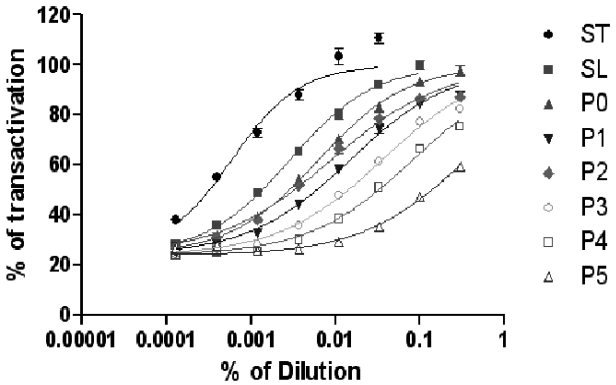

Fig. 4. Luciferase activity induced on MELN cell line by the sample. Curves are percent of transactivation (mean of four measures and standard deviation) of luciferase gene according to the concentration of tested compound. Percent of transactivation is calculated compared to maximal activity obtained with estradiol at 10^{-8} M. Sampling points are as for Fig. 2

3.7. Relationships between removal efficiency and growth of the plant

A calculation, taking into account their harvest and their content in nutrients, gives exportation by the plants out of the system, for the experimentation period, of about 25 kg for nitrogen, 6 kg for phosphorus and 20 kg for potassium - first estimation from the figures given above.

Besides, the nutrients brought to the system by the pigs feeding can be estimated to 320 kg for nitrogen, 80 for phosphorus and 210 for potassium.

It is well known that many other mechanisms than the absorption and removal by harvest of the vegetables are efficient for removing nutrients, for instance nitrification/denitrification for nitrogen and sedimentation for phosphorus. It appears that this is also true for potassium, the adsorption on the organic matter being the most probable hypothesis.

3.8. Taking gaseous emission into consideration

As the nitrogen input is very high compared to spreading, this kind of system can achieve a recycling of the nutrient on a surface much less than spreading: 12 m² pig^{-1} compared to 600 m² pig^{-1} when the slurry is used on crops in the field. However, less nitrous oxide emissions are expected from this system compared to spreading because of two reasons: very small nitrous oxide emissions were observed in the vermifilter [9], a compartment that receives the highest nitrogen rate; nitrogen inputs are very regular, while during spreading, the nitrogen input of one year is concentrated on some minutes, generally with highly bioavailable nitrogen that is more favorable to a perturbation of the nitrogen cycling within the ecosystem.

As for the piggery, the ammonia concentration is reduced by 74% [18]. Even if the gaseous emissions have not yet been tested on all the elements of the prototype, the budget is expected to be in favor of a flushing system following by an extensive treatment of the effluent.

4. Conclusions

Recycling water for using it in a semi-close system (external supply by rain and eventual spreading of excess) changed the stoichiometry of nutrients but did not lead to a continuously increase in nutrient concentrations or pollutants, provided that the treatment system be sufficiently dimensioned. Through the increase in nutrients, the organic matter produced was richer in nutrients.

Despite a nitrogen input much higher than spreading of the slurry (around 50 times higher), it is expected from this system a lower risk of perturbation of the environment than spreading the slurry, because most of the nutrients are recycled, the inputs are small all the time, and because the liquid is highly diluted, inducing a small risk in the case of leakages.

The demonstration plant experienced in Guernévez showed that, as far the micro-organisms and the endocrine disruptors are concerned, they are sufficiently eliminated through a combination of various compartments, associating various biological functions, to give convenient water for flushing and not leading to a progressive enrichment in pathogens.

The association of biological compartments to build an effluent treatment system was also sufficiently robust in order that the piggery could function continuously with the flushing. But proposing a future design, which will avoid completely the release of an excess of water during the rainy season, still necessitates the achievement of a dynamic modeling of the water balance.

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