Fate of antibiotics, steroid hormones and multiple endocrine activities during biological treatment of swine manure under anaerobic and aerobic/anoxic conditions

S. Combalbert[1], M. J. Capdeville[2], V. Bellet[3], R. Rajagopal[4], J. C. Motte[1], P. Balaguer[3], F. Béline[4], N. Bernet[1], H. Budzinski[2], G. Hernandez-Raquet[1,5]

[1]INRA- UR050 Laboratoire de Biotechnologie de l'Environnement- Avenue des étangs, F-11100 Narbonne, France (E-mail*: bernet@supagro.inra.fr*).
[2]Université Bordeaux I, EPOC-LPTC, F-33405 Talence, France (E-mail : *h.budzinski@epoc.u-bordeaux1.fr*).
[3]INSERM, U 896-IRCM, F-34197 Montpellier, France (E-mail : *Patrick.Balaguer@montpellier.unicancer.fr*)
[4]IRSTEA, UR GERE, F-350444 Rennes, France (E-mail : *beline@irstea.fr*)
[5]Université de Toulouse, INSA, UPS, INRA UMR792, CNRS UMR5504, Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, 135, avenue de Rangueil, F-31077, Toulouse, France (E-mail: *hernandg@insa-toulouse.fr*).

Abstract
Little information exists on the fate of antibiotics, hormones and their associated endocrine activity during manure treatment processes. Thus, the aim of this study was to assess the fate of such emerging pollutants and multiple endocrine activities during manure treatment coupling anaerobic digestion and aerobic/anoxic conditions under mesophilic conditions. The thermophilic anaerobic digestion was also studied. The endocrine activities tested include estrogenic (ER), dioxin-like (AhR), peroxisome proliferator receptor gamma (PPARγ), pregnane X (PXR) and androgenic (AR) activities. Our results showed that antibiotics and steroid hormones were recalcitrant to biodegradation under anaerobic conditions in both mesophilic and thermophilic temperatures. In contrast, antibiotic and hormone removal reached between 76 to 95 % when anaerobic digestion was coupled to aerobic/anoxic treatment. In absence of anaerobic predigestion, hormone removal in aerobic/anoxic conditions was about 40 %; it suggests that the anaerobic predigestion could favour the further elimination of hormones in the anaerobic/anoxic process. The estrogenic activity was mainly removed in the aerobic/anoxic (71%) compartment. The dioxin-like activity appeared to be more recalcitrant to degradation. No others endocrine activities were detected.

Keywords
Antibiotics; hormones; anaerobic digestion; emerging pollutants, endocrine disruption, manure.

INTRODUCTION

Swine manure concentrates a wide spectrum of pollutants from pig breeding activities. For example, pigs produce high quantities of natural steroid hormones, which are involved in the communication between cells. Swine breeding is the main consumer of veterinary antibiotics used to treat bacterial infections. After their action in the organism, hormones and antibiotics are excreted via urines and faeces. Thus, these compounds will be concentrated in manure were they have been detected even after long periods of manure storage under anaerobic conditions. So, hormones and antibiotics can be disseminated in natural ecosystems through manure spreading practices. In the ecosystem, hormones have been associated to endocrine disruption phenomena such as feminisation of wild fish populations while antibiotics have been associated to the development of antibiotic resistance in bacteria (Desbrow et al., 1998; Ding et al., 2010).

Manure can also be contaminated by other compounds like polycyclic aromatic hydrocarbons (PAH), dioxin and furans mainly through the surrounding air or by compounds like nonylphenol detergents used for cleaning purposes. These pollutants may also induce endocrine activities through the estrogen receptors, but also through other pathways which are potentially linked with the estrogenic response (Tabb and Blumberg, 2006). These potential activities on the endocrine system can also be found in manure and can be disseminated by manure spreading practices.

Little information exists on the elimination of hormones, antibiotics and endocrine activities contained in swine manure mainly due to analytical limitations caused for the complex matrix of manure. In other matrices, such as wastewater, sludge, or soils controversial results have been reported on the fate of these pollutants. Some studies reported high removal rates of hormones and antibiotics in anaerobic system treating sewage sludge (Carballa et al., 2007); but other studies reported low elimination of hormones during anaerobic digestion (Andersen et al., 2003). Similarly, the reported elimination of several antibiotics varies from 18% to 100%, in different wastewater systems applying aerobic processes (Zorita et al., 2009). In other studies realised in aerobic or anaerobic environment, no removal of ciprofloxacin was reported, whatever the experimental conditions tested; while tetracycline and doxycycline were slightly removed (77 days) and the elimination of erythromycin and clarithromycin was relatively fast (Chenxi et al., 2008). Hence, further research in needed on the fate of such pollutants in complex matrices such as swine manure.

This study is a complete investigation on the fate of steroid hormones hormones (estrone, 17α and 17β estradiol, estriol, 17β-testosterone and progesterone), antibiotics (monensine MN, marbofloxacin MFX, lincomycin LCM, sulfadiazine SFZ, and oxytetracycline OTC) and endocrine activities from swine farms under anaerobic and aerobic/anoxic processes, optimized to produce methane and remove nitrogen from swine manure.

MATERIAL AND METHODS

Conditions for manure treatment

Three conditions were tested for their abilities to remove hormones, antibiotics and endocrine activities: In the first conditions, anaerobic digestion was coupled to the aerobic/anoxic treatment of manure (called coupled anaerobic-aerobic/anoxic treatment). In the second conditions, manure was treated under aerobic/anoxic conditions (called non-coupled aerobic/anoxic treatment). In the last experimental conditions, manure was treated under thermophilic anaerobic conditions using two different inocula (called anaerobic thermophilic treatment R1 and R2). All the reactors were fed with stored manure collected in a farm located in the Brittany region, France.

Chemical and endocrine activity analysis

Total and volatile (VS) solids, chemical oxygen demand, ammonium-nitrogen and Total Kjedahl Nitrogen (TKN) were measured according to standard methods. The biogas production was continuously measured and analysed by gas chromatography (GC). Temperature, pH and oxydo-reduction potential were measured on line.

The extraction and analysis of steroid hormones from manure was performed according to Muller et al. (2008) and Combalbert et al. (2010). Hormones were quantified by GC-MS on a GC autosytem XL coupled to a Turbo Mass Gold mass spectrometer (Perkin Elmer Waltham, MA, USA). Antibiotics were extracted as described by Capdeville (2011) and analysed by LC-MS/MS using a LC 1200 Agilent with a SB-Zorbax C18 coupled to a tandem mass spectrometer 6410A Agilent.

For the measurement of endocrine activities present in manure, an additional extraction was performed in manure samples in order to maximize the extraction of overall estrogenic potency (according to Combalbert et al., 2010). MELN, HAhLP, HG5LN-PXR and HGELN-PPARγ cell lines, transfected with specific responsive genes, were used for endocrine activity measurements according to the conditions previously described (Pillon et al., 2005; Le Maire et al., 2006).

RESULTS AND DISCUSSION

Reactor performances

The nitrogen (TKN) removal measured in the aerobic/anoxic processes reached 74 % in the coupled system and 63 % in the non-coupled system. The anaerobic tanks displayed methane yields of 0.31,

0.77 and 0.51 NL CH_4.gVS remove.d^{-1} and a methane production of 0.12, 0.28 and 0.17 $m^3.m^{-3}.d^{-1}$ for respectively, the coupled system, the thermophilic tanks R1 and R2. These data reflect the well-functioning of aerobic/anoxic and anaerobic reactors.

Elimination of hormone and antibiotics

The fate of steroid hormone in the coupled anaerobic-aerobic/anoxic system (A), in the non-coupled system (B) and in the anaerobic thermophilic processes (C) is shown in Figure 1 while the fate of antibiotics is shown in Figure 2. The average total hormone concentration in the feeds was 25 µg.L-1; this value stayed unchanged after anaerobic treatment, demonstrating that hormones were not removed under anaerobic conditions. Nevertheless, the hormone distribution changed during this step, particularly E1 level decreased whereas E2 increased (data not shown). For the other compounds, the variations observed in hormone concentrations were not significant. A similar behavior was observed for the antibiotics studied (Fig. 2) which displayed no-significant removals after anaerobic treatment. In contrast, at the end of the aerobic/anoxic process the total hormone concentration and antibiotics levels were reduced of about 81% and 85%, respectively. It is in agreement with previous data showing that the aerobic conditions are more efficient for emerging pollutant removal. The non-coupled aerobic/anoxic treatment (Fig. 1B) allowed to remove about 40 % of the total hormone and antibiotic concentrations. It was surprising to note that this process, without anaerobic pre-digestion step, was less efficient to remove these pollutants that the coupled treatment. It is possible that the pre-digestion step, by modifying the structure of the solid matrix of manure, improved the further elimination of hormones and antibiotics in the coupled system. In the thermophilic experiments (Fig. 1C), whatever the inocula used, no hormone removal was observed. A similar behavior was observed for all antibiotics studied (data not shown).

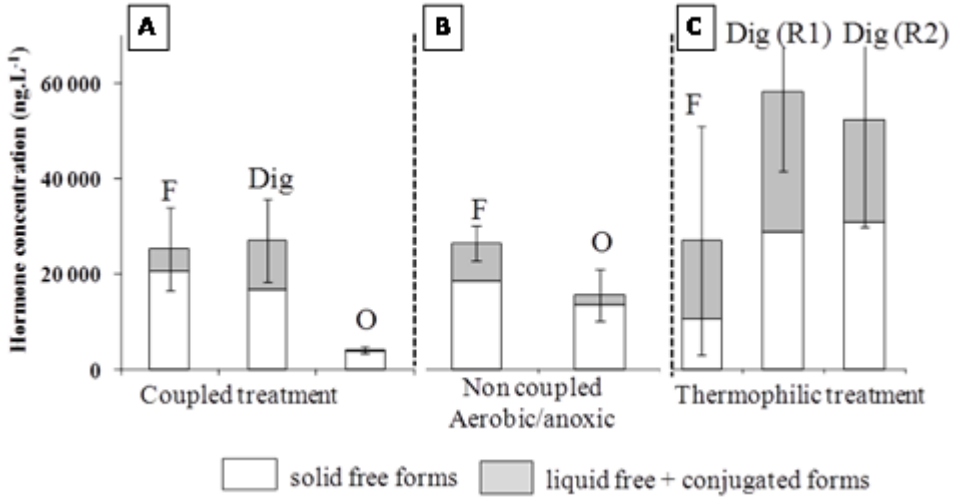

Figure 1. Fate of steroid hormones (A, B, C) during manure treatment. Concentrations in each compartment of the studied systems: Feed (F), Digester (Dig) and outlet (O) of the three treatment processes.

The monitoring of the endocrine activities was realized in the coupled anaerobic-aerobic/anoxic treatment which was the most efficient to eliminate hormones and antibiotics. Our results showed that the theoretical estrogenic activity, deduced from the hormone abundance determined by chemical analysis, was much lower than the estrogenic activity measured by the *in vitro* test. It suggests the presence in manure of other compounds able to activate the ERα and inducing an estrogenic response. The measured estrogenic activity was essentially present in the solid fraction of the manure. An important fraction of the measured estrogenic activity was removed in the anaerobic digester (41 %) and this removal continued during the aerobic/anoxic treatment, which removed 66 % of the measured estrogenic activity. Our results are consistent with the existing literature that reported up to 80 % of estrogenic activity removal in aerobic processes (Furuichi et al., 2006; Ermawati et al., 2007).

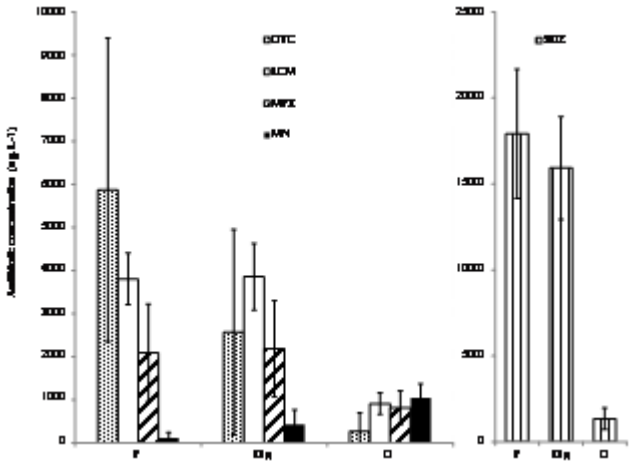

Figure 2. Fate of antibiotics in the coupled treatment of manure.

CONCLUSIONS

We showed that hormones and antibiotics were partially removed (up to 80 % of the initial load) during the aerobic/anoxic process, depending on the compound considered. By contrast, these compounds were not removed under anaerobic mesophilic or thermophilic conditions. AR, PPARγ, PXR activities were never detected, neither in the manure used to feed the reactors, nor after manure treatment. Estrogenic and dioxin-like activities seemed to be less recalcitrant to the anaerobic digestion (about 40 % of removal) and may be due to compounds less persistent than hormones.

ACKNOWLEDGEMENTS

The authors would like to thank the French National Agency for Research (ANR) and theFrench Agency for the Environment and Energy Management (ADEME) which funded the DIPERPHA project (ANR-07-SEST-006-01).

REFERENCES

Capdeville M.J. 2011. Study of the biogeochemical cycles of emerging contaminants in aquatic ecosystems. PhD Thesis. University of Bordeaux, France.

Carballa, M., Omil, F., Ternes, T. and Lema, J. M., 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Res*. **41**, 2139-2150.

Chenxi W., Spongberg A.L., Witter J.D. 2008. Determination of the persistence of pharmaceuticals in biosolids using liquid-chromatography tandem mass spectrometry. *Chemosphere* **73**, 511–518.

Combalbert, S., Pype, M. L., Bernet, N., Hernandez-Raquet, G. 2010. Enhanced methods for conditioning, storage, and extraction of liquid and solid samples of manure for determination of steroid hormones by solid-phase extraction and gas chromatography-mass spectrometry. *Anal. Bioanal. Chem*. **398,** 973-984.

Desbrow C., Routledge E.J., Brighty G.C., Sumpter J.P., Waldock M. 1998. Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. *Environ Sci Technol*. **32**:1549–1558

Ding C., He J. 2010. Effect of antibiotics in the environment on microbial populations. *Appl. Microbiol Biotechnol* **87**, 925–941.

Ermawati, R., Morimura, S., Tang, Y.Q., Liu, K., Kida, K., 2007. Degradation and behavior of natural steroid hormones in cow manure waste during biological treatments and ozone oxidation. *J. Biosci. Bioeng*. **103**, 27-31.

Furuichi, T., Kannan, K., Suzuki, K., Tanaka, S., Giesy, J.P., Masunaga, S., 2006.Occurrence of estrogenic compounds in and removal by a swine farm waste treatment plant. *Environ. Sci. Technol*. **40**, 7896-7902.

Le Maire, A., Bourguet, W., Balaguer, P., 2010. A structural view of nuclear hormone receptor: endocrine disruptor interactions. *Cell Mol. Life Sci*., **67**, 1219-1237.

Muller, M., Rabenoelina, F., Balaguer, P., Patureau, D., Lemenach, K., Budzinski, H., Barcelo, D., De Alda, M.L., Kuster, M., Delgenes, J.P. and Hernandez-Raquet, G. 2008. Chemical and biological analysis of endocrine-disrupting hormones and estrogenic activity in an advanced sewage treatment plant. *Environ. Toxicol.Chem*. **27**, 1649-1658.

Pillon, A., Boussioux, A. M., Escande, A., Ait-Aissa, S., Gomez, E., Fenet, H., Ruff, M., Moras, D., Vignon, F., Duchesne, M. J., Casellas, C., Nicolas, J. C. & Balaguer, P. 2005 Binding of estrogenic compounds to recombinant estrogen receptoralpha: application to environmental analysis. *Environ. Health Perspect*. **113**, 278–284.

Tabb, M.M. and Blumberg, B. (2006) New modes of action for endocrine-disrupting chemicals. *Mol. Endocrinol*. **20**, 475-482.

Zorita S, Martensson L, Mathiasson L (2009) Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. *Sci Total Environ*. **407**, 2760–2770.