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Biodegradation by activated sludge and toxicity of tetracycline into a semi-industrial membrane bioreactor

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ABSTRACT

Much attention has been devoted recently to the fate of pharmaceutically active compounds such as tetracycline antibiotics in soil and water. Tetracycline (TC) biodegradability by activated sludge derived from membrane bioreactor (MBR) treating swine wastewater via CO_2-evolution was evaluated by means of modified Sturm test, which was also used to evaluate its toxicity on carbon degradation. The impact of tetracycline on a semi-industrial MBR process was also examined and confronted to lab-scale experiments. After tetracycline injection in the pilot, no disturbance was detected on the elimination of organic matters and ammonium (nitrification), reaching after injection 88% and 99% respectively; only denitrification was slightly affected. Confirming the ruggedness and the superiority of membrane bioreactors over conventional bioreactors, no toxicity was observed at the considered level of TC in the pilot (20 mg TOC L^{-1}), while at lab-scale sodium benzoate biodegradation was completely inhibited from 10 mg TOC L^{-1} TC. The origin of the activated sludge showed a significant impact on the performances, since the ultimate biodegradation was in the range −50% to −53% for TC concentrations in the range 10–20 mg TOC L^{-1} with conventional bioreactor sludge and increased to 18% for 40 mg TOC L^{-1} of TC with activated sludge derived from the MBR pilot. This confirmed the higher resistance of activated sludge arising from membrane bioreactor.

1. Introduction

Brittany concentrates 57% of the French pigs' production. The public is becoming increasingly concerned with the livestock industries potential impact on water and air quality, which are damaged by an excess of different chemical compounds. The European project "Zero Nuisance Piggeries' proposes the implantation of a global management of piggeries for the treatment of swine manure in a semi-industrial scale" (Prado et al., 2007).

The system combines a flushing technology of the piggeries; this technology conducts to Dilute Swine WasteWater (DSWW). The flush is followed by a mechanical liquid/solid separation (centrifugation) of the DSWW and a biological treatment by reaction/separation coupling (submerged membrane bioreactor). Regular flushing helps to prevent anaerobic decomposition of the manure, being the main process responsible for the release of odour creating compounds. The bioreactor aims at reducing organic and nitrogen content of the DSWW, while the membrane withholds pathogens and allows the reuse of water for flushing purposes. Membrane bioreactors are used in urban wastewater (Yeom et al., 1999; Shim et al., 2002), industrial wastewater and agricultural wastewater (Cicek, 2003). But only a small part of semi-industrial and industrial processes reuses effectively the treated water.

The occurrence and fate of pharmaceutically active compounds in the natural environment has been recognized as one of the emerging issues in environmental chemistry (Halling-Sorensen et al., 1998; Sarmah et al., 2006). Amongst them, tetracycline (TC) is an important broad-spectrum antibiotic, widely prescribed in pigs' production. Several authors (Halling-Sorensen, 2001; Loke et al., 2002; Sengelov et al., 2003) described non-negligible concentrations of tetracycline found in pig slurry (up to 5 mg L^{-1}). The fate of tetracycline and antibiotics in general in a membrane bioreactor is not widely documented. Several authors studied the interaction between activated sludge processes and pharmaceutically active compounds (Halling-Sorensen, 2001; Göbel et al., 2005; Kim et al., 2005; Joss et al., 2006; Radjenovic et al., 2007). The fate of pharmaceuticals has been demonstrated highly dependant to process parameters (Clara et al., 2005; Kim et al., 2005; Joss et al., 2006).The possibility to control the Sludge Retention Time (SRT) in MBR is better than in Classical Activated Sludge System (CASS). Moreover, due to the higher biomass concentration, MBR are less sensitive to fluctuation than CASS (Radjenovic et al., 2007).

During passage through Activated Sludge (AS) treatment plants, antibiotics removal may occur by hydrolysis, biodegradation and/or sorption to sludge. Sorption to sludge leads to an overestimation

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Nomenclature

AS	activated sludge	MF	microfiltration
Biod%	percentage of biodegradation	MLSS	mixed liquor suspended solid (ML^{-3})
$Biod_{res}$	percentage of residual biodegradation	PVDF	polyvinyl fluoride
COD	chemical oxygen demand (ML^{-3})	SPE	solid phase extraction (T)
CTP	conventional treatment plant	SRT	solid retention time (T)
DSWW	dilute swine wastewater	SVU	sealed vessel unit
HPLC	high phase liquid chromatography	TC	tetracycline
HRT	hydraulic retention time (T)	$ThCO_2$	theorical CO_2 production (ML^{-3})
MBR	membrane bioreactor	TN	total nitrogen (ML^{-3})
m_{CO_2P}	mass of CO_2 produced (ML^{-3})	TOC	total organic carbon (ML^{-3})
m_{CO_2PE}	endogen CO_2 production (ML^{-3})	TS	total solids (ML^{-3})
m_{CO_2theo}	theorical CO_2 production (ML^{-3})	TU	trapped unit
m_{CiRC}	carbon mass of the referent compound (M)	VSS	volatile suspended solid (ML^{-3})
m_{CiTC}	carbon mass of the target compound (M)		

of the activated sludge treatment plants efficiency for antibiotics removal and to the release of persistent molecules after biomass death. CO_2-evolution test (OECD 301 B, 1992), formerly known as "Modified Sturm test", allows the evaluation of the biodegradability of antibiotic pollutants by activated sludge via the measure of the produced carbon dioxide. This lab-scale test was carried on 17 antibiotics where the inoculum was derived from municipal sewage treatment (Gartiser et al., 2007) showing that only penicillin had a potential for biodegradation in these conditions. However, the behaviour of tetracycline with another inoculum is not predictable.

In order to understand the impact of MBR acute contamination by tetracycline, a semi-industrial MBR was contaminated in this work. In parallel, the susceptibility of tetracycline to be degraded by AS derived from MBR treating DSWW via CO_2-evolution was evaluated. Modified Sturm tests were also used to evaluate the toxicity of TC on easily biodegradable compound biodegradation. Lab-scale and pilot-scale results were critically confronted to describe the fate of tetracycline into a semi-industrial MBR.

2. Methods

2.1. Pilot scale experiments (Biosep®)

A pilot scale experiment using a setup able to treat up to 0.4 m^3 day^{-1} of swine wastes (2.4 m^3 day^{-1} of DSWW) was conducted in Brittany at the Agriculture Applicated Research Centre in Finistere (Prado et al., 2007). A flow-sheet diagram is presented in Fig. 1. The process consists in a centrifugal apparatus, a raw slurry storage tank (1 m^3), an anoxic tank (8 m^3), an aerobic tank (12 m^3), a submerged membrane (microfiltration – MF) tank (2 m^3), a treated water storage tank (10 m^3), two flush tanks (1 m^3 each) and composting equipment. MF tank is provided of 20 polyvinylide fluoride (PVDF) membrane units of 1.4 m^2 each (in reactor 24.4 m^2).

The antibiotics contamination study was carried out under the following experimental conditions:

- the average membrane flux was 9 L m^{-2} h^{-1},
- the membrane area was 27.4 m^2,
- the SRT was 30 days,
- the HRT was 3 days,
- the average food/microorganisms ratio was 0.088 kg COD kg^{-1} of SS day^{-1},
- the average volumetric load was 0.96 kg COD m^{-3} day^{-1} and
- the operational temperature varied from 15.9 °C to 19.7 °C (summer time).

Tetracycline hydrochloride (>96% – HPLC, Fluka–Sigma–Alldrich – St Quentin Fallavier, France) was daily added directly in the anoxic tank. To calculate the quantity, the posology of TC was consulted. In case of respiratory affections, a pig receives 50 mg of TC kg^{-1} and per day during one week. If 72 animals weighing approximately 100 kg each are treated, the addition of TC is around 360 g day^{-1}. Therefore, 100 g of TC day^{-1} were added into the anoxic tank, representing 27% of the total posology, which keeps realistic. It is noticeable that, in France, since January the 1st from the year 2006, it is strictly forbidden to use antibiotics as growth factor. Antibiotics usage is restricted to therapeutic use; however, they are often administrated to a group of pigs. Therefore, the contamination lasted only 5 days and the total quantity introduced in the system was 500 g, corresponding to a TC concentration of approximately 40 mg L^{-1}, namely 22 mg TOC L^{-1} (12 m^3 total water volume in the process).

2.2. Lab-scale experiments (OECD 301 B, norm ISO 9439)

2.2.1. Biodegradation tests

Modified Sturm test (28-days aerobic degradation) was conducted following the method developed by the OECD. To determine heterotrophic activity the CO_2 uptake rate test was applied. Biomass to be characterized was placed into a sealed vessel unit (SVU). The SVU is a 1 L vessel continuously aerated with air CO_2-free (system is composed by six units). Gas from SVU was continuously transferred in CO_2 trapped unit (TU). CO_2 produced by microbial activity was trapped by a solution of barium hydroxide ($Ba(OH)_2$) which precipitate as barium carbonate in presence of CO_2. The remaining barium hydroxide was titrated with 0.05 N standard HCl in presence of phenolphthalein. The CO_2-free air production system consisted of an air compressor, two 200 ml gas wash bottle filled with 4 M NaOH, followed by one 200 ml gas wash bottle filled with 0.0125 M $Ba(OH)_2$. The CO_2-free air was passed on to an air distributor with one input and 6 output channels and through PE-tubes to the SVU. Two $Ba(OH)_2$ traps were connected to each SVU. The 6 reactors were prepared as described on Table 1; the mineral medium described by OECD 302 B was used for dilutions.

Due to the instability of barium hydroxide its concentration was checked with HCl standard solution when traps were changed. Calculations of the mass of CO_2 produced were determined from these parameters. The mass of CO_2 produced (m_{CO_2P}) in SVU reactor was calculated as follows:

$$m_{CO_2P} = (C_{Ba(OH)_2} \cdot V_{Ba(OH)_2} - C_{HCl} \cdot V_{HCl}) \cdot 44 \quad \text{(i)}$$