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Short communication

Biodegradation and biosorption of tetracycline and tylosin antibiotics in activated sludge system

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ABSTRACT

The aim of this study was to determine the fate of veterinary antibiotics entering biological treatment process. Due to the prevalence of their respective antibiotic family usage in livestock, tetracycline and tylosin were selected. Using modified Sturm test (OECD 301-B), their biodegradation were compared to that of a referent pollutant, sodium benzoate, well-known for its high biodegradability. Biodegradation rates were −28 and −35% for tetracycline and 4 and −5% for tylosin showing an absence of biodegradability. OECD 301-B inhibition tests showed a potential toxicity of both molecules on activated sludge inoculum derived from membrane bioreactor. Tetracycline presented good adsorbability while tylosin remained mostly present in the soluble phase. The Langmuir maximum adsorption capacity ($C_{s,max}$) was found to be 72 and 7.7 mg g^{-1} for tetracycline and tylosin, respectively. Adsorption was therefore the most favourable fate for tetracycline entering a biological process. Conclusions on tylosin case were more controversial.

1. Introduction

Largely used in livestock, veterinary pharmaceutically active compounds are now identified as environmental issue. Public concern on their environmental fate and occurrence increased in recent years. Among veterinary pharmaceuticals, antibiotics are widely prescribed with a prevalence of the tetracycline family; 50% of the antibiotics sold in France in 2004 [1] were derived from tetracycline. The other antibiotics frequently used in livestock are aminoside, β-lactamine, macrolide, polypeptide, sulfamide and trimetoprime. Tylosin antibiotic belong to the macrolide family representing 7.8% of the total veterinary antibiotic sold in France in 2004 [1]. Tetracycline and tylosin are both broad-spectrum antibiotics widely used in pig production, tetracycline is used in case of respiratory affection and tylosin is recommended in case of diarrhoea. Antibiotics are proscribed as growth factor in France since 2006. However, their usage as therapeutics is still very large as far as they are nearly always used in group [2]. Moreover the dosage of veterinary antibiotics is high with among 40 mg kg^{-1} day^{-1} during 10 days and 10 mg kg^{-1} day^{-1} during 15 days for tetracycline and tylosin, respectively. In many cases, only part of the treatment is actually metabolised [3,4], the part left is found back as active form in pigs' excreta.

The concentration of pig production in a restricted area leads to exceed cropland capacity to receive piggeries wastewater as agronomic rates. As a results pigs industry involves environmental burdens on water and air quality, which finally increased pressure for the installation of water treatment plants. Swine wastewater is commonly treated by biological way with activated sludge processes and anaerobic or aerobic digestion processes [5]. Treatments by system including activated sludge such as membrane bioreactors have proved their efficiency for the decrease of organic and nitrogen loads of swine wastewater [6]; however, antibiotics excreted as active form may enter the system and results in an acute contamination during a therapeutic usage. Different studies on antibiotics fate [7–10] in bioreactor highlighted three different ways for antibiotics disappearance, the most favourable case is biodegradation, another way is accumulation on biomass defined as biosorption, which leads to the release of molecules after biomass death, and a third way is hydrolysis.

CO_2-evolution tests (OECD 301-B), formerly known as modified Sturm tests [11], are commonly used for the evaluation of the biodegradation potential of non-volatile molecules via the measurement of the produced carbon dioxide. In this test, the ratio molecules/biomass is high and the biodegradation qualified as "easy". Further tests could be done in order to evaluate the "inherent" biodegradability [12], which means the potential biodegradation in a specific environment but would not be relevant to simulate an acute pollution of a bioreactor.

Biosorption of non-biodegradable molecules on activated sludge can be compared to adsorption on an inactive adsorbent

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and Freundlich and Langmuir absorption models can be used to describe the phenomenon. The Freundlich model (1939) assumes a heterogeneous adsorbent surface with an exponential distribution of adsorption sites. It leads to the determination of K_f and n, Freundlich coefficient and linearity parameter, respectively and no restriction due to possible molecule–adsorbent interactions exist. As far as the adsorption capacity has no upper limit, this model is confined to diluted solutions. K_f and n are limited to the couple molecule–adsorbent at a given temperature. The Langmuir model (1918) describes the molecule–adsorbent interactions with a limited number of adsorption sites and no interaction between the molecules. It leads to the determination of K_l and $C_{s,max}$, the Langmuir coefficient and the maximum solid concentration. Freundlich coefficients for tylosin and oxytetracycline can be found in the available literature [13–15], contrarily to Langmuir coefficients for relevant environmental conditions.

Previous work showed that nearly 90% of tetracycline was eliminated from the soluble phase in a membrane bioreactor; however, no conclusion on the tetracycline fate was possible at this stage [16]. This paper proposes to evaluate biodegradation and biosorption of tetracycline and tylosin in presence of activated sludge at lab-scale. Modified Sturm test was used for biodegradation evaluation. Biosorption was evaluated by batch tests in order to determine Langmuir and Freundlich constants for each molecule. The purpose was to predict the most probable route for tetracycline and tylosin antibiotics through activated sludge processes facing an acute contamination.

2. Materials and methods

2.1. Chemical and reagents

Tetracycline hydrochloride (>96%, HPLC) and tylosin tartrate (>98% UV) were obtained from Fluka–Sigma–Aldrich (St. Quentin Fallavier, France).

Citric acid (anhydrous, 99%), Na_2EDTA (99%), trifluoroacetic acid (99.5%) and Na_2HPO_4 (99%) were purchased from Acrôs Organics (Noisy-le-grand, France). Methanol and acetonitrile (ACN) were both HPLC grade from Fisher Scientific (Illkirch, France) and tetrahydrofuran (THF–HPLC grade, 99.7%) was purchased from Prolabo VWR (Fontenay-sous-Bois, France). Standards were made with ultra pure water (Purelab Options–Q7/15, Elga, 18.2 MΩ cm).

Conditioning and extraction buffer was EDTA–McIlvaine buffer (50:50), prepared by mixing 150 mL of 0.1 M EDTA (ethylenediaminetetraacetic acid), 90 mL 0.2 M citric acid, 60 mL 0.4 M Na_2HPO_4. Extraction buffer pH was adjusted to 4 by adding H_3PO_4 if needed.

2.2. Biodegradation tests

Modified Sturm tests (28 days aerobic degradation) were conducted according to the OECD 301-B method. 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 inoculum concentration was 30 mg L^{-1}. The SVU was a 1 L vessel continuously aerated with air CO_2-free (the system was composed by six units, Fig. 1). Gas from SVU was continuously transferred in a CO_2 trap unit. CO_2 produced by microbial activity was trapped in a solution of barium hydroxyde ($Ba(OH)_2$) which precipitate as barium carbonate in presence of CO_2. The remaining barium hydroxide was titrated with 0.05N standard HCl in the presence of phenolphtaleine. 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 $Ba(OH)_2$ (Fig. 1). The CO_2-free air was passed on to an air sparger with one input and six output channels and through PE-tubes to the SVU. Two $Ba(OH)_2$ traps were connected to each SVU. The six reactors were prepared as described in Fig. 1 using the mineral medium given in OECD 301-B for dilutions.

The mass of CO_2 produced (mCO_2) and the biodegradation ratio (nCO_2) were determined as follows:

$$n_{CO_2} = n_{Ba(OH)_2} - \frac{n_{HCl}}{2} = (C_{Ba(OH)_2} \cdot V_{Ba(OH)_2}) - \left(C_{HCl} \cdot \frac{V_{HCl}}{2}\right) \quad (1)$$

$$M_{CO_2} = n_{CO_2} \cdot M_{CO_2} = n_{CO_2} \cdot \frac{44}{12} \quad (2)$$

SVU1 called blank inoculum test allowed to evaluate the CO_2 production if no carbon was added. CO_2 production in SVU1 corresponded to the measured endogenous CO_2 production (mCO_{2endo}). SVU3 corresponded to the abiotic test to evaluate the CO_2 production if no activated sludge was added to the reactor. CO_2 production in SVU1 and SVU3 were taken into account for CO_2 production calculations in the reactors 2, 4, 5 and 6.

The ultimate biodegradation (%) of the target compound was calculated as follows:

$$\text{Biod}(\%) = \frac{m_{CO_2} \cdot 100}{\text{Th}_{CO_2\text{Tot}}} \quad (3)$$

$$\text{Th}_{CO_2} = m_{C_i} \frac{44}{12} \quad (4)$$

With Th_{CO_2} corresponding to the total theoretical CO_2 formation produced by total oxidation of the material and m_{Ci} corresponding to the initial carbon mass in the reactor (mg TOC L^{-1}). The ratio 44/12 was the conversion factor of carbon to carbon dioxide.

2.3. Total organic carbon (TOC) determination

TOC was analysed via a TOC-meter (OI-Analytical 1010). Sample analysis included several steps. At the first step, the sample was acidified with sulfuric acid to reach a pH lower than 2 and served with gas to remove the inorganic carbon. Carbon was then oxidized and released as CO_2, which was then determined by an infrared detector.

2.4. Biosorption tests

For biosorption, a common isotherm experiment was applied. A known amount of activated sludge (about 0.5 g) was suspended in 500 mL identical batch reactors where various concentrations of the target compounds (tetracycline and tylosin) were added. All reactors were placed in thermostated baths at 25 °C. Initial samples and samples after 3 and 24 h were taken and analysed by HPLC with UV detection. The equilibrium was considered reached after 24 h.

Freundlich coefficients were determined by means of the Frendlich model:

$$C_s = K_f C_w^n \quad (5)$$

With C_s and C_w the equilibrium target compound concentrations on the biomass (mg g^{-1}) and in solution (mg L^{-1}) respectively, K_f the Freundlich parameter (L g^{-1}) and n the linearity parameter.

Langmuir coefficients were determined using the Langmuir equation:

$$C_s = \frac{C_{s,max} K_l C_w}{1 + K_l C_w} \quad (6)$$

With C_s and C_w the equilibrium target compound concentrations on the biomass (mg g^{-1}) and in solution (mg L^{-1}), respectively, K_l the Langmuir parameter (L mg^{-1}) and $C_{s,max}$ the maximum adsorption capacity.

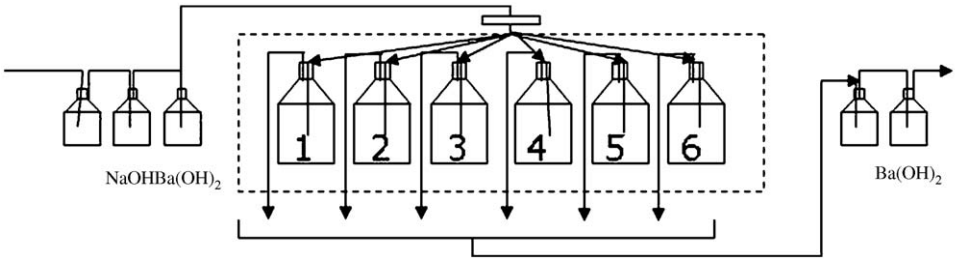

Fig. 1. Diagram of Sturm tests experimental design. Sealed vessel unit (SVU) 1, blank inoculum; SVU 2, reference (sodium benzoate, 47 mg L^{-1} TOC); SVU 3, abiotic test; SVU 4 and 5, target molecule (40 mg L^{-1} TOC); SVU 6, inhibition control test (sodium benzoate 47 mg L^{-1} TOC + target molecule 40 mg L^{-1} TOC).