

AEROBIC BIODEGRADABILITY ASSESSMENT OF PHARMACEUTICAL WASTEWATER FROM INDUSTRIAL PRODUCTION OF ANTIBIOTICS

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ABSTRACT – Antibiotics are considered emerging environmental issues and the presence of its traces in the environment can induce the development of resistant pathogens and promote allergies. Biological treatment is the most widely used method for industrial wastewater treatment, but it has been proved inefficient for the removal of all hazardous constituents of pharmaceutical products. In this context, the aim of this study is to evaluate the potential of aerobic biodegradation of organic matter present in real antibiotics production effluent using the method proposed by Zahn - Wellens. The organic matter degradation was monitored through periodic analysis of total organic carbon (TOC). After 28 days, the reactor achieved more than 80% of TOC removal, characterizing the wastewater as biodegradable, according to the used methodology.

1. INTRODUCTION

Emerging pollutants are groups of compounds without specific legal regulations whose toxic effects to the environment and human health coupled with high occurrence make them subject to future regulations (MIRALLES-CUEVAS *et al.*, 2013). This group includes various types of globally widespread organic compounds, such as pesticides, dyes, pharmaceuticals, personal care products, polymers and plastics. Most of the pharmaceuticals used are excreted in an unchanged or only partially metabolized form and end up as such in municipal waste water and in the environment.

Antibiotics are chemical, natural or synthetic pharmaceuticals that can eliminate or prevent the multiplication of bacteria. These drugs have been extensively used in human and veterinary medicine to treat diseases caused by bacteria and, in some cases, to prevent bacterial infections. They are also used as growth promoters in animals that are part of the food industry, and as pesticides for controlling bacterial infections in crop fields, particularly cereals. Therefore, they are abundant contaminants in the environment (KIM *et al.*, 2013). The effluents from the production of antibiotics are considered emerging environmental problems due to their refractory characteristics and toxicity to the environment, even in low concentrations. The presence of

traces of such pharmaceuticals in the environment can induce the development of antibiotic-resistant pathogens, triggering serious problems for human and animal health (MASCOLO *et al.*, 2010). It is estimated that about half of the pharmaceutical effluent produced in the world is thrown into water bodies without any treatment (DEEGAN, 2011). When these wastewaters are treated, there may be employed inefficient techniques for the degradation of recalcitrant and dangerous compounds present.

Biological treatment consists in decomposing the water-soluble and non-volatile organic matter through microorganism's oxidation and/or fermentation in controlled and specially designed reactors. There are two types of biological treatment: aerobic, in which microorganisms degrade organic substances through oxidation processes with the use of oxygen as the electron acceptor; and anaerobic, in which anaerobic microorganisms decompose organic substances through oxidation with electron acceptors different than oxygen (sulfur; nitrate, etc.) or fermentation processes. Biological treatment is the most common and economical method of wastewater treatment used, however, it has been proved ineffective for the removal of all potentially hazardous constituents of pharmaceutical effluents (MONTEAGUDO *et al.*, 2013; PÉREZ-MOYA *et al.*, 2010).

In another hand, advanced oxidations processes are widely used for treatment of toxic or non-biodegradable wastewaters, but the intensity and duration of those reactions influence directly on the treatment costs and mineralization efficiency. Hence, special attention must be paid to the biodegradability determination of the mixture of intermediates generated during advanced oxidation treatment of recalcitrant and dangerous wastewaters, which relies on biodegradability tests (BALLESTEROS MARTÍN *et al.*, 2010). Therefore, biodegradability is a key parameter in the hazard assessment of chemicals and wastewaters. High biodegradability implies a reduced tendency to bioaccumulate or to persist in the environment (STOLTE *et al.*, 2012).

In order to assess the biodegradability of wastewater, several methods (Zahn-Wellens test, BOD₅/COD ratio, respirometry test, *Pseudomonas putida* bioassay) were proposed by international organizations, such as OECD and ISO. These biodegradation assessments are divided into three major groups: tests on ready biodegradability, tests on inherent biodegradability and simulation tests. Zahn-Wellens is an inherent biodegradability test that evaluates the potential biodegradability of water-soluble, non-volatile organic substances exposed to high concentrations of microorganisms and nutrients in 28 days (BALLESTEROS MARTÍN *et al.*, 2010; PAGGA, 1997).

The Zahn-Wellens test forecasts the degradation behavior of a wastewater in an activated sludge treatment plant, since the experimental conditions are similar to this process. Nevertheless, humble biodegradability results obtained in an inherent biodegradability test are considered sufficiently indicative for poor biodegradability, but positive results are not necessarily predictive of biodegradability under real environmental conditions, a significant overestimation of the removal extent was reported in literature (MASCOLO *et al.*, 2010). Ballesteros Martin *et al.* (2010) compared four biodegradability tests (*Pseudomonas putida* bioassay, Zahn-Wellens test,

BOD₅/COD and respirometry assay) to determine the biodegradability enhancement of a treated pesticides mixture taking into account repeatability and precision of each biodegradability test. The authors concluded that *P. putida* and Zahn–Wellens tests showed higher repeatability and precision.

In this context, the aim of this study is to evaluate the potential of aerobic biodegradation of organic matter present in real antibiotics production wastewater using the method proposed by Zahn – Wellens (OECD, 1992) with the use of recirculation sludge from the activated sludge reactor of the municipal Wastewater Treatment Plant (WWTP) of Ribeirão Arrudas in Belo Horizonte, as inoculum.

2. MATERIAL AND METHODS

2.1. Biomass

The sludge used as biomass source was collected at the recirculation step of the biological reactor in activated sludge system of the WWTP of Ribeirão Arrudas in Belo Horizonte, Brazil. The sludge was stored in the fridge at 5°C until used and pre-conditioned to the experimental conditions by sedimentation and several tap water washing prior to use in order to concentrate the biomass and reduce the TOC background. Sludge biomass concentration $(250 \pm 4) \cdot 10^{-1} \text{ g} \cdot \text{L}^{-1}$ was determined gravimetrically (APHA-AWWA-WEF, 1992) by measuring volatile suspended solids (VSS).

2.2 . Wastewater

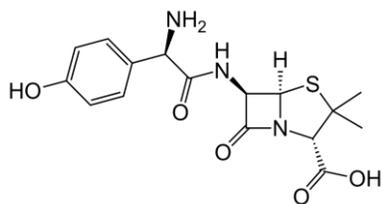
The wastewater sample used in this work was obtained at an antibiotics production plant in a pharmaceutical industry in Brazil. The effluent is an aqueous mixture of cleaning waters, cleaning products, antibiotics, solvents and intermediates. At the time of sample collection, the industry produced amoxicillin, so this antibiotic was monitored after the biodegradation assessment. The physical-chemical characteristics of the pharmaceutical wastewater were assessed and are reported in Table 1.

2.3. Chemicals

Sodium hydroxide and sulfuric acid were used in the biological reactor in order to keep the pH in a neutral threshold (6,5 – 7,5). All the chemicals used for the wastewater characterization analysis and for the preparation of the mineral nutrients solutions were at least >98% pure (analytical grade). The amoxicillin used in HPLC determinations was provided by the pharmaceutical industry.

Table 1 – Physical-chemical characteristics of the investigated pharmaceutical wastewater

Parameter	Value	Unit
pH	7,8	
Conductivity	30,6 – 32,1	$\mu\text{S}\cdot\text{cm}^{-1}$
Dissolved oxygen	2,8 - 3,5	$\text{mg O}_2\cdot\text{L}^{-1}$
Turbidity	63,3 - 69,3	NTU
TOC	2365 – 2393	$\text{mg C}\cdot\text{L}^{-1}$
Total carbon	2403 – 2428	$\text{mg C}\cdot\text{L}^{-1}$
Inorganic Carbon	36 - 38	$\text{mg C}\cdot\text{L}^{-1}$
COD	5900 – 6140	$\text{mg O}_2\cdot\text{L}^{-1}$
BOD ₅	3500 - 3800	$\text{mg O}_2\cdot\text{L}^{-1}$
BOD ₅ /COD	0,61	
Nitrate	1,37 - 1,58	$\text{mg NO}_3^-\cdot\text{L}^{-1}$
Nitrite	0,87 - 0,92	$\text{mg NO}_2^-\cdot\text{L}^{-1}$
Sulfate	14 - 15,3	$\text{mg SO}_4^{2-}\cdot\text{L}^{-1}$
Phosphate	3,9 - 4,1	$\text{mg PO}_4^{3-}\cdot\text{L}^{-1}$
Fluoride	Not detected	$\text{mg F}\cdot\text{L}^{-1}$
Chloride	45,6 - 50,5	$\text{mg Cl}\cdot\text{L}^{-1}$
Bromide	1,9-2,0	$\text{mg Br}\cdot\text{L}^{-1}$
Alkalinity	233,1	$\text{mg CaCO}_3\cdot\text{L}^{-1}$
TSS	297 - 346	$\text{mg}\cdot\text{L}^{-1}$
VSS	172 - 184	$\text{mg}\cdot\text{L}^{-1}$
Amoxicillin	150-180	$\text{mg}\cdot\text{L}^{-1}$



2.4. Analytical techniques and procedures

The organic matter degradation was monitored through periodic sample collection, filtration with quantitative filter paper 125mm C40 to remove the suspended solids (sludge) and subsequent analysis of total organic carbon (TOC) in TOC-V CPN equipment (Shimadzu). The specific degradation of amoxicillin after the biodegradability experiments was followed by HPLC (Agilent Technologies Model 1260 Infinity) equipped with a reverse-phase Zorbax Eclipse Plus® C18 (4.6 X 150 mm, 5.0 μm). The mobile phase was methanol:water (55:45, v/v) at isocratic elution. The flow was 0.750 $\text{mL}\cdot\text{min}^{-1}$ and the monitored wavelength was 210 nm. The aliquots were filtered through 0.20 μm syringe filters (Millex-GN, 25mm, Millipore) before HPLC injection. The injection volume was 20 μL and the total run time was 10 minutes.

2.5. Aerobic biodegradation

The aerobic biodegradability assays were performed accordingly to the Zahn-Welles test methodology (OECD, 1992) with high nutrient and biomass concentration. The biodegradation was also assed adapting the method for the use of the high organic carbon wastewater (TOC ~2400 mg·L⁻¹). The organic matter degradation was monitored through periodic analysis TOC. Test A (called diluted wastewater) was performed using a diluted wastewater so that the initial TOC was in the organic matter threshold imposed by Zahn-Wellens method (initial TOC ~ 217 mg·L⁻¹). Test B (called pure wastewater) was performed with the pharmaceutical wastewater without prior dilution (the mineral nutrients solution used in the reactor produced a small dilution) and initial TOC was around 1720 mg·L⁻¹.

A micro and macro nutrients mineral solution was prepared accordingly to the Zahn-Wellens methodology. 500 mL of this nutrients solution was added to each reactor. In order to obtain around 0,6 g·L⁻¹ of biomass in the reactors, 50 mL of the pre-conditioned sludge was added to each vessel. The control and blank experiments were prepared using glucose as carbon source (initial TOC ~ 200 mg·L⁻¹), which is highly biodegradable, and distilled water, respectively. Then, the mineral nutrients and the activated sludge were also added. Two litters Erlenmeyer vessels were used as reactors and the test was performed under aeration with the use of aquarium aerators and kept in the dark (aluminum sheet exterior cover) at room temperature 23–29 °C for 28 days. Samples were taken at appropriate regular time intervals and TOC was determined. At each sampling, loss of volume due to evaporation was mitigated with distilled water and NaOH and H₂SO₄ solutions were used to keep the pH in the reactors in a neutral threshold (6,5 – 7,5).

The percentage of biodegradation (D_t) at time t was determined by the equation 1:

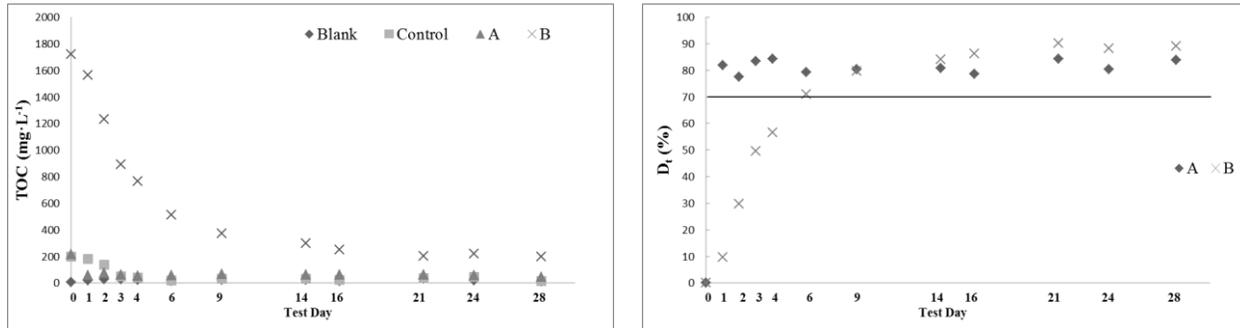
$$D_t = \left[1 - \left(\frac{C_t - C_B}{C_A - C_{BA}} \right) \right] \times 100 \quad (1)$$

where C_A and C_{BA} are the TOC (mg·L⁻¹) in the sample and in the blank, respectively, measured 3 h after starting the experiment, and C_t and C_B are the TOC (mg·L⁻¹) in the sample and in the blank, respectively, measured at the sampling time t . The wastewater samples are considered biodegradable by Zahn-Wellens methodology when D_t is higher than 70%.

3. RESULTS AND DISCUSSION

Figure 1 shows the average evolution of the measured TOC in all the reactors investigated during the 28 days of biodegradation by Zahn-Wellens methodology. It is possible to note that the diluted wastewater (A) TOC decreased faster than the pure wastewater (B). The percentage of biodegradation (D_t) for the diluted wastewater (A) and for the pure wastewater (B) was determined by equation 1 and is represented in Figure 1b. The wastewater samples are considered biodegradable when D_t is higher than 70%. According to this test, both diluted and pure

wastewaters can be considered biodegradable effluents. It can be noted that the diluted wastewater achieved more than 80% of biodegradation after the first day of reaction, while the raw wastewater needed 6 days to achieve biodegradability.



(a)

(b)

Figure 1(a) – TOC evolution in the blank, control, A- diluted wastewater - and B - pure wastewater reactors during the 28 days of biodegradation by Zahn-Wellens methodology.

Figure 1(b) – Zahn-Wellens percentage of biodegradation (D_t) for the diluted wastewater (A) and for the pure wastewater (B).

HPLC monitoring (Figure 2) showed that more than 80 % of the amoxicillin present in the pure wastewater (B) was destructed after the biodegradation reaction (28 days).

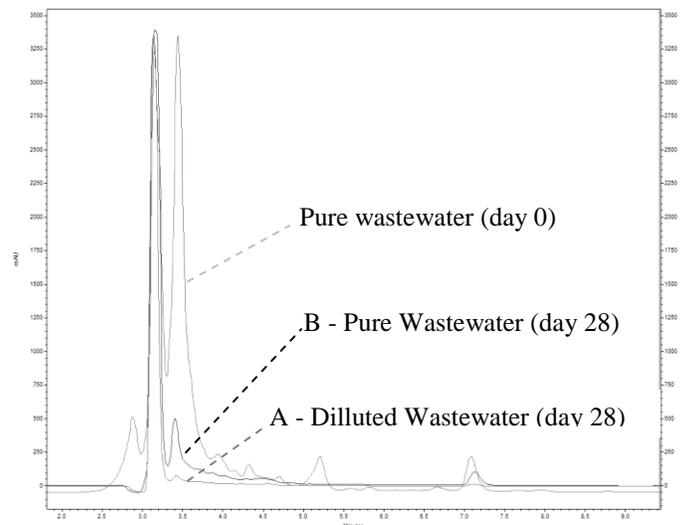


Figure 2 – HPLC chromatograms of the pure wastewater at day 0 and at day 28 for the diluted wastewater (A) and for the pure wastewater (B).

It is noteworthy that even though the used methodology classifies this wastewater as biodegradable, positive results are not necessarily predictive of biodegradability under real treatment conditions. Some researches, such as Mascolo *et al.* (2010), reported that a significant overestimation of the removal extent can be assumed by this method. A real wastewater treatment plant may not receive diluted effluents, may not have all the nutrients added in this experiment and may not have all the residence time proposed by Zahn-Wellens methodology.

Moreover, the organic matter that was not biodegraded in the 28 days experiment can be more toxic and more dangerous than the raw wastewater. Furthermore, the use of biological treatment for antibiotics containing wastewater can induce the development of antibiotic-resistant pathogens, whose resistance genes can be transmitted through many generations, triggering serious problems for human and animal health.

4. CONCLUSIONS

It can be concluded that the real antibiotics production wastewater investigated in this work is considered biodegradable by the Zahn-Wells test (both diluted and raw wastewater) and that biomass could degrade more than 80 % of the antibiotics present in the raw wastewater. However, it is noteworthy to say that significant overestimation of the removal extent can be assumed by this method and that the use of biological treatment for antibiotics containing wastewater can induce the development of antibiotic-resistant pathogens, triggering serious problems for human and animal health.

5. ACKNOWLEDGEMENT

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