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REMOVAL OF ANTIBIOTICS CONTAINING SIMULATED WASTEWATER BY BIOLOGICAL-PHOTOCHEMICAL PROCESS

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ABSTRACT

The occurrence of the antibiotics in surface waters after biological treatment become public health problems because of the development of antibioresistant microorganisms. Thus, the aim of this work is to treat wastewaters containg hat are difficult to biodegrade. The biological method is generally used as a means of treatment to treat wastewaters containing either amoxicillin (AMX) or ceftriaxone (CTX) but also the combination of the biological and photochemical treatment. The methodology used in this work is the treatment of the simulated wastewaters by the biological treatment using activated sludge followed by a photochemical treatment UV-C at a wavelength of 254 nm. The obtained results showed that the biological treatment led to a degradation yield of the COD (71 % for AMX and 38 % for CTX) leading to a decay in the ratio COD/BOD_5. It passes from 6,94 to 4,20 for CTX and from 5,58 to 3,82 for AMX. The combination of the treatment processes allowed a COD degradation yield of 100 % of the two antibiotic simulated wastewaters.

Keywords : *Zahn-Wellens, photochemistry, amoxicillin, ceftriaxone.*

RÉSUMÉ

Élimination d'antibiotiques contenant des eaux usées simulées par procédé biologique-photochimique

La présence des antibiotiques dans les eaux de surface après des étapes de traitement biologique constitue un véritable problème de santé publique du fait du développement des microbes antibiorésistants. Ainsi, l'objectif de ce travail est de traiter les eaux usées contenant l'amoxicilline (AMX) et le ceftriaxone (CTX) par l'association des traitements biologique et photochimique. La méthodologie employée est le traitement biologique par boues activées suivi d’un traitement photochimique ultraviolet UV-C de longueur d’onde 254 nm. Les résultats obtenus montrent que le traitement biologique a conduit à un abattement de la DCO (71 % pour l’AMX et 38 % pour le CTX) conduisant à une décroissance du rapport DCO/DBO_5 en passant pour la solution de CTX de 6,94 à 4,20 et de 5,58 et de 3,82 pour la solution de AMX. La combinaison des deux procédés a conduit à un taux d’élimination de 100 % de la DCO de ces solutions synthétiques en antibiotiques.

Mots-clés : *Zahn-Wellens, photochimie, amoxicilline, ceftriaxone.*

I - INTRODUCTION

The pharmaceuticals used for human’s care are unfortunately found in surface water, groundwater and drinking water [1 - 3]. This becomes major a concern because it represents a potential hazard for the aquatic organisms [4]. Among these pharmaceuticals, antibiotics are the most detected product in the environment because of their non-biodegradable character [5] and They do not undergo metabolic inactivation [6, 7]. To overcome this problem, biological treatment is used as a solution to remove these organic pollutants. Unfortunately, these antibiotics are detected in water systems after conventional biological wastewater treatments [8]. The presence of antibiotics in the environment leads to a proliferation of bacterial strains resistant to these antibiotics [9]. This would render these drugs ineffective against the treatment of these bacteria, leading to health problem [10, 11]. This situation is more acute in our country, where the hospital wastewater are rejected in the environment without any treatment. In this work, our focus will be made on the antibiotics amoxicillin and ceftriaxone which have been found to be the most prescribed medicine to the sick in Côte d’Ivoire. Thus, the aim of this work was to remove the two antibiotics from a simulated wastewater by a biological-photochemical process. The biological test used in this study is the Zahn-Wellens (Z.W) test and the photochemical process used is the UV/H_2O_2 process. The UV-C lamp used has a wavelength of 254 nm.

II - EXPERIMENTAL PART

II-1. The Zahn-Wellens test

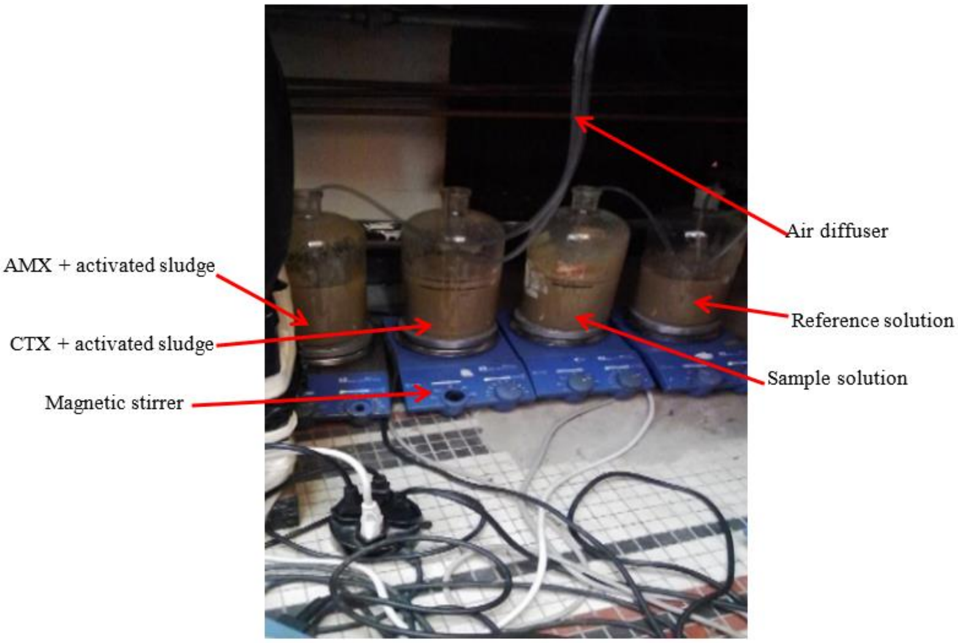
The Zahn-Wellens test is the method used in this work to remove biodegradable substances by microorganisms for 28 days. The equipment used for the Zahn-Wellens test consists of 2-liters bottles, carefully washed and sterilized in oven at 105°C for 30 minutes, magnetic stirrers, magnetized bars, air diffusers (HX-406A) and activated sludge. A mixture containing the test substance, mineral nutrients and a relatively large proportion of aqueous activated sludge is stirred and aerated at 20-25°C in dark or in diffuse light for up to 28 days. For this work, four types of solutions were prepared:

- Solution 1 is simulated waste water containing 1 g/L amoxicillin (AMX) from the Bailly-Creat laboratory, 1,5 g / L of activated sludge and nutrients.
- solution 2 is simulated waste water containing 1g/L of ceftriaxone (CTX) from LDP TORLAN laboratory, 1,5g/L of activated sludge and nutrients.
- solution 3 is the reference solution containing 1g/L of sodium acetate, 1,5g/L of activated sludge and nutrients.
- solution 4 is the control solution containing 1,5g/L of activated sludge and nutrients.

The nutrient solution was prepared according to the methodology based on Zahn-Wellens test (OECD 302B). The experimental conditions of the Zahn-Wellens test are shown in ***Table 1***. ***Picture 1*** shows the experimental setup of the Zahn-Wellens test used in this work.

Table 1 : *Experimental conditions for the Z.W method*

Parameters	**Standards (OECD 301-302)**	**This experiment (amoxicillin)**	**this experiment (ceftriaxone)**
Chemical oxygen demand (mgO_2/L)	100 - 1000	1250	1284
Disolved oxygen (mgO_2/L)	> 2	$4,5 \pm 0,1$	$4,5 \pm 0,1$
Concentration of the sludge in the solution (g/L)	0,2 – 1	$1,3 \pm 0,2$	$1,3 \pm 0,2$
Potential hydrogen, pH	6,5 - 8	6,5 - 8	6,5 - 8
Temperature (°C)	20 - 25	25	25
Sample volume (L)	2- 5	2	2



Picture 1 : *Biological treatment device by the method of Zahn Wellens*

The degradation rate of the organics during 28 days was determined with the following ***Equation*** :

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

$D_t(\%)$ = Percentage of degradation at time t
C_t = COD value in the test suspension or reference solution at time t (mgO_2/L)
C_A = COD measured in the test suspension or reference solution after 3 h ± 30 min incubation (mgO_2/L)
C_B = COD measured in the control solution at time t (mgO_2/L).
C_{BA} = COD measured in the control solution after 3 h ± 30 min of incubation (mgO_2/L).

For the Zahn-Wellens test, the COD and BOD_5 were determined. The examined samples were aerated for 28 days at temperature 25°C. Organic substances degradation process was monitored by determined COD of the samples on day 1, 2, 3, 4, 5, 6, 7, 11, 13, 19, 21, 26 and 28 of the experiment. The process was additionally controlled through air oxygen and pH. The COD was determined by the pre-measured COD tubes of the HACH product. To determine the value of this parameter, 2 ml of sample are withdrawn and introduced into a COD tube of the product HACH and heated in a digester at

150 ° C for 120 minutes. After cooling, the value of the COD is read directly on spectrophotometer DR6000 (HACH) at a wavelength of 620 nm. For the zeroing, a reference solution was used by adding to a COD tube 2 mL of distilled water. The BOD_5 was determined by the "manometric" method using the HACH VELP SCIENTIFICA oxitop (HACH). This method was developed by [12]. The principle is based on the measurement of the pressure decay related to oxygen consumption by microorganisms during the oxidation of the organic matter. From the pressure change measured by a pressure gauge, the BOD_5 value is automatically given. In this work, it was determined before the beginning of the experiment and after the Zahn-Wellens test.

II-2. The photochemical test

The equipment for the photochemical processing includes a UV-C lamp (λ = 254 nm), a magnetic stirrer, a magnetic bar, a pH probe, a water bath and a quartz tube to protect the lamp and a double-walled quartz system to cool the lamp during the experiment. All this device was put in dark room to protect it from light.

II-3. The combination Zahn Wellens-photochemical process

After 28 days of Zahn-Wellens experiment, 500 mL of the test solutions were filtered by the ordinary filter to retain the sludge. After the addition of 0.206M hydrogen peroxide, each sample was irradiated with the UV-C lamp (λ =254 nm) for 10h. The organics of the degradation rate was monitored by determining the COD of the sample at 2h intervals.

III - RESULTS AND DISCUSSION

III-1. Biological treatment (Zahn-Wellens test)

The COD degradation rate of the AMX and CTX solutions during the Z.W test is given in ***Figure 1***. This ***Figure*** shows for the reference solution, a rapid increase of the degradation rate that reaches 76 % after 3h of treatment. After that, the COD degradation rate increases slowly from the second day to the seventh day reaching, a value of 99 % which remains almost constant until the 28^{th} day. For the pharmaceuticals, there is a very sluggish evolution of the COD degradation rate during the first seven days of incubation ***(Figure 1)***. The degradation rate reaches 20 % and 17 % for AMX and CTX respectively. After the seventh day, the COD degradation rate increase to reach 71% and 38% respectively for AMX and CTX.

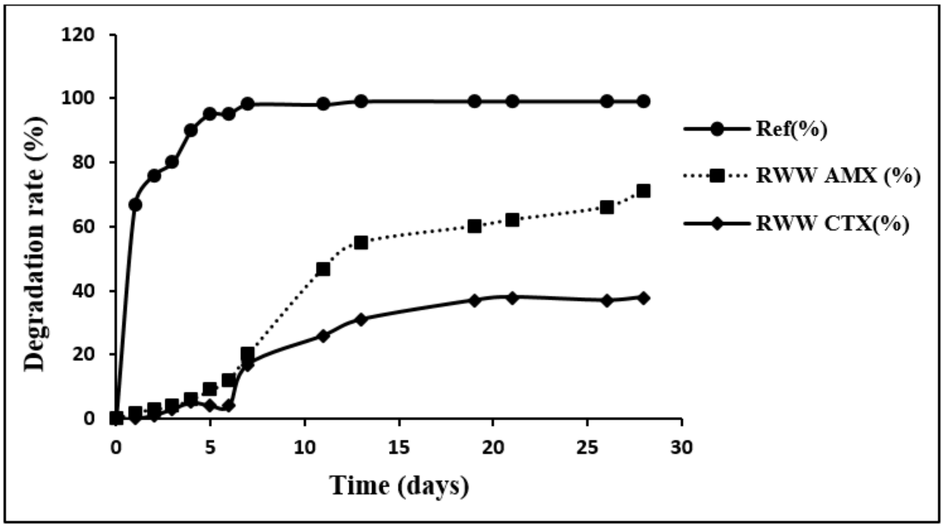


Figure 1 : *AMX and CTX wastewater degradation rates versus time*

Z.W: pH = 6.5-8, T = 25°C, [activated sludge] = 1,3 ± 0,2 g / L, [AMX] = [CTX] = 1g / L.

It is worth noting that in Z.W test, the reference solution is very important since it helps to check the correct functioning of the experimental system as well as the quality of the activated sludge [13]. The reference solution used is sodium acetate as recommended by OECD guideline 301F. According to this directive, the test is considered valid if the reference solution (sodium acetate) reaches a degradation rate of about 68 % in 14 days of the experiment. In the OECD guideline 302B, it is recommended that after 14 days of incubation, the reference solution should reach a degradation rate of about 70 %. In this work, the reference solution reached a degradation rate of 99 % after 14 days of incubation ***(Figure 1)***. This shows the good degradation power of the activated sludge used [13]. It has also been pointed out in literature [13] that the Zahn-Wellens test is considered valid, if after 10 days of incubation, the reference solution reaches a degradation rate of 70 %. In this work, the reference solution (sodium acetate) had a degradation rate of about 97 % after 10 days of incubation. For the synthetic wastewater containing AMX or CTX, the graph of the degradation rate of these organic compounds presents three parts ***(Figure 1)***. The first part starts from the first day to the seventh day of incubation. During this period, there is a slow degradation yield of the organics. This slow degradation yield of the organics by the microorganisms corresponds to their adaptation phase [14]. The second phase begins from the 7th to the 13th day of incubation. During this second phase, microorganisms begin to degrade considerably the organic pollutants. This is shown in ***Figure 1*** by an increase in the degradation curve for that time period. This phase corresponds to the microorganism growth phase that is manifested by the production of biomass [14]. The degradation rate of CTX is lower than that of AMX ***(Figure 1)***. The third part starts from the 13th day to the 28th day. During this phase, the degradation rate goes very slowly. This is probably due to the decrease in the amount of microorganisms and a decrease in the concentration of the substrate [15]. Thus, after 28 days, the degradation yield of the solution containing AMX reached to 71 % and that containing CTX reached to 38 %. This low degradation rate of CTX is due to its toxicity to microorganisms [14]. In fact, AMX seems to be more sensitive for microbial degradation than CTX. All these findings are in accordance with literature [16 - 18] where at laboratory scale a biological degradation rate of AMX reached 75 % [18]. These results show that AMX and CTX are considered non-biodegradable [19].

III-2. Biological-photochemical process

Solutions containing 1g/L of AMX or 1g/L of CTX were separately treated by the Zahn-Wellens technique for 28 days. After 28 days of experiment, each solution was filtered and treated photochemically for 10 hours in the presence of 0.206 M hydrogen peroxide at a pH of 7.2. The obtained results are shown in ***Figure 2***.

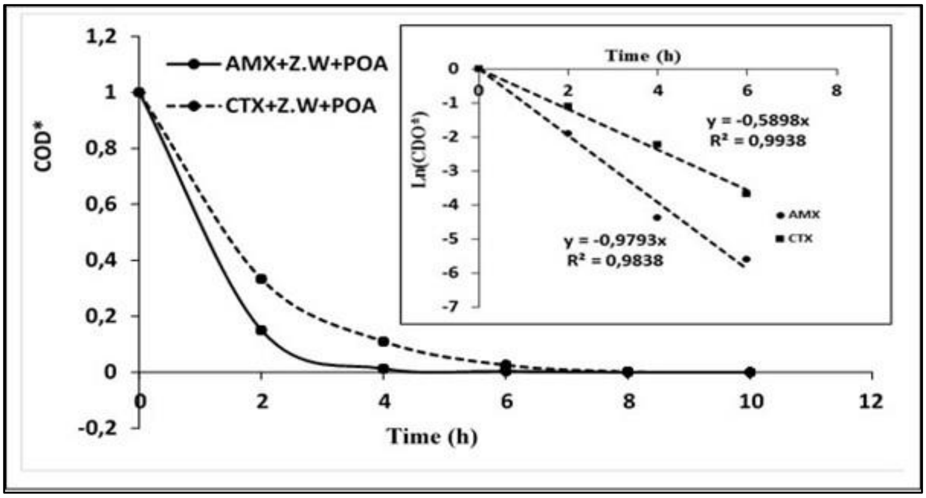


Figure 2 : *Evolution of the normalized COD versus time during the photochemical process. In insert the evolution of the graph Ln (COD *) = f (t) for the two types of solutions.*

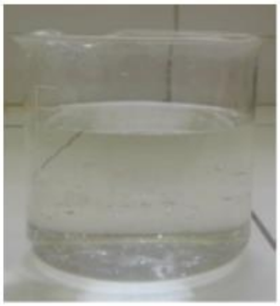
Figure 2 shows that the COD decreases rapidly independently of the antibiotics. Thus the degradation process of AMX is kinetically higher than that of CTX. After 10h of the photoreaction, a 100 % abatement of the COD was achieved regardless of the antibiotics. In the insert of ***Figure 2***, Ln

(COD *) is plotted against the organics treatment time. In this inserted figure, straight lines have been obtained independently of the organics. This result indicates that the photochemical oxidation kinetic for both the organic compounds is a pseudo first order kinetic. The kinetic constants determined are 0,9838 h^{-1} and 0,5898 h^{-1} for AMX and CTX respectively. This result indicate that the photochemical treatment of AMX is easier than CTX. Indeed, the high degradation rate of the organics could be related to the involvement of the hydroxyl radicals generated through the irradiation of hydrogen peroxide [20]. Knowing the non-biodegradability character of the two organics, this technique seems to be effective for their degradation. In the same way, it is found in literature that phenol and other biorecalcitrant compounds underwent a degradation rate more than 90 % [21]. In this work and in the biological treatment step, part of the antibiotics are transformed into biodegradable organic compounds or removed from the solution since the ratio COD/BOD_5 decreases as shown in table 2. The fact that COD/BOD_5 remains higher than 3 ***(Table 2)*** indicates that the wastewater still contains biorecalcitrant pollutants after the biodegradation step. At that step, one can indicate that the digestion of AMX by the microorganism leads to intermediates, probably to less oxygenated organics which are easy to degrade. But during the 10 hours of the photochemical step, the production of the hydroxyl radicals and possibly radical compounds allowed the experiment total degradation of the two antibiotics [20, 22].

Table 2 : *COD/BOD_5 ratio before and after Z.W test*

Solutions	**Before biological treatment (BBT)**	**After biological treatment (ABT)**
	COD / BOD_5	COD / BOD_5
CTX	6,94	4,20
AMX	5,58	3,82

During the biological treatment, the color of the solution is followed ***(Picture 2 and picture 3)***. Before biological treatment, the simulated wastewater is colorless either with AMX or CTX and after the biological treatment, the colors of the solutions are respectively bright yellow and faint yellow for AMX and CTX indicating the formation of subproduct of these antibiotics.



A- *Solution before treatment Z.W*

B : *Solution after treatment Z.W*

Picture 2 : *Color of the AMX solution before and after Zahn-Wellens treatment*

A-*Solution before treatment Z.W*

B : *Solution after treatment Z.W*

Picture 3 : *Color of the CTX solution before and after Zahn-Wellens treatment*

IV - CONCLUSION

CTX and AMX are two antibiotics known as hardly biodegradable compounds. But the biological treatment (Z.W) made it possible to obtain partially biodegradable solutions after 28 days. Thus, the biological technique is not sufficient to treat completely solution containing chemicals such as AMX and CTX known to biorecalcitrant chemicals. Photolysis associated to the biological treatment allowed a total degradation i.e. a COD abatement rate of 100 % for each of the simulated wastewaters containing respectively AMX and CTX. Thus, coupling the biological treatment to photolysis could be one of the most effective alternatives to treat effectively and efficiently the so-called difficult biodegradable compounds.

REFERENCES

[1] - VIKAS CHANDER, BHAVTOSH SHARMA, VIPUL NEGI, RAVINDER SINGH ASWAL, PRASHANT SINGH, RAKESH SINGH, RAJENDRA DOBHAL, *Journal of Xenobiotics*, 6 (2016) 5774

[2] - RANDHIR P DEO, *Curr Envir Health Rpt*, 1 (2014) 113 - 122

[3] - XUELIAN BAI, ALEX LUTZ, ROSEMARY CARROLL, KRISTEN KETELES, KENNETH DAHLIN, MARK MURPHY, DAVID NGUYEN, *Chemosphere*, 200 (2018) 133 - 142

[4] - TOMAS BRODIN, SUSANNA PIOVANO, JERKER FICK, JONATAN KLAMINDER, MARTINA HEYNEN AND MICAEL JONSSON, *Phil. Trans. R. Soc. B*, 369 (2014) 20130580

[5] - PAOLA GRENNI, VALERIA ANCONA, ANNA BARRA CARACCIOLO, *Microchemical Journal*, 136 (2018) 25 - 39

[6] - K. KÜMMERER, *Chemosphere*, 75 (2009) 435 - 441

[7] - D. J. YU, X. L. YI, Y.F. MA, B. YIN, H. L. ZHUO, J. LI, Y. F. HUANG, *Chemosphere*, 76 (2009) 915 - 920

[8] - ADRIANA OSINSKA, EWA KORZENIEWSKA, MONIKA HARNISZ and SEBASTIAN NIEST ¸EPSKI, *Appl. Sci*., 9 (2019) 387

[9] - A. C. PAWLOWSKI, W. WANG, K. KOTEVA, H. A. BARTON, A. G. MCARTHUR, G. D. WRIGHT, *Nat. Commun*., 7 (2016) 13803

[10] - T. SCHWARTZ, W. KOHNEN, B. JANSEN, U. OBST, *Microbiol. Ecol*., 43 (2003) 325 - 335

[11] - T. SCHWARTZ, H. VOLKMANN, S. KIRCHEN, W. KOHNEN, K. SCHON-HOLZ, B. JANSEN, U. OBST, *Microbiol. Ecol*., 57 (2006) 158 - 167

[12] - D. H. CALDWELL and W. F. LANGELIER, *Sewage Works Journal*, 20 (2) (1948) 202 - 218

[13] - MILENA ELEONORE LAPERTOT, CESAR PULGARIN. Chemosphere, 65 (2006) 682 - 690

[14] - M. CLEUVERS, *Toxicol. Lett*., 142 (2003) 185 - 194

[15] - HATEM DHAOUADI, Traitement des Eaux Usées Urbaines Université Virtuelle de Tunis, (2008)

[16] - B. GRANDJEAN, Principe des traitements biologiques [cours en ligne]. Université Laval, Canada. Disponible sur http://www .gch.ulaval .ca/bgrandjean /gch13201 /p4.pdf, (2006)

[17] - A. MORSE, A. JACKSON, Water Air and Soil Pollution, 157 (1 - 4) (2004) 117 - 132

[18] - ANDREAS LÄNGIN, RADKA ALEXY, ARMIN KÖNIG, K. KÜMMERER, *Chemosphere*, 75 (2009) 347 - 354

[19] - EWELINA PLUCIENNIK-KOROPCZUK , SYLWIA MYSZOGRAJ, CEER, 28 (1) (2018) 077 - 086

[20] - Y. PENRU, A. R. GUASTALLI, S. ESPLUGAS and S. BAIG, A *Chemistry*, 233 (2012) 40 - 45

[21] - MOJTABA POURAKBAR, GHOLAMREZA MOUSSAVIN and SAKINE SHEKOOHIYAN, *Ecotoxicology and Environmental Safety*, 125 (2016) 72 - 77

[22] - O. LEGRINI, E. OLIVEROS, A. M. BRAUN, *Chemical Reviews*, 93 (2) (1993) 671 - 698