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Abatement of Amoxicillin and Doxycycline in Binary and Ternary Aqueous Solutions by Gas-phase Pulsed Corona Discharge Oxidation

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Abstract

The presence of pharmaceutical compound residues in water bodies is becoming an increasingly serious problem. Various pharmaceuticals have been detected in raw municipal wastewaters, after wastewater treatment plant processes, and even in drinking water. Many common pharmaceuticals are bio-accumulating and they can have a harmful impact on aquatic and terrestrial organisms.

In this work, pulsed corona discharge technology (PCD) is studied as a potential method for antibiotic compounds abatement. Two antibiotics, amoxicillin and doxycycline, were chosen as test pharmaceutical compounds. The aim of the study was to investigate the transformation kinetics of binary solutions (water - single antibiotic compound) and ternary solutions (water - two antibiotic compounds) of the compounds and to optimize operational parameters for improved oxidation performance. Ternary solutions were investigated to obtain data on transformation kinetics when two competing pharmaceutical molecules are present in the solution. The experiments showed that reactions of doxycycline oxidation are always first order reactions. Reaction of amoxicillin oxidation has second order in the case of experiments with binary solution in alkaline medium. In other cases, it has first order. The transformation products formed were identified and monitored based on liquid chromatography mass spectrometer analysis. OH-amoxicillin, amoxicillin pencilloic acid, OH-doxycycline and 2-OH-doxycycline had the largest peaks areas. All studied compounds and all transformation products can be easily oxidized by PCD. Approximately 1 kWh/m^3 and 0.5 kWh/m^3 delivered energy is enough for oxidation of great part of amoxicillin and doxycycline respectively. Low frequency, 50

pps, and high (pH=12) are preferable for oxidation of both antibiotics from the energy consumption point of view.

Keywords. Pharmaceuticals; Wastewater treatment; Cold plasma; AOPs; ozone.

1. Introduction

Pharmaceutical compounds were first detected in natural water bodies in the 1970s. At the time, these compounds, i.e. active pharmaceutical ingredients (API), were not considered hazardous as their concentrations were insignificant. However, a continuously growing and ageing of population, improving quality of life and greater use of pharmaceuticals in rearing of livestock have led to increased consumption of pharmaceutical compounds, which in turn has resulted in greater accumulation of pharmaceutical compounds in the environment. Furthermore, an absence of legislation specifically addressing the discharge of pharmaceuticals-containing wastewaters into ground water and surface water bodies has also contributed to continuous growth of these compounds in the natural environment [1] [2].

Pharmaceutical compounds are developed to be highly bioactive in order to react with receptors in humans and animals and produce the desired medicinal effect. They are usually toxic towards health-threatening organisms such as bacteria, fungi and parasites. A significant amount of lower animals have receptor systems similar to humans and higher animals. Furthermore, many of the organism groups affecting human and animal health and targeted by pharmaceuticals play an important role in natural ecosystems. Therefore, pharmaceuticals may impact significantly on aquatic and terrestrial organisms [3].

A number of studies have reported on the impact of pharmaceuticals on environmental health [4] [5] [6] and there are special requirements for environmental risk assessment of the effects of human and veterinary medicines on aquatic and terrestrial organisms. Such requirements were first established in 1980 by the US Food and Drug Administration (FDA), and similar requirements were adopted in the EU in 1997. These risk assessments investigate potential negative effects on daphnids, fish, algae, bacteria, earthworms and plants [3].

One of the main sources of pharmaceuticals contamination in natural waters is wastewater effluent from municipal treatment plants treating hospital effluents and human wastes [7]. Municipal wastewater treatment

plants are generally not equipped to deal with complex pharmaceuticals, as most plants consist of primary and secondary treatments (mainly activated sludge systems) without tertiary treatment processes able to efficiently remove medical drugs [8] [9]. The concentration of pharmaceuticals in natural water bodies is in the range of ng/L to μg/L [10].

Ozonation can be applied for removal of pharmaceuticals from water [11], but the high cost of ozone makes this method economically unviable [12] [13] [14]. The necessity for the novel water treatment methods is thus clear.

The present work investigates, economically and as regards environmental safety, the feasibility of ultra-short gas-phase pulsed corona discharge (PCD) oxidation at ambient conditions for pharmaceuticals removal from water. PCD oxidation is a chemical-free method that generates hydroxyl radicals and ozone as the main oxidation species for oxidation of various organic molecules. PCD has been shown to be an effective method for oxidation of pharmaceuticals by Panorel et al. [15] [16], who studied PCD oxidation for removal of compounds such as paracetamol, ibuprofen, β-estradiol, indomethacin and salicylic acid.

Many antibiotics are refractory substances [17] that can pass through biological treatment facilities without degradation, thus ending up in water bodies [18] [19]. The compounds investigated in this work were chosen based on risk quotient (RQ), as proposed by Verlicchi [19]; namely, the ratio between the average concentration of pharmaceuticals in a secondary effluent and the corresponding predicted no-effect concentration (PNEC) [20].

Possible oxidation reaction pathways of these compounds have been presented by Klauson et al. [21] [22]. Most research into degradation of the studied antibiotics by advanced oxidation processes (AOP) has considered UV photolysis, ozonation, the Fenton reaction and combinations of these methods. Moreira et al. [23] and Elmolla and Chaudhuri [24] report extremely low degradation of amoxicillin under direct single photolysis, and similar results are reported for doxycycline degradation [25]. However, amoxicillin and doxycycline are easily oxidized in processes where ozone is involved [23] [26] [25]. Although ozonation is an effective approach, its use brings additional costs as the ozone has be generated separately in the ozone generator and transferred to the wastewater treatment unit, and the residual ozone gas has to be recovered. The

Fenton process is the most effective method of commonly used AOPs [27] [28] [29] but has the drawback of sludge formation when ferrous iron chemical is added to the system.

This work focuses on investigation of process parameters with the aim of increasing the energy efficiency of the oxidation process. Process parameters considered include pulse repetition frequency, treatment time, and pH value.

There is a considerable body of work about the effect of carbonate species (HCO_3^-, CO_3^{2-}), natural organic matter and other organic compounds on oxidation by AOPs. It is well known that such species act like a OH-radicals scavenger and have a negative influence on oxidation [30] [31]. Some work has also been presented in which real waste water is used as a solvent for antibiotics [32]. The study found a decrease in AOP efficiency with surface water and water from a wastewater treatment plant compared with ultra-pure water as a solvent. Although the effects of various pharmaceutical compounds in water on AOP efficiency have been investigated in numerous studies, the work has tended to focus on a single parent pharmaceutical compound, and the combined effect of the presence of antibiotics has received little attention. The present work makes an attempt to evaluate the influence of presence of one antibiotic on degradation of another antibiotic in terms of energy efficiency as well as from the point of view of possible intermediate products of oxidation.

Although pharmaceutical oxidation products are usually less pharmacologically active than the parent compound, it has been shown that advanced oxidation processes can lead to the production of oxidation products with enhanced toxicity compared to the parent compound [33] [34] [35]. Thus, it is important to study not only the concentration of the parent compound, but also the transformation products formed when pharmaceuticals are oxidized.

2. Materials and methods

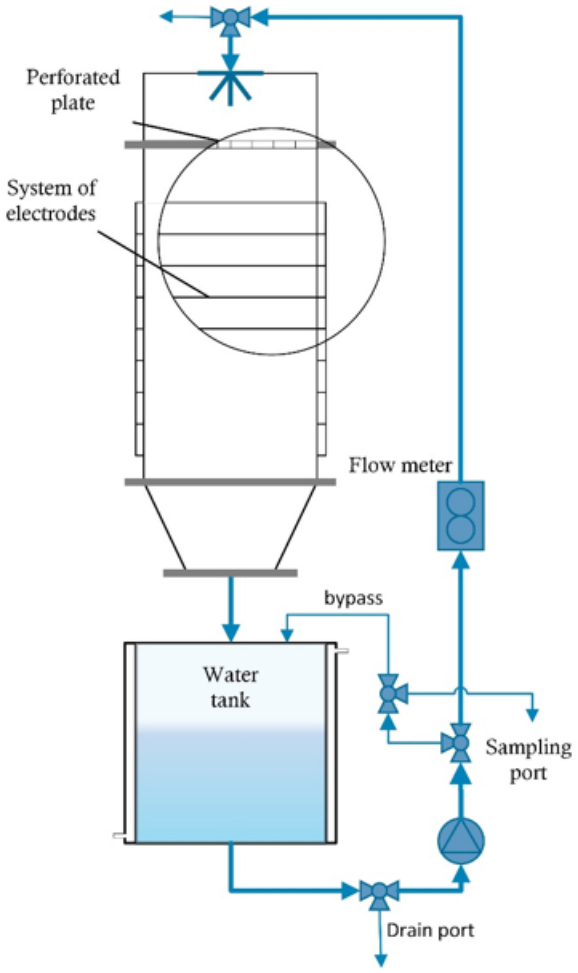
Two commercial antibiotics, amoxicillin and doxycycline, were used as test compounds. Both antibiotics were supplied by Sigma Aldrich and the purity of the antibiotics exceeded 99% (analytical grade). Liquid chromatography eluents were prepared with MilliQ water and LC-MS grade acetonitrile (VWR). Formic acid (98%, Merck) was used as an additive.

The experimental system comprised a pulsed corona discharge reactor and a high voltage pulse generator with the setup illustrated in Figure 1. The chamber of the PCD reactor consists of two grounded vertical plate electrodes and a high voltage wire electrode located parallel between two plate electrodes. The dimensions of the plates are 210 mm in width and 1000 mm in height. The plates are located 34 mm from each other. The total plasma volume is 7.14×10^6 mm^3. The high voltage electrodes are placed at a distance of 29 mm from each other. The diameter of the wire electrode is 0.5 mm. The chamber is covered by a transparent acrylic glass frame. An oscilloscope, Agilent 54622D, was used for determination of the pulse parameters. The energy of a single pulse was calculated using the following equation:

$$W_p = \int_0^{T_p} U(t)I(t)dt \quad (1)$$

where, W_p is the energy of a single pulse, J; T_p is the duration of the voltage pulse, ns; U(t) and I(t) are waveforms of voltage and current respectively.

Figure 1 Experimental setup of 100 W PCD reactor



The energy of a single pulse produced by the generator is 0.12 J at 22 kV and 180 A in the amplitude peak at 100 ns duration, which corresponds to average energy density of 16.8 J/m^3 in the plasma volume [12].

Aqueous solutions of the test compounds were circulated from the reservoir tank through the reactor by a pump. The solution is fed to the top of the reactor, where it is spread with a perforated plate and falls by gravity between the electrodes. The solution contacts the electrodes and is treated by the oxidants.

The flow rate of the re-circulating water was 4.5 L/min, which ensured sufficient trickling and adequate mixing. The key studied experimental parameters were

pH level and pulse repetition frequency. All experiments were carried out at ambient pressure and temperature. Deionized water was used in the solutions.

Table 1 Corresponding energy delivered with treatment time

Delivered energy dose (kWh/m^3)	Treatment time for 50 pps (min)	Treatment time for 200 pps (min)	Treatment time for 500 pps (min)
0	0	0	0
0.05	5	-	-
0.1	10	2.5	-
0.2	20	5	-
0.4	40	10	4
1	100	25	10
1.6	160	40	16
2.4	240	60	24

Table 2 List of experiments

		Frequency	pH changing	№ of experiments	max SD
1	DXC	500 pps	4.3 → 3.3	2	0.0497
2	DXC	500 pps	11.8 → 11.7	2	0.0532
3	DXC	200 pps	4.2 → 3.4	4	0.0387
4	DXC	200 pps	11.8 → 11.7	4	0.0406
5	DXC	50 pps	4.0 → 3.5	4	0.0351
6	DXC	50 pps	11.9 → 11.7	4	0.0389
7	AMX	500 pps	6.6 → 3.3	3	0.0412
8	AMX	500 pps	11.8 → 11.7	3	0.0426
9	AMX	200 pps	6.3 → 3.4	4	0.0299
10	AMX	200 pps	11.8 → 11.7	4	0.031
11	AMX	50 pps	6.7 → 4.0	4	0.0309
12	AMX	50 pps	11.9 → 11.7	4	0.0312
13	mix DXC	200 pps	4.3 → 3.9	3	0.0352
14	mix DXC	200 pps	12.2 → 12.1	3	0.036
15	mix DXC	50 pps	4.7 → 4.4	3	0.03
16	mix DXC	50 pps	12.2 → 12.1	3	0.032
17	mix AMX	200 pps	4.3 → 3.9	3	0.049
18	mix AMX	200 pps	12.2 → 12.1	3	-
19	mix AMX	50 pps	4.7 → 4.4	3	0.048
20	mix AMX	50 pps	12.2 → 12.1	3	-

The experiments were done with constant initial concentration of antibiotics of 50 ppm with two different initial pH levels, neutral and alkaline (in the presence of NaOH). Such relatively high concentration was chosen to make possible to investigate the behavior of tested compounds during PCD treatment. It should be noted that media without the NaOH additive are henceforth referred to as “neutral”. The compounds were tested separately with binary and ternary solutions. Ternary solutions were prepared by mixing of 50 ppm of

both antibiotic compounds. Initial solution concentration was chosen high enough to obtain accurate data on oxidation rates of the substance. As pulse repetition frequency is one of the primary parameters that determines the efficiency of the process, three different pulse repetition frequencies of 50 pps, 200 pps and 500 pps were used. The samples were taken with treatment time as indicated in Table 1.

The total number of experiments is 20, as listed in Table 2. Each experiment was repeated several times. All plots and calculations were based on mean values of compound concentrations. Standard deviation was calculated to estimate the reproducibility of experiments. The maximum values of standard deviation for each experiment are shown in Table 2. As the table shows, the maximum standard deviation is around 0.05 indicating quite high experiment reproducibility. The concentration of pharmaceuticals was measured at least four times after each experiment. The accuracy of these measurements was around ±0.3 ppm.

The concentration of pharmaceuticals was measured by high performance liquid chromatography (HPLC). A Kinetex 2.6 µm C18 100A 150x4.60 mm column was used for analysis of the studied sotablelutions. The specific HPLC parameters for each compound are shown in Table 3.

Table 3 Analysis methodology and parameters used in HPLC analysis

	Amoxicillin	Doxycycline
Eluent	95% phosphate buffer solution (0.01 mol/L) and 5% acetonitrile	methanol, acetonitrile, 0.01M oxalic acid solution in volumetric proportion of 2:3:5 respectively
Column temperature	25 °C	25 °C
Retention time	around 4 min	around 3 min
Wavelength	229 nm	350 nm
Eluent flow rate	0.6 ml/min	0.8 ml/min
Injection volume	20 µl	20 µl

Analysis of the transformation products of doxycycline and amoxicillin was performed using liquid chromatography coupled to an ion trap mass spectrometer (Agilent 1100 series LC/MSD Trap) equipped with an electrospray ionization interface. The analysis was conducted in both positive and negative mode using full scan and auto MS/MS modes. The neutral samples were injected without prior sample treatment and the pH of the alkaline samples was adjusted to 3 with formic acid before injection. For chromatographic separation, an XBridge C_{18} column (2.1 × 50 mm, 3 µm, Waters) and an Atlantis T3 C_{18} column (2.1 × 100 mm, 3 µm,

Waters) were used for doxycycline and amoxicillin respectively. The flow rate was 0.4 mL/min and MilliQ water with 0.1 % formic acid and acetonitrile with 0.1 % formic acid were eluents A and B, respectively. The gradient applied in the doxycycline method was: 0-1 min, 5 % B; 1-25 min, 5-95 % B; 25-26 min, 95 % B; 26-27 min, 95-5 % B; 27-35 min, 5 % B. The gradient applied in the amoxicillin method was: 0-1 min, 0 % B; 1-24 min, 0-60 % B; 24-25 min, 60-0 % B; 25-35 min, 0 % B. The injection volume was 30 µL. The LC instrument was equipped with a variable wavelength UV detector adjusted to 275 nm in the doxycycline method and 229 nm in the amoxicillin method. The source parameters in the doxycycline method were: capillary voltage of ±3.5 kV, gas temperature 350°C, gas flow 8 L/min, nebulizer 40 psi. Parameters for the amoxicillin method were the same with the exception that the capillary voltage was ±4.5 kV.

Further evaluation of the transformation products was carried out with a quadrupole time-of-flight mass spectrometer (Bruker MicrOTOF) equipped with an electrospray ionization interface. The analysis was carried out in positive mode in full scan mode. An Agilent series 1200 LC was used for chromatographic separations. The chromatographic method was the same as for the ion trap spectrometry.

3. Results and discussion

The present work studied the effect of pH and pulse repetition frequency on energy consumption during the oxidation process of each model compound. The main parameter to determine the efficiency of the PCD treatment was energy efficiency (ε, g/kWh):

$$\varepsilon = 0.5C_0/E \quad (2)$$

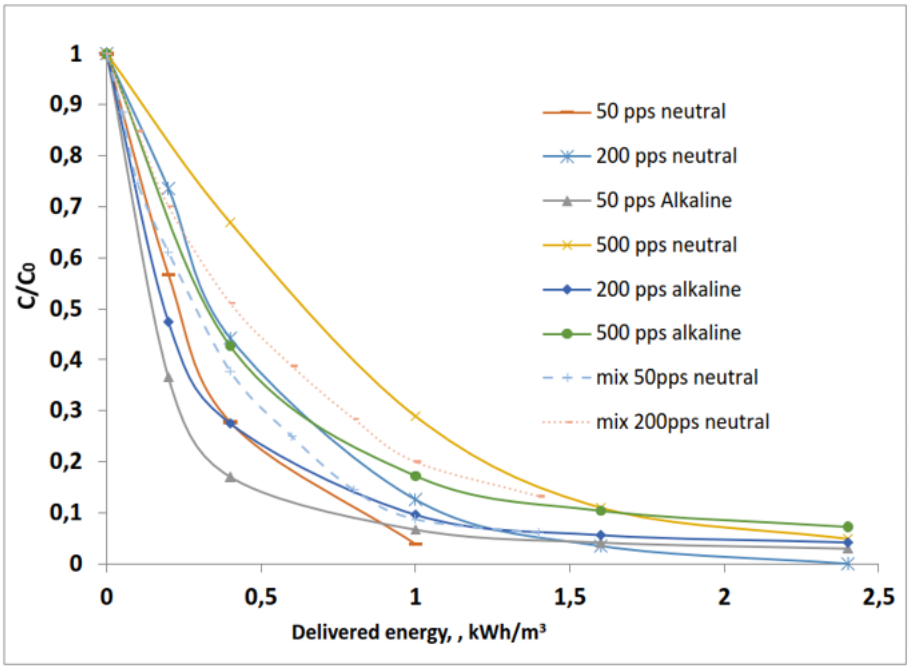
where, E is the delivered energy dose, corresponding to the treatment time required for 50% compound degradation, kWh/m^3; C_0 is the initial concentration of the compound, mg/L. In other words, the energy efficiency shows how much energy was consumed to oxidize a half of initial concentration of tested compound. To make sure, that amoxicillin and doxycycline do not degrade them self and do not react with each other in aqueous solution, a several controlling experiments were done. Binary solution of each antibiotic was pumped through the reactor without power supply for 5 hours. Samples were taken after each hour.

Changes in concentration of both antibiotics were not detected. The same run was carried out for ternary solution. The concentration of doxycycline remained in the same initial level. Decreasing in amoxicillin concentration was observed. It dropped at the beginning by 16 % and remained on the same level during 5 hours. We assumed that happened due to acidic condition only, and not because of chemical reaction with doxycycline. Explanation of concentration decreasing is given in chapter 3.3.

3.1 Amoxicillin oxidation

Figure 2 shows the oxidation results for amoxicillin. The experiments were carried out in neutral and alkaline conditions. The binary test solution was prepared with one compound, in this particular case with amoxicillin only, and the ternary solution contained both amoxicillin and doxycycline. During the treatment in alkaline conditions, the pH remained constant, around 12. In the experiments without sodium hydroxide, the pH dropped from neutral to acidic, Table 2. In treatment of the ternary solution, detection of amoxicillin in the alkaline condition failed. It would thus appear that the implemented analysis method does not give reliable results in the presence of doxycycline and sodium hydroxide.

Figure 2 Amoxicillin relative concentration vs delivered energy



Description of the reaction kinetics is quite demanding due to the unknown concentration of the oxidants and their share in the reaction. However, using data from the experiments, the reaction order and reaction rate constant can be determined by integration method. Functions $\ln(C/C_0)$ and $1/C$ versus treatment time were

plotted to determine whether the reaction is first or second order. The linear dependence of logarithmic amoxicillin degradation in a neutral medium confirmed the first order reaction with respect to the concentration in both binary and ternary solutions, which is agreement with results presented by Dogan and Kidak [36], who also observed a first order reaction. In the alkaline medium, the reaction is a second order reaction (plot 1/C gave the best fitting results). The first order reaction rate constant (k_1, min^{-1}) was determined as the slope of $ln(C/C_0)$ versus treatment. The second order reaction rate constant (k_2, L mg^{-1} min^{-1}) was determined as the slope of 1/C versus time. Calculated reaction rate constants are given in Table 4. As amoxicillin could not be detected at alkaline conditions, the reaction order was not calculated for the experiment with ternary solution in an alkaline medium. It is clearly seen that the reaction rate increases with increase in frequency, as expected.

Table 4 Reaction rate constants; k_1, min^{-1}; k_2, L mg^{-1} min^{-1}

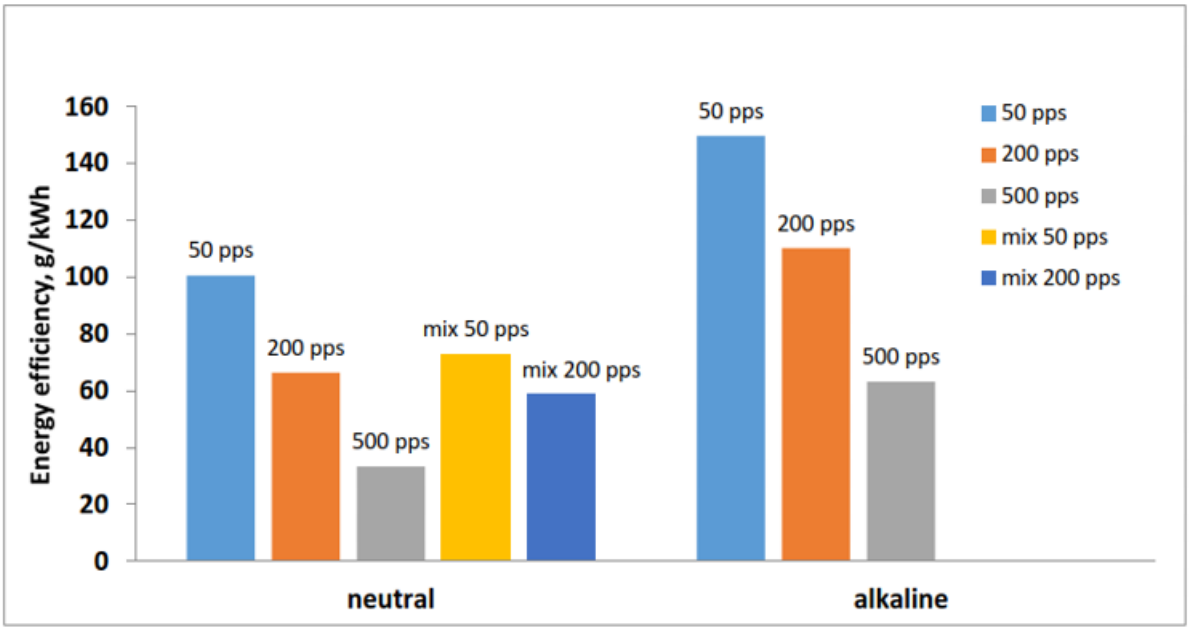
Frequency, pps	Binary solution								Ternary solution							
	Amoxicillin				Doxycycline				Amoxicillin				Doxycycline			
	neutral		alkaline		neutral		alkaline		neutral		alkaline		neutral		alkaline	
	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2
50	0.0328	-	-	0.0031	0.0974	-	0.161	-	0.024	-	n/a	n/a	0.0894	-	0.0738	-
200	0.0850	-	-	0.0096	0.2933	-	0.311	-	0.0633	-	n/a	n/a	0.175	-	0.1832	-
500	0.1307	-	-	0.0137	0.3328	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

From Figure 2, it can be concluded that amoxicillin is easily oxidized by PCD. The highest degradation was observed at the beginning of the PCD treatment. After energy delivery of 1 kWh/m^3, the great part of amoxicillin was oxidized. It can further be observed that the lowest pulse repetition frequency leads to the fastest degradation rate. In an alkaline medium, the oxidation is more energy-efficient at the very beginning of the treatment than with neutral solutions. It is clearly seen from Figure 2 that, at constant frequency, the oxidation of amoxicillin is enhanced at higher pH. This result is especially noticeable at higher frequency, when influence of OH-radicals is more significant comparing with ozone, and since the alkaline medium contains more hydroxyl radicals, the oxidation efficiency is higher at high pH. With pulse repetition frequency of 50 pps, alkaline conditions are still preferable but the influence of pH is smaller. At the lowest frequency, 50 pps, amoxicillin seems to almost oxidize fully at delivered energy around 1 kWh/m^3. On the other hand, the treatment time at lower pulse repetition frequency becomes longer. In this case, ozone has more time to accumulate during the pauses between pulses and has more time for reaction with target compounds.

Consequently, at low frequency, ozoneplays a more significant role in the oxidation. Furthermore, the effect of pH decreases at the end of the oxidation, which is clearly seen from Figure 2.

Energy efficiency of amoxicillin oxidation was calculated according to equation (2) after degradation of 50 % of the initial amount of the test compound (see Figure 3). Energy efficiency decreased with high pulse repetition in both alkaline and neutral solutions. The highest energy efficiency was obtained in alkaline solution at pulse repetition frequency of 50 pps with 149.75 g/kWh, indicating that 149.75 g of amoxicillin can be oxidized per 1 kWh. The minimum energy efficiency, 33.46 g/kWh, was detected at 500 pps frequency in neutral media. For comparison, Jin et al. [37] obtained a maximum 6.025 g/kWh for degradation of amoxicillin in aqueous solution with contact glow discharge electrolysis. With PCD treatment, even the worst result gave 5.5 times better efficiency, showing the considerable potential benefits from PCD utilization.

Figure 3 Energy efficiency of amoxicillin degradation



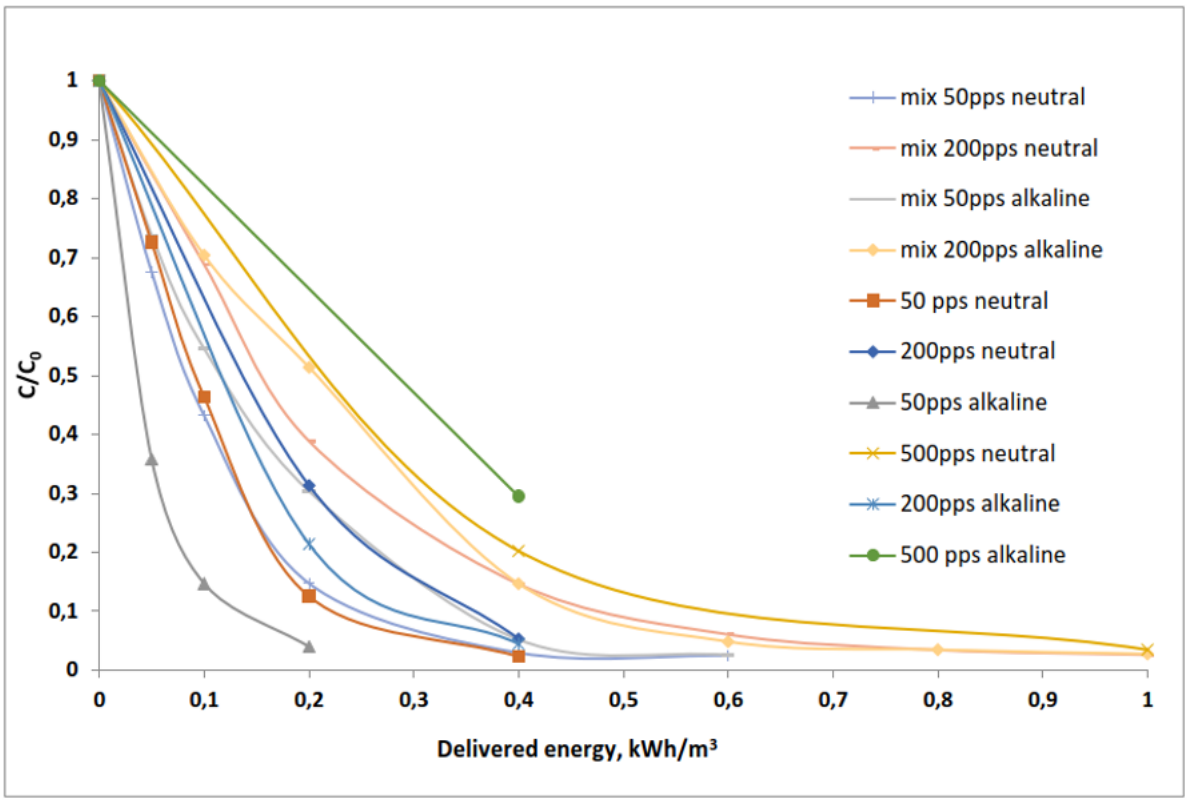
When treatment of the binary solution is compared with ternary solution treatment, it can be concluded that oxidation of amoxicillin in the presence of doxycycline occurs more slowly. The reaction rate constant decreased by 26-27 % for the ternary solution irrespective of frequency. Moreover, more energy was required for oxidation of the same amount of amoxicillin than in treatment of the binary solution, most likely due to competitive reactions of oxidants with doxycycline. Energy efficiency of amoxicillin degradation in ternary solution decreased from 100.62 g/kWh to 72.86 g/kWh at frequency 50 pps, and from 66.4 g/kWh to 58.79 g/kWh at 200 pps. It should be noted here, that the inhibitory effect of doxycycline on amoxicillin degradation

is more significant at lower pulse repetition frequency. Comparison of amoxicillin behavior during PCD treatment under alkaline and neutral conditions was not carried out. As mentioned earlier, the implemented analytical method did not enable detection of amoxicillin in an alkaline medium in the presence of doxycycline.

3.2 Doxycycline oxidation

Figure 4 presents the oxidation of doxycycline in binary and ternary solutions in neutral and alkaline conditions. It is important to note, that the term "neutral" means absence of NaOH, practically the pH of doxycycline solutions was around 4.

Figure 4 Doxycycline relative concentration vs delivered energy



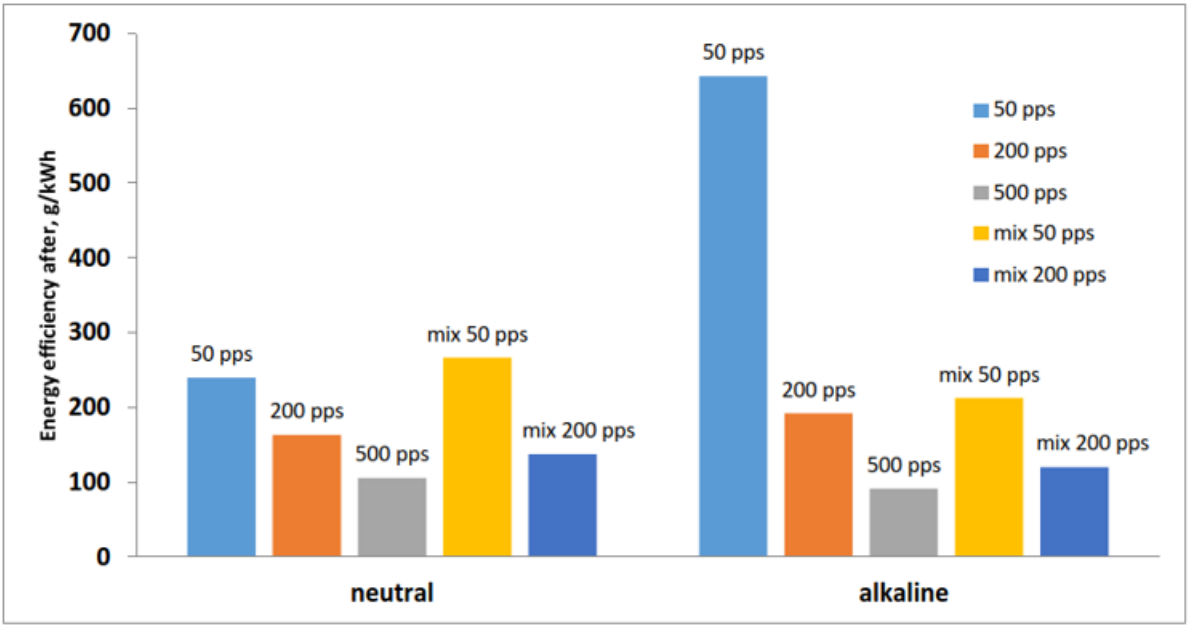
Doxycycline can be oxidized with lower energy consumption than amoxicillin, and at delivered energy above 0.4 kWh/m^3, doxycycline concentrations reached the detection limit of the HPLC analysis method used.

In the range of 0 to 0.4 kWh/m^3, the trends of the obtained oxidation curve are very similar as with amoxicillin. However, unlike amoxicillin degradation, the doxycycline degradation is a first order reaction in both alkaline and neutral media. Because of the fast doxycycline oxidation at a frequency of 500 pps under alkaline conditions, it was possible to take only two samples, and therefore, the reaction rate constant could not be

determined in this case. As with the amoxicillin experiments, in alkaline conditions the pH remained constant in doxycycline solutions, around pH 12. However, without NaOH, the initial pH was around 4.5 and dropped to 3.5 after PCD oxidation (see Table 2). Calculated reaction rate constants are shown in Table 4. It is clearly seen that the reaction rate constant increases with increasing pulsed repetition frequency in both binary and ternary solutions irrespective of pH. Comparing results for the binary and ternary solutions, it can clearly be seen that the reaction rate constant is higher with binary solutions. For neutral conditions, the difference between the values of reaction rate constant in ternary and binary solutions is around 8.2 % at 50 pps (k_1=0.0974 min^{-1} in binary solution against k_1=0.0894 min^{-1} in ternary solution) and 40 % at 200 pps. (k_1=0.2933 min^{-1} in binary solution against k_1=0.175 min^{-1} in ternary solution). For alkaline conditions, this difference is 54 % (k_1=0.161 min^{-1} in binary solution against k_1=0.0738 min^{-1} in ternary solution) and 41 % (k_1=0.311 min^{-1} in binary solution against k_1=0.1832 min^{-1} in ternary solution) at 50 pps and 200 pps respectively. The presence of amoxicillin significantly slows down the doxycycline oxidation process. In general, high pH enhances the oxidation, but it is important to note that in the case of ternary solution treatment in alkaline media, the doxycycline oxidation reaction rate constant is a little bit lower than with neutral media. This outcome is observed only at 50 pps.

Energy efficiency of amoxicillin oxidation was calculated according to equation (2). The calculation results are presented in the Figure 5.

Figure 5 Energy efficiency of doxycycline degradation



As in the case of amoxicillin oxidation, lower pulse repetition frequencies are preferable from the energy consumption point of view. As in the case of experiments with amoxicillin, the highest energy efficiency (643.11 g/kWh) was obtained in alkaline condition in binary solution at pulse repetition frequency of 50 pps. This value is four times higher than the similar value for amoxicillin treatment (643.11 g/kWh against 149.75 g/kWh). This proves that oxidation of doxycycline is much more easily comparing with amoxicillin. It can be seen from the Figure 5, that with increasing of frequency, energy efficiency decreasing. It is interesting observation, that at higher frequency, the influence of pH on energy efficiency become insignificant. The results presented in on the Figure 5 suggest, that energy efficiency at 200 pps is almost the same in neutral and alkaline conditions, and higher at 500 pps in neutral condition. Better result in energy efficiency in neutral condition at 500 pps is surprising. However, it could be explained by insufficient quantity of samples, only two sample were taken during treatment at 500 ppm. Obviously, two sample are not enough for accurate analysis.

Comparing energy efficiency of treatment in binary and ternary solutions, we can see from the Figure 5, that low frequency is still preferable in the case of ternary solution. Of course, the absolute value is lower compared to binary solution. The most interesting and unexpected finding is that, in ternary solution energy efficiency performance is higher in neutral media.

As stated earlier, the initial concentration of the treated solutions was 50 ppm. However, HPLC analysis suggests that the initial concentration of the untreated samples was lower than prepared. The difference is particularly noticeable with acidic and alkaline conditions. The ternary solution is an acidic medium due to the presence of doxycycline. It is known that amoxicillin starts to oxidize under acidic conditions and amoxicillin penicilloic acid appears [38]. It is reasonable to assume that this is the cause of the lowered concentrations found in the HPLC analysis. Further (see chapter 3.3) analysis showed the presence of amoxicillin penicilloic acid in untreated solution

3.3. *Identification of transformation products*

As stated earlier, the initial concentration of the treated solutions was 50 ppm. However, HPLC analysis suggests that the initial concentration of the untreated samples was lower than prepared. The difference is particularly noticeable with acidic and alkaline conditions. The ternary solution is an acidic medium due to

the presence of doxycycline. It is known that amoxicillin starts to oxidize under acidic conditions and amoxicillin penicilloic acid appears [38]. It is reasonable to assume that this is the cause of the lowered concentrations found in the HPLC analysis. Further analysis showed the presence of amoxicillin penicilloic acid in untreated solution

To investigate the transformation products formed during PCD oxidation, LC-ESI-ion trap analyses were carried out. Oxidation of both doxycycline and amoxicillin leads to the formation of several transformation products. Identification of the major transformation products was based on their fragmentation patterns using ion trap MS and MS^2 analyses in both positive and negative mode and their exact mass recorded by a time-of-flight mass spectrometer (Table 5).

Table 5 Accurate mass observed by LC-ESI-TOF-MS of amoxicillin and doxycycline transformation products

Product	Calculated exact mass	Experimentally determined mass	Error, ppm
AMX-C1	382.10673	382.1091	4.4
AMX-C2	384.12238	384.1220	0.9
DXC-C1	461.15546		8.4
DXC-C2	477.15037		6.1

The relative rates of transformation of the antibiotics and the transformation products were determined by measuring the areas of the peaks in the UV chromatograms for doxycycline and its products and by measuring the areas of the extracted ion chromatograms for amoxicillin and its major products. Dividing the current peak area by the largest peak area enables the behavior of the content ratio of the tested compound and its degradation products during PCD treatment to be seen. Figures 6 and 7 show the change in the content ration of amoxicillin and doxycycline and their transformation products for ternary solution respectively.

Figure 6 Changing of the content ratio of amoxicillin and its major oxidation products

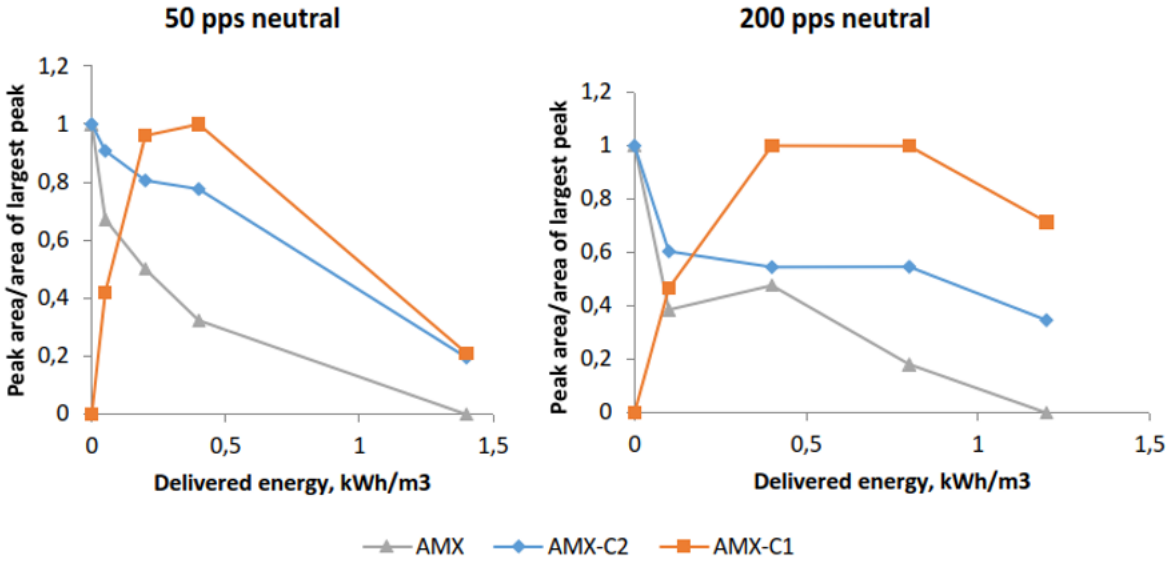
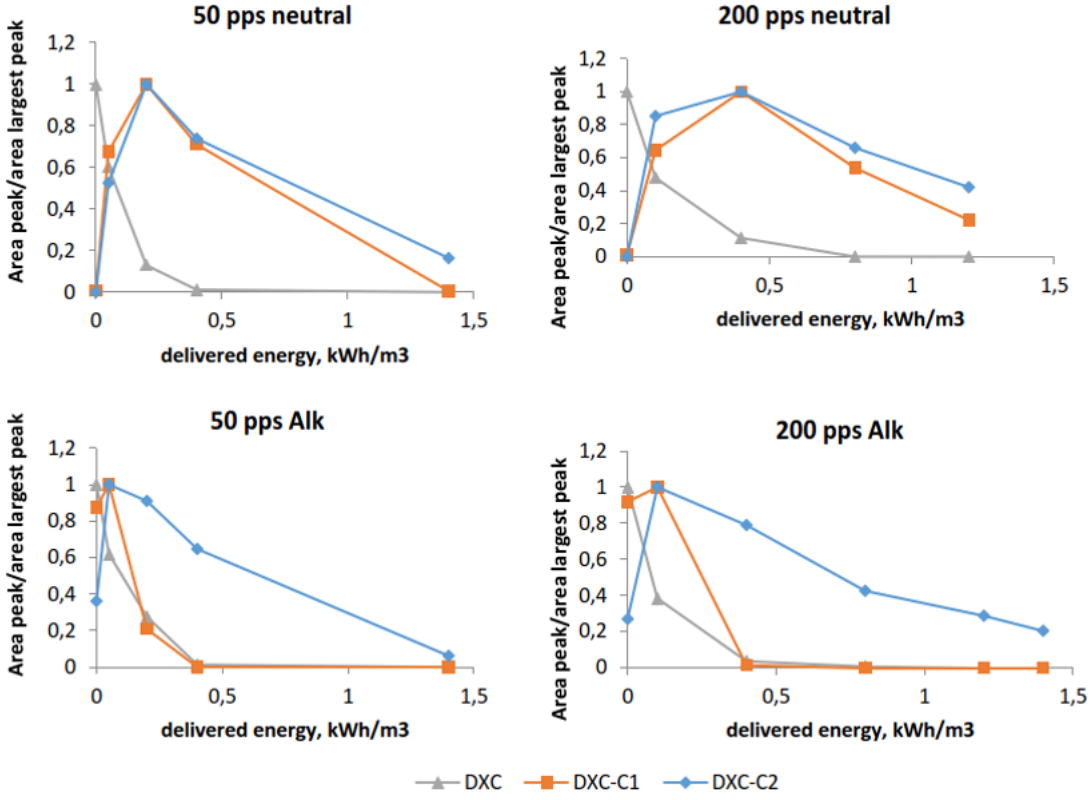


Figure 7 Changing of content ratio of doxycycline and its major oxidation products



Qualitative analysis of trenary solution showed five transformation products of amoxicillin (Figure 8). The identification was based on observed molecular mass and the fragmentation patterns. The exact masses were recorded for the products OH-amoxicillin (AMX-C1) and amoxicillin penicilloic acid (AMX-C2) and used for structural determination. The structure of the transformation product AMX-C4 has previously been reported by Trovó et al. [39]. Figure 6 shows the change in the content ratio of amoxicillin and its major (with

the largest peak areas) oxidation products. As can be seen from the figure, along with amoxicillin there are some amount of amoxicillin penicilloic acid in the sample without treatment (Delivered energy = 0 kWh/m^3). This is largely due to acidic conditions in ternary solution, and, as was mentioned before amoxicillin starts to hydrolyze under acidic conditions and it leads to amoxicillin penicilloic acid formation. The presence of both compounds decreases continuously until the end of the treatment. At the same time, there is now OH-amoxicillin in the 0 sample, it appears when treatment starts and its quantity increases up to certain point, where the value peaks as the hydroxyl radicals start to react with amoxicillin. Finally, all three compounds decompose by the end of the treatment.

Figure 8 Proposed oxidation pathway of amoxicillin

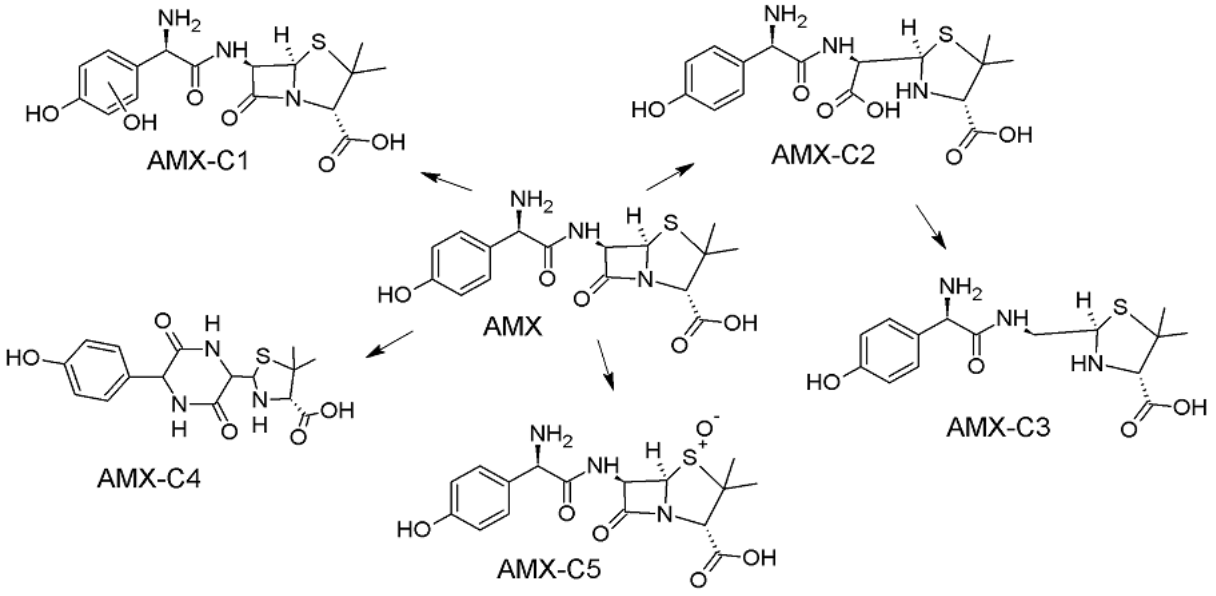
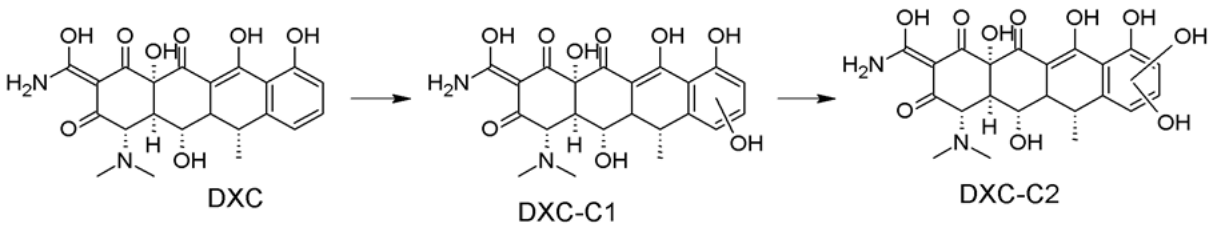


Figure 9 Proposed oxidation pathway of doxycycline



The transformation products of both antibiotics were detected in neutral samples. In alkaline samples, only doxycycline oxidation products were detected. Qualitative analysis of amoxicillin oxidation intermediate products in alkaline samples did not give reliable results. The implemented analytical method requires modification to enable detection of amoxicillin and its oxidation products in alkaline media.

Qualitative analysis of the binary solution showed the same transformation products of amoxicillin. The difference is there is no amoxicillin penicilloic acid in the 0 sample. Both amoxicillin penicilloic acid and OH-amoxicillin appear during the experiment, and their quantity increases at the beginning of the treatment and then drops.

For doxycycline, two major transformation products, OH-doxycycline (DXC-C1) and 2-OH-doxycycline (DXC-C2), were detected (Figure 9). The products were identified based on their exact mass, which suggests the addition of one and two OH groups respectively. The OH groups were most probably added to the aromatic rings. Figure 7 presents the behavior of the content ration of doxycycline ant its degradation products during PCD treatment of ternary solution with and without the presence of NaOH. Binary solution of doxycycline was also studied and gave approximately the same results in that the relative change in compounds was the same. As can been seen from Figure 7, in the absence of NaOH, only doxycycline is found at the beginning of the treatment, while other compounds start to appear later due to the reaction of hydroxyl radicals with doxycycline. It is clearly seen that the concentration of intermediate products increases unless there is enough primary component. In other words, as long as we have excess of primary compound in the system, we can observe an increasing of concentration of intermediate products. When the required amount of primary component in the system is no longer available, the intermediate products start to oxidize as well. In experiments with NaOH, some amount of 2-OH-doxycycline and some OH-doxycycline is found at the beginning of the treatment. As against treatment in neutral media, doxycycline and OH-doxycycline oxidize quite fast under alkaline condition, whereas the 2-OH-doxycycline is more resistant to oxidation.

4. Conclusion

The studied antibiotic compounds, amoxicillin and doxycycline, are relatively easy to oxidize with PCD and pulsed corona discharge oxidation is thus an effective degradation approach for these compounds, achieving high rate of degradation in a short period of time with low energy consumption.

The energy efficiency of oxidation of all the compounds increases with reducing pulse repetition frequency. At low frequency, ozone accumulates during pauses between the pulses. Thus, the in situ concentration of ozone increases, it has more time to react with target compounds and the role of ozone in the oxidation becomes more significant.

High pH is preferable for oxidation at the beginning of the process; however, as the treatment progresses, with decreasing of primary compound, the effect of pH becomes less significant.

The reaction of amoxicillin is first order in a neutral medium and second order in an alkaline medium. The doxycycline oxidation reaction is a first order reaction in both neutral and alkaline media. However, the reaction rate constant is higher at alkaline conditions.

Five and two intermediate compounds were detected during oxidation of amoxicillin and doxycycline respectively. OH-amoxicillin, amoxicillin pencilloic acid, OH-doxycycline and 2-OH-doxycycline had the largest peaks areas. All products were oxidized by the end of the treatment. The behavior of all intermediate products was similar to that of the original compounds, i.e. better degradation under alkaline conditions, and low pulse repetition frequency is to be preferred from the energy efficiency point of view.

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