Non-thermal plasma brush used for degradation of antibiotics - fundamental studies on high-throughput four-cone plasma system. 21-26 May 2023, Kyoto, Japan

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Abstract:

The presence of sublethal doses of antibiotics in aquatic environment contributes to start and spread of multidrug resistance among pathogenic bacteria. To ensure an efficient way to remove drugs from liquid wastes, an effective non-thermal plasma-based system generating four cones of plasma, operating with a constant flow of antibiotics-containing water solution, has been developed and studied. The removal efficiency of the drugs was determined along with mechanisms responsible for deactivation of pharamceuticals. **Keywords:** wastewater treatment, cold atmospheric pressure plasma, drugs

1. Introduction

Nalidixic acid (NAL) was the first synthetically obtained antibiotic from the quinolone group and so far found numerous applications. It is commonly applied to combat Gram-negative bacteria, and its effectiveness depends on the dosege used [1]. The highest susceptibility towards nalidixic acid was observed in *Aeromonas hydrophila*, *Clostridium* spp. and *Haemophilus* spp., therefore this pharmaceutical is most commonly provided to treat urinary tract infections. Beside medical sector, NAL is used in veterinary, agriculture and slaughter animal breeding [2].

Unmetabolized nalidixic acid remains in a biologically active form and diffuses into the waterways together with urine. Thus, high residuals of this drug tend to be detected in contaminated water reservoirs. For instance, a maximal NAL concentration reaching 20.5 ng L⁻¹ was determined in Karst river system in Kaiyang (Southwest China) [3]. The observed environmental pollution with NAL inexorably leads to the isolation of bacterial strains belonging to *Salmonella* spp., *Escherichia* spp., and *Enterobacter* spp. of elevated resistance to this drug [4-6].

The present study focused on application of an innovative continuous flow reaction-discharge system that generates four cones of plasma to purify flowing NAL aquous solution of diverse concentrations. The four cones of non-thermal plasma system, called the plasma brush, was driven by a high-voltage generator with pulsemodulating radio frequencies (pm-rf). Changes in the physicochemical properties of NAL solutions flowing through the plasma brush system were verified by measurements of pH, electrical conductivity, and concentration of Total Organic Carbon (TOC) and Total Nitrogen (TN). To investigate the plasma-liquid interactions besides the observed physicochemical changes, the concentration of particular reactive nitrogen species (RNS), i.e., NO_2^- , NO_3^- , that could be involved in the degradation processes of NAL was determined.

2. Reagents and Solutions

Nalidixic acid sodium salt was purchased from Sigma-Aldrich (CAS: 3373-05-8, Israel). The studied antibiotic solution with a concentration of 1000 mg L^{-1} was prepared in de-ionized water. Afterwards, the NAL solutions of concentrations 30, 15, and 5 mg L^{-1} were prepared by proper dilutions of the stock solution.

Plasma brush system

To provide effective, ecological, and economical technology for lowering multi-drug resistance in an aqueous environment, an innovative high throughput nonthermal plasma-based system, called the plasma brush that generated four cones of plasma, has been developed, optimized, and protected by international patent application (PCT/PL2022/050027). The main part of the system, presented in Fig. 1, consists of a 3-D printed head (1) distributing helium working gas into four attached brass electrodes (2), connected to a common electrical circuit (3). The head of the plasma brush is placed above the quartz chamber with a tributary of antibiotic solutions attached (4). The two-channel peristaltic pump introduced into the system the NAL solution with a flowing rate of 3.0 mL min⁻¹, which acted as a liquid electrode to ignite four plasma cones. The high-voltage generator with pm-rf was applied, and discharge parameters were set at the frequency of modulation of 1700 Hz and duty cycle of 40%.



Fig. 1. The plasma brush system dedicated to degradation of constantly flowing nalidix acid (NAL) solutions. The highlight parts: 1 - 3D-printed plasma brush head; 2 - four brass electrodes; 3 - electrical circuit; 4 - flowing solution of NAL acting as a liquid electrode.

3. Assessment of physicochemical changes in NAL solutions following plasma brush treatment

The collected samples of NAL in three different concentrations after degradation in plasma brush systems were analyzed in terms of changes in pH and electrical conductivity. Regarding pH measurements, HI 98103 pH Checker (Hanna-Instruments, Romania) was used. The electrical conductivity measurements were conducted with the HI 98308 electrical conductivity tester (Hanna-Instruments, Romania) by immersing the head of the tester in a volume of 200 times diluted samples. The results were collected from three independent experiments and presented as mean values with SD. For statistical analysis, the NAL solutions treated in the plasma brush systems were compared with the non-treated ones.

It is important to study whether the solutions treated by the cold plasma system demand additional steps to decolourize them or if they are suitable to release into the aqueous environment. The colour of NAL solution before and after the plasma brush treatment was analyzed with the multiparameter bench photometer for environmental testing HI 83206-01 (Hanna Instruments, Romania). The measurements were done according to the manufacturer's protocol in three independent measurements. The collected results were presented as means with SD.

Nitrite (NO_2^{-}) and nitrate (NO_3^{-}) concentrations were measured with the aid of Portable Photometers for nitrite model HI 93708-0 and nitrate HI 93728-0 (Hanna Instruments, Romania). Before the experiments the samples were diluted two times to reach a final volume of 10,00 mL and prepared according to manufacturer's protocols with the aid of dedicated reagents. The results were collected from three independent studies and presented as means \pm SD.

4. Results and discussion

The first and most noticeable difference in the physicochemical properties of NAL solutions after treatment with the plasma brush was a deviation in pH (Fig. 2). The pH values significantly dropped together with lowering the admixture of NAL in the plasma-treated reaching lowest group, the value of 4.94 ± 0.05 . The decreased pH in the antibiotic solutions after non-thermal plasma treatment was also observed in our previous studies and is mainly linked with the formation of HNO2 and HNO3 following plasma-liquid interactions [7,8]. electrical conductivity The measurements of the plasma-treated solutions allowed for quantitative analysis of the present negative and positive ions, which can be created due to the plasma-liquid interactions. For the group of the plasma brush-treated NAL solutions, a significant increase in the electrical conductivity was observed only for a concentration of 30 mg L⁻¹ NAL. In the rest of cases, the conductivity slightly increased compared to the untreated solutions, and the elevation was more remarkable for a higher concentration of NAL.

Electrical conductivity and pH in Nalidixic acid after plasma brush treatment



Fig. 2. The electrical conductivity and pH measurements in Nalidixic acid solutions after plasma brush teatment.

The performed analysis of plasma-treated NAL solutions in terms of water colour indicated that the collected samples turned colourless for the NAL concentration of 15 and 5 mg L⁻¹ (70 \pm 1 and 34 \pm 1 PCU, respectively), which characterizes liquids safe to drink. However, for the highest NAL concentration the plasma brush treatment resulted in higher PCU values 140 \pm 1, which is observed for waters not recommended for drinking, but safe to release to the environment. From that perspective, it was confirmed that the plasma brush-treated NAL solutions could be further processed or

released without extra purification or decolourization, which positively impacts operationa costs. Α concentration of carbon and nitrogen assessed by TOC and TN analyses allowed for studying the impact of the plasma brush treatment on NAL solutions. The results showed a statistically significant increase in TOC concentration in each NAL solution treated in the plasma brush system (Fig. 3). It is worth highlighting that the highest increase was observed for 30 and 15 mg L⁻¹ of NAL concentration (**** p < 0.0001), while for the concentration of 5 mg L⁻¹, the increase was not so abundant (** p < 0.007). The main reason behind the increased TOC concentration, following the plasma brush decomposition, is most likely linked with the impact of helium gas flow, formin aerosols that lowered water contribution. Additionally, the increased TOC content can be explained by the formation of carboxylic acids as a result of the reaction between degradation products of NAL and carbon dioxide from the atmosphere [9]. The degradation process, driven by the plasma brush has a strong effect on the contribution of nitrogen in NAL solutions (Fig. 3). For all plasma-treated solutions, a statistically significant increase in TN concentration was observed (**** p < 0.0001). Moreover, the TN contribution decreases with a lower concentration of NAL solutions, most likely linked with the plasma-liquid interactions, leading to the formation of certain reactive nitrogen species [8].

Total Organic Carbon (TOC) and Total Nitrogen (TN) concentration in Nalidixic acid after plasma brush treatment



Nitrogen (TN) concentrations, determined in Nalidixic acid (NAL) solutions, treated by plasma brush system.

The qualitative and quantitative analysis of nitrite and nitrate concentrations in plasma brush-treated NAL solutions was performed to verify this statement (**Fig. 4**). The generation of NO_2^- ions was detected in NAL

solutions treated by plasma brush systems. In more detail, NO₃⁻ concentration exceeded 42 mg L⁻¹ for NAL concentrations of 30 and 15 mg L⁻¹ and insignificantly dropped to 30 mg L⁻¹ regarding the lowest NAL concentration studied. On the other hand, the presence of NO₃⁻ ions was also confirmed. However, the NO₃⁻ concentration elevated from 14.18 ± 0.01 (30 mg L⁻¹ of NAL) to $23.33 + 0.52 \text{ mg } \text{L}^{-1}(5 \text{ mg } \text{L}^{-1} \text{ of NAL})$, showing an opposite trend compared to the presented results for NO₂⁻ ions. The observed situation likely results from the preferable generation of NO2⁻ ions as a result of plasma brush interactions with a higher contribution of NAL in wastes. The determined concentrations of NO₂⁻ and NO₃⁻ ions as the main representatives of RNS can be responsible for the degradation efficacy of the presented high-throughput plasma brush system.





Fig. 4. The nitrite (NO₂⁻) and nitrate (NO₃⁻) contributions, determined in Nalidixic acid (NAL) subjected to plasma brush teatment.

5. Conclusion

The herein presented innovative plasma-based reactiondischarge system, named the plasma brush, is dedicated to effective and high-throughput degradation of antibiotics solutions. Here, a plasma brush system was adopted for the degradation of NAL from water solutions in three concentrations of this drug, *i.e.*, 30, 15, and 5 mg L⁻¹. Considering the observed changes in physicochemical

properties of NAL solutions before and after the plasma brush treatment, a significant drop in pH shall be recognized post the degradation process. On the other hand, a significant increase in electrical conductivity of 30 mg L⁻¹ NAL solution, resuting from the plasma brush irradiation indicates to the abundance of plasma-liquid interactions, most likely leading to the degradation of NAL. The performed studies on TOC and TN contents confirmed changes in the composition of NAL solutions after the plasma brush treatment. On the other hand, the increased contribution of carbon and nitrogen, indicated the presence of degraded intermediates. We may conclude that generation of RNS leads to decomposition of antibiotic molecules. It is also worth to notice that purification of NAL solutions in the plasma brush systems seems convenient in terms of rescaling up for the requirements of wastewater treatment plants and shall be considered a highly efficient and cost-effective technique for degrading antibiotics before releasing liquid disposals into wastewaters.

6. Patents

The construction and application of the plasma brush system is protected under the polish patent application no. P.440185 and international patent application in PCT mode no. PCT/PL2022/050027.

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8. References

 N. Aggarwal, R. Kumar, C. Srivastva, P. Dureja, J.M. Khurana, J. Agric. Food Chem., 58, 3056 (2010).

[2] B. Callens, M. Cargnel, S. Sarrazin, J. Dewulf, B.

Hoet, K. Vermeersch, P. Wattiau, S. Welby, Prev. Vet. Med., 157, 50 (2018).

- [3] S. Zou, F. Huang, L. Chen, F. Liu, Water Sci. Technol. Water Supply, **18.6**, 1 (2018).
- [4] A.L. Krueger, S.A. Greene, E.J. Barzily, O. Henao, D. Vugia, S. Hanna, S. Meyer, K. Smith, G. Pecic, D.

Hoefer, P.M. Griffin, Foodborne Pathog. Dis., **11**, 5 (2014).

[5] B. Preethi, V. Shanthi, K. Ramanathan, Appl. Biochem Biotechnol., **177**, 528 (2015)

[6] H.M. Nirbhavane, U.S. Bagde, Afr. J. Microbiol. Res., 9, 49 (2015)

[7] A. Dzimitrowicz, M. Caban, D. Terefinko, P. Pohl, P.

Jamroz, W. babinska, P. Cyganowski, P. Stepnowski, E. Lojkowska, W. Sledz, A. Motyka-Pomagruk, Sci. Rep., **12**, 7354 (2022)

[8] D. Terefinko, M. Caban, A. Motyka-Pomagruk, W.

Babinska, P. Pohl, P. Jamroz, P. Cyganowski, W. Sledz,

E. Lojkowska, P. Stepnowski, A. Dzimitrowicz, Chem. Eng. J., **452**, 4, (2023). [9] C. Sarangapani, D. Ziuzina, P. Behan, D. Boehm, B.F. Gilmore, P.J. Cullen, P. Bourke, Sci. Rep., 9, 3955 (2019).