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**DOI**

[10.1002/cctc.202001225](https://doi.org/10.1002/cctc.202001225)

**Publication date**

2020

**Document Version**

Final published version

**Published in**

ChemCatChem

**Citation (APA)**

Yayci, A., Dirks, T., Kogelheide, F., Alcalde, M., Hollmann, F., Awakowicz, P., & Bandow, J. E. (2020). Microscale Atmospheric Pressure Plasma Jet as a Source for Plasma-Driven Biocatalysis. *ChemCatChem*, 12(23), 5893-5897. <https://doi.org/10.1002/cctc.202001225>

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# Microscale Atmospheric Pressure Plasma Jet as a Source for Plasma-Driven Biocatalysis

Abdulkadir Yayci,<sup>[a]</sup> Tim Dirks,<sup>[a]</sup> Friederike Kogelheide,<sup>[b]</sup> Miguel Alcalde,<sup>[c]</sup> Frank Hollmann,<sup>[d]</sup> Peter Awakowicz,<sup>[b]</sup> and Julia E. Bandow\*<sup>[a]</sup>

The use of a microscale atmospheric pressure plasma jet ( $\mu$ APPJ) was investigated for its potential to supply hydrogen peroxide in biocatalysis. Compared to a previously employed dielectric barrier discharge (DBD), the  $\mu$ APPJ offered significantly higher  $\text{H}_2\text{O}_2$  production rates and better handling of larger reaction volumes. The performance of the  $\mu$ APPJ was evaluated with recombinant unspecific peroxygenase from *Agrocybe aegerita* (rAaeUPO). Using plasma-treated buffer, no side reactions with other plasma-generated species were detected. For long-term treatment, rAaeUPO was immobilized, transferred to a rotating bed reactor, and reactions performed using the  $\mu$ APPJ. The enzyme had a turnover of  $36,415 \text{ mol mol}^{-1}$  and retained almost full activity even after prolonged plasma treatment. Overall, the  $\mu$ APPJ presents a promising plasma source for plasma-driven biocatalysis.

## Main text

Peroxygenases perform a range of hydrogen peroxide ( $\text{H}_2\text{O}_2$ )-dependent oxyfunctionalization reactions such as enantioselective hydroxylations and epoxidations.<sup>[1]</sup> Their high selectivity and operational stability makes peroxygenases appealing enzymes for biocatalysis. However, like all heme-containing

enzymes, peroxygenases suffer from inactivation by  $\text{H}_2\text{O}_2$ .<sup>[2]</sup> Several approaches exist to generate  $\text{H}_2\text{O}_2$  gradually *in situ*, thereby lowering the working concentration of  $\text{H}_2\text{O}_2$  and mitigating the issue of inactivation as well as dilution of the reaction solution.<sup>[3]</sup>

One approach makes use of atmospheric pressure plasmas.<sup>[4]</sup> In general, plasmas are generated by high electric fields, accelerating free electrons that collide with atoms or molecules in the gas phase, which leads to the formation of metastable as well as excited species and ions.<sup>[5]</sup> The short-living reactive species react with each other or with surrounding gas atoms or molecules to eventually yield less reactive species.<sup>[6]</sup> One of the species produced in plasma-treated aqueous liquids is  $\text{H}_2\text{O}_2$  that can then be used to drive peroxygenase-based biocatalysis.

We previously showed that the  $\text{H}_2\text{O}_2$  in plasma-treated buffers can drive biocatalysis with the *in vitro*-evolved, recombinant UPO from *Agrocybe aegerita* (rAaeUPO).<sup>[4]</sup> When enzyme and substrate were treated together with plasma, however, the yield of product was significantly reduced as compared to a catalysis scheme in which plasma treatment and subsequent biocatalysis were uncoupled. Since the enzyme was quickly inactivated by plasma treatment with a dielectric barrier discharge (DBD), immobilization was used to prolong the lifetime of the enzyme. However, immobilization did not significantly improve product formation under direct treatment. Since only small volumes ( $\mu\text{L}$  scale) could be treated, mixing of the reaction solution during plasma treatment was not feasible, thereby limiting substrate supply to the enzyme and presumably turnover.

In this study, we addressed the aforementioned shortcomings by going into the micro-scale. In a first experiment, the influence of the surrounding gas on the  $\text{H}_2\text{O}_2$  production rate of a DBD was investigated. An in-house built DBD,<sup>[7]</sup> employing comparable electrode geometry and plasma parameters as the previously used Cinogy PlasmaDerm source,<sup>[8]</sup> was placed in different atmospheres and used to treat  $110 \mu\text{L}$  of phosphate buffer. Immediately after treatment, the  $\text{H}_2\text{O}_2$  concentration was measured with a colorimetric assay. Using an argon atmosphere to ignite the plasma gave the highest  $\text{H}_2\text{O}_2$  concentration as compared to synthetic air or nitrogen (Figure 1). In argon atmosphere, after 5 min  $13.7 \text{ mM}$  of  $\text{H}_2\text{O}_2$  were measured whereas only  $3 \text{ mM}$  and  $4.7 \text{ mM}$   $\text{H}_2\text{O}_2$  were observed under synthetic air and nitrogen, respectively, under otherwise identical conditions (Figure 1). It is worth mentioning that the  $\text{H}_2\text{O}_2$  accumulation was linear during the 5 min treatment in all cases.

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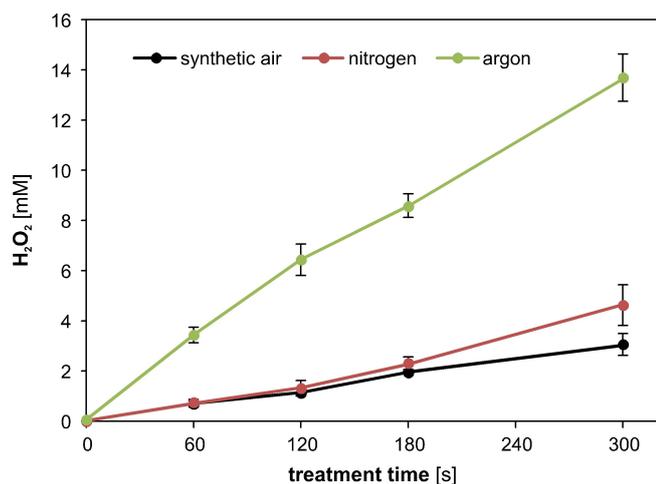
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Supporting information for this article is available on the WWW under <https://doi.org/10.1002/cctc.202001225>

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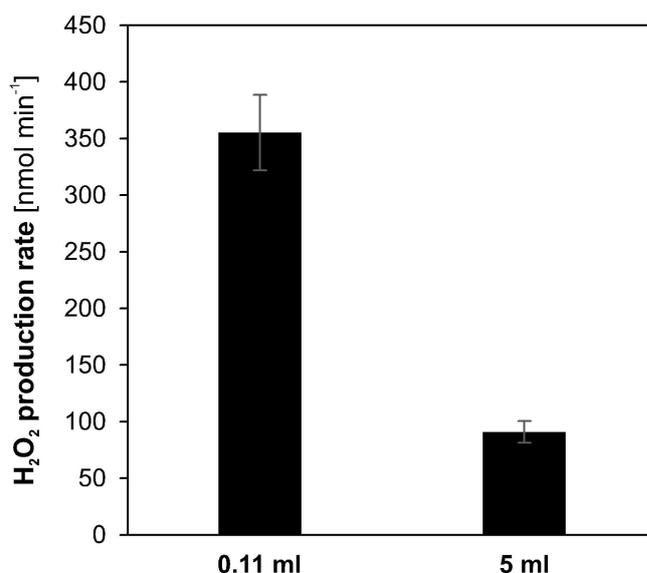
**Figure 1.** H<sub>2</sub>O<sub>2</sub> production with the DBD in different gas atmospheres. 110  $\mu$ L of phosphate buffer (100 mM, pH 7) were treated for the indicated times and analyzed photometrically. N = 3, STD.

Since argon is an atomic gas, the collision of accelerated electrons and gas particles may not lead to molecular vibration or rotation and therefore increases the formation of metastables and ions, which in turn increases H<sub>2</sub>O<sub>2</sub> production.

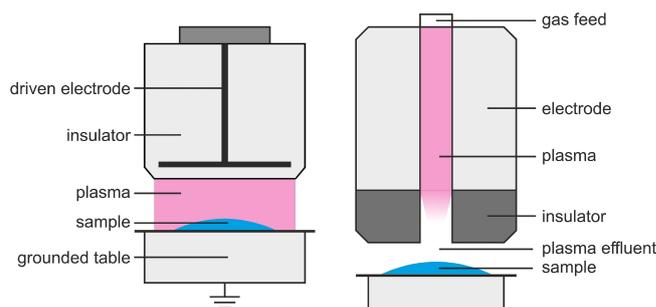
Next, we tested whether the high production rate of H<sub>2</sub>O<sub>2</sub> under argon atmosphere persists when treating larger volumes of liquid. Using the same setup, 5 mL of phosphate buffer were treated and analyzed. The absolute production rate of H<sub>2</sub>O<sub>2</sub>, i.e. the total amount of H<sub>2</sub>O<sub>2</sub> molecules produced per minute, for 5 mL was significantly lower than for 110  $\mu$ L, indicating that the surface-to-volume ratio influences H<sub>2</sub>O<sub>2</sub> generation (Figure 2). Nevertheless, the DBD setup used here did not allow for a wider vessel to be treated in a pure argon atmosphere so that no further experiments were conducted to investigate the scalability of biocatalysis driven by this source.

Since the DBD geometry posed some restrictions on the sample volume and distance to the source as well as H<sub>2</sub>O<sub>2</sub> production rate, a different kind of plasma source was tested, namely a microscale atmospheric pressure plasma jet ( $\mu$ APPJ).<sup>[9]</sup> A comparison of both plasma geometries is shown in Figure 3. In the  $\mu$ APPJ, the plasma is ignited between two electrodes with a vertical noble gas flow (in this case He) that disperses the created reactive species. While in a DBD setup the sample is in direct contact with the plasma, with the  $\mu$ APPJ the sample is only exposed to the plasma effluent, i.e. the reactive species transported by the gas flow.<sup>[10]</sup> Since the plasma is remote from the treated sample, the  $\mu$ APPJ enables treatment over a broad range of sample volumes and formats.

Previous studies with the  $\mu$ APPJ showed that by employing water vapor in the feed gas the generation of H<sub>2</sub>O<sub>2</sub> is greatly enhanced.<sup>[11]</sup> Therefore, we first measured the H<sub>2</sub>O<sub>2</sub> production rate and whether it improved with increased water vapor content. A part of the feed gas was passed through a bubbler containing deionized water and mixed with the remaining dry feed gas before going through the electrode. When 10% of the



**Figure 2.** H<sub>2</sub>O<sub>2</sub> production rates of the in-house built DBD for different volumes treated in an argon atmosphere. Samples of 110  $\mu$ L and 5 mL were treated with the DBD for 2 and 15 min, respectively, in an argon atmosphere and analyzed photometrically. The production rate of H<sub>2</sub>O<sub>2</sub> is shown as absolute values (amount per minute), accounting for both treated volume and time. N = 3, STD.

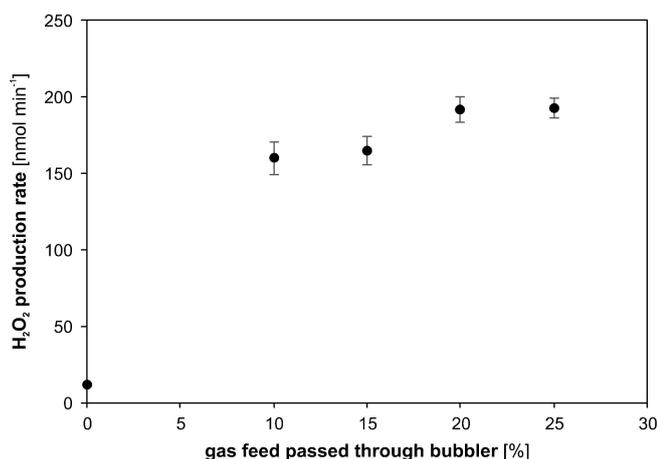


**Figure 3.** Comparison of both plasma devices used in this work. Left: DBD. Right:  $\mu$ APPJ. Images modified from Kuchenbecker *et al.*<sup>[8]</sup> and Golda *et al.*<sup>[9]</sup>

total feed gas flow were passed through the bubbler, the H<sub>2</sub>O<sub>2</sub> production rate already increased significantly (Figure 4).

The absolute H<sub>2</sub>O<sub>2</sub> production of the  $\mu$ APPJ with 25% relative humidity was comparable to that of the DBD in argon and approx. 15 $\times$  higher than that of the DBD in ambient air (see Figure 2 and Table S1 for details). When more than 25% of the gas feed were routed through the bubbler, the plasma did not ignite anymore when applying the standard voltage of 230 V<sub>pp</sub>. We therefore used 25% of water-treated He for all subsequent experiments. H<sub>2</sub>O<sub>2</sub> concentration also increased linearly with the treatment time, showing that the production rate stays constant (Figure S1). Adding minor amounts of oxygen to the feed gas had no significant effect on H<sub>2</sub>O<sub>2</sub> production (Figure S2).

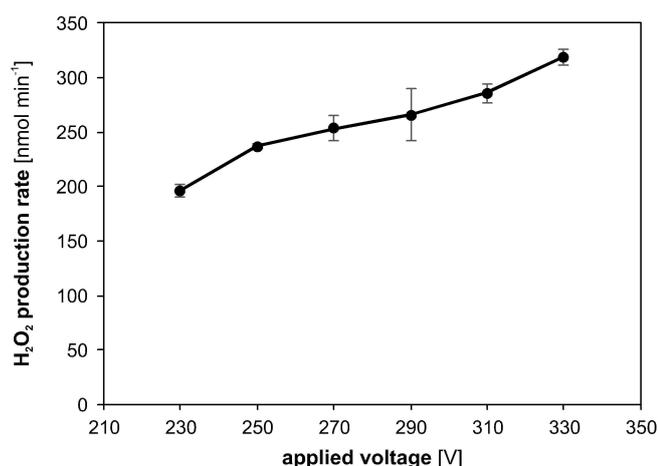
In a biocatalysis setting, it would be beneficial to tailor the plasma and thereby the supplied amount of H<sub>2</sub>O<sub>2</sub> to match the needs and limitations of the enzyme employed. We therefore



**Figure 4.** H<sub>2</sub>O<sub>2</sub> production by the  $\mu$ APPJ at different water vapor ratios. The  $\mu$ APPJ gas feed was partially passed through a bubbler containing water before being introduced into the electrode gap. 200  $\mu$ L of buffer (pH 7) were treated in a microwell plate for 2 min and the H<sub>2</sub>O<sub>2</sub> concentration determined. The production rates of H<sub>2</sub>O<sub>2</sub> are shown as amount per minute, accounting for both treated volume and time. N=3, STD.

tested whether H<sub>2</sub>O<sub>2</sub> production can be tuned by modifying the voltage. Generation of H<sub>2</sub>O<sub>2</sub> was found to correlate linearly with the applied voltage, which corresponds to input power (Figure 5). The ability to quickly tune H<sub>2</sub>O<sub>2</sub> production rates is a decisive advantage over some other *in situ* methods, e.g. enzymatic H<sub>2</sub>O<sub>2</sub> generation systems.

After having established that the  $\mu$ APPJ produces suitable amounts of H<sub>2</sub>O<sub>2</sub>, we first checked whether plasma-treated buffer was a reasonable resource to drive biocatalysis. The investigated reaction was the well-known hydroxylation of ethylbenzene (ETBE) to (*R*)-1-phenylethanol ((*R*)-1-Phol) by *rAaeUPO*.<sup>[12]</sup> This enzyme showed remarkable turnover numbers (TONs) and enantioselectivity in previous studies and was used

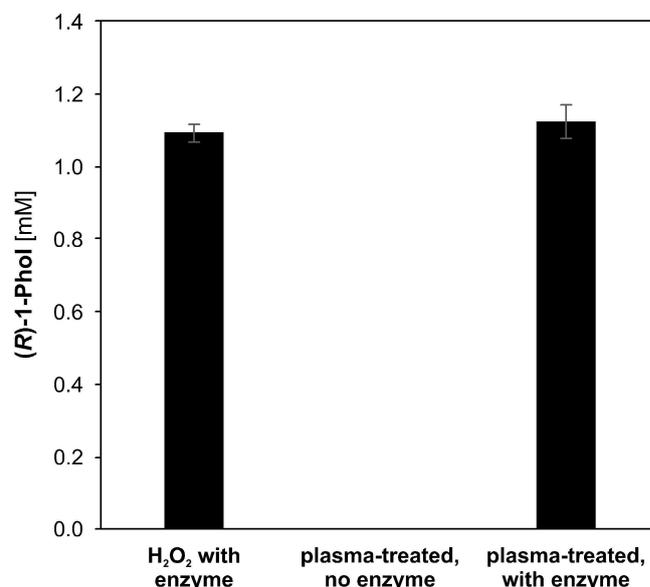


**Figure 5.** H<sub>2</sub>O<sub>2</sub> production by the  $\mu$ APPJ at different voltages. In a microwell plate, 200  $\mu$ L of phosphate buffer (pH 7) were treated with the  $\mu$ APPJ for 2 min at minimal distance to the jet nozzle, using 25% relative humidity in the gas feed. N=3, STD.

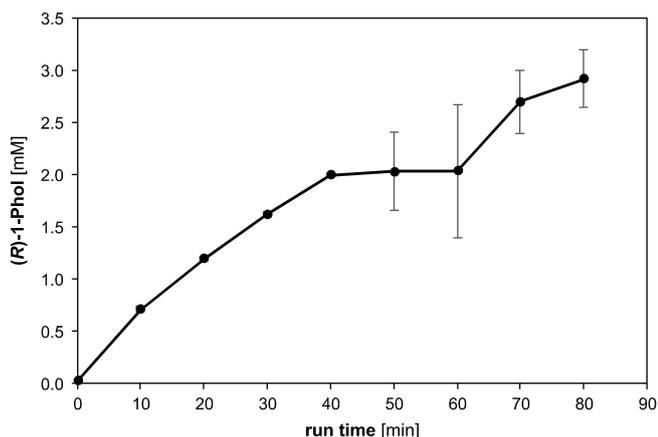
in conjunction with plasma before.<sup>[4]</sup> After treating 5 mL of buffer with the  $\mu$ APPJ for 15 min, ETBE and *rAaeUPO* were added and allowed to react for 15 min. Both H<sub>2</sub>O<sub>2</sub> produced with the  $\mu$ APPJ and a diluted H<sub>2</sub>O<sub>2</sub> stock solution with the same concentration as in plasma-treated buffer yielded approx. 1.1 mM product (92% ee) after enzymatic conversion, showing that other possible side products of plasma treatment, like peroxyxynitrite, nitrite, or nitrate [9], did not have a negative effect on the reaction (Figure 6). No overoxidation of 1-Phol to acetophenone was observed in GC chromatograms. Also, no background activity was observed for plasma-treated buffer without the enzyme.

In order to couple plasma treatment and catalysis while retaining enzyme activity, *rAaeUPO* was then immobilized by covalent binding to support beads. The beads were transferred to a small-scale rotating bed reactor that provides mixing through rotation, allowing for high substrate flow through the bead layer. The rotating bed reactor was placed into a suitable vessel filled with 5 mL of buffer containing 50 mM of ETBE and treated with the  $\mu$ APPJ. Aliquots were withdrawn and analyzed by GC, revealing that after an initial phase with high conversion, the turnover rate declines at around 3 mM of (*R*)-1-Phol (Figure 7). In this setup, a TON of 23,037 mol<sub>(*R*)-1-Phol</sub> mol<sup>-1</sup><sub>*rAaeUPO*</sub> was achieved.

Generally, turnover stalling can be explained by three major causes: reduced enzyme activity, substrate depletion, or product inhibition.<sup>[13]</sup> After 80 min of treatment, the immobilized enzyme was extracted and checked for activity *ex situ*. Only a negligible loss of activity was found, showing that enzyme activity was not the reason for decreased turnover (Figure S3). Next, the performance of the enzyme was tested in the same rotating bed reactor system when 4 mM of racemic 1-Phol,



**Figure 6.** Biocatalysis using buffer treated with the  $\mu$ APPJ. 5 mL of buffer were treated for 15 min with the  $\mu$ APPJ and subsequently mixed with ETBE and *rAaeUPO* (50 mM and 50 nM, respectively). Reactions were run for 15 min, extracted, and analyzed by GC. N=3, STD.



**Figure 7.** Conversion of ETBE by *in situ* treatment with the  $\mu$ APPJ. Immobilized *rAaeUPO* was transferred into a rotating bed reactor and placed in a narrow vessel containing 5 mL of buffer with 50 mM ETBE. Plasma treatment was performed as described above, with 25% of the gas feed passed through a bubbler. At the indicated intervals, aliquots were withdrawn, subsequently extracted, and analyzed by GC. N = 3, STD.

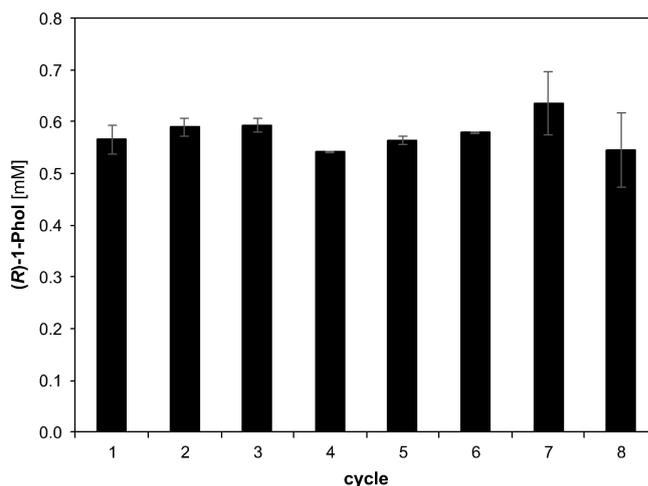
approximately corresponding to the extrapolated final concentration shown in Figure 7, were added from a stock solution. Enzyme activity decreased to  $\sim 61\%$  (Table S2), indicating that high concentrations of the product 1-Phol indeed affected turnover. However, the increase in product concentration from 70 to 80 min of treatment in Figure 7 corresponds to only  $\sim 30\%$  of the initial activity, showing that product inhibition is not the only cause for decreased turnover.

The  $\mu$ APPJ is operated with 1.4 slm gas flow. Because the effluent hits the surface of the treated solution, it seemed likely that substrate evaporation limits catalysis. While the overall buffer volume stayed constant for long periods of time ( $< 10\%$  loss after 30 min), the volatile substrate ETBE appeared to evaporate. Since ETBE is hydrophobic and only dissolves into the liquid at quite low concentrations, the majority of the substrate floats on top of the reaction solution in droplets, even when the reaction mixture is stirred. These droplets were observed to quickly evaporate due to the high gas flow to the surface which could negatively affect the outcome of the experiment presented in Figure 7 in which the substrate is added at high concentrations at the beginning of the reaction and may be depleted at prolonged treatment times.

Consequently, direct biocatalysis using the rotating bed reactor was repeated while replacing the entire reaction solution after every cycle of 10 min (Figure 8).

The obtained (R)-1-Phol concentration stayed constant over 8 cycles (total of 80 min accumulated treatment time), indicating that both substrate depletion as well as product inhibition were alleviated. The TON for this system was  $36,415 \text{ mol}_{(R)\text{-1-Phol}} \text{ mol}_{rAaeUPO}^{-1}$  which is comparable to previously published results.<sup>[14]</sup> Here, however, enzyme activity was not exhausted so that much higher TONs are to be expected.

In summary, we showed that a  $\mu$ APPJ is a suitable plasma source for plasma-driven biocatalysis. Compared to the DBD, the  $\mu$ APPJ is advantageous because larger volumes can be



**Figure 8.** ETBE conversion by direct treatment with the  $\mu$ APPJ effluent and substrate replenishment. Plasma treatment was performed at 25% relative humidity. Conversion was set up using immobilized *rAaeUPO* in the rotating bed reactor in 5 mL of buffer containing 50 mM ETBE. After taking an aliquot for GC analysis, the buffer solution with ETBE was renewed. Each cycle corresponds to a plasma treatment time of 10 min. N = 3, STD.

effectively treated and biocatalysis with direct treatment using the plasma effluent is now feasible. However, the  $\mu$ APPJ used here requires the use of expensive feed gases, such as helium or argon, which considerably increases the cost of running the system. Jet-based plasma sources that operate in ambient air have successfully been designed and would be beneficial for biocatalysis, combining cost-effective operation and favorable source geometry.<sup>[15]</sup>

At this point, the solubility of hydrophobic substrates needs to be addressed as well to make this system truly valuable for preparative scale.

## Experimental section

### Plasma sources

The DBD was used essentially as described before at 24 kV<sub>pp</sub> and 300 Hz.<sup>[16]</sup> A detailed account of the DBD can be found elsewhere.<sup>[7]</sup> To provide different gas atmospheres, a lateral gas flow was applied at 2 slm. When 5 mL were treated, a stainless steel wire loop was placed inside the glass vessel and connected to the ground.

The  $\mu$ APPJ was operated at 230 V<sub>RMS</sub> and 13.56 MHz with a combined flow of 1.4 slm He.<sup>[17]</sup> The gas feed was split and partially routed through a bubbler containing deionized water at room temperature. Both lines were merged in a T-piece before entering the electrode chamber.

### H<sub>2</sub>O<sub>2</sub> measurements

Immediately after treatment, samples were withdrawn and diluted to an appropriate concentration with deionized water. To 200  $\mu$ L of the diluted sample, 12.5  $\mu$ L of the reagents 1 and 2, supplied by a commercially available kit, were added and left to react for 5 min (Spectroquant H<sub>2</sub>O<sub>2</sub>, Merck, Germany). Absorption was measured at 455 nm and concentration determined using a calibration curve.

## Enzyme preparation

*In vitro*-evolved, recombinant rAaeUPO was purified essentially as described before.<sup>[18]</sup> The supernatant of a *Pichia pastoris* expression culture was subjected to microfiltration and single-step ion exchange chromatography, yielding the purified protein. Immobilization was carried out as described previously.<sup>[4]</sup> Briefly, HA403 M beads (Resindion, Binasco, Italy) were washed twice with water and incubated with phosphate buffer (pH 7) containing 0.5% glutaraldehyde. After 1 h, beads were washed three times with buffer and enzyme was added at 0.5 nmol per 100 mg beads. Immobilization was allowed to proceed overnight at 8 °C. Binding efficiency was checked by measuring enzyme activity in the supernatant and was > 80% in all cases. Roughly 150 mg of protein-loaded beads were then transferred to a rotating bed reactor that was build in-house by 3D-printing (dimensions:  $\varnothing$ 2 cm  $\times$  0.7 cm). The reactor was designed with a snap-on lid to enable extraction of the enzyme and reuse of the reactor. The final concentration of rAaeUPO in 5 mL of buffer was approx. 125 nM (see Table S3 for details).

## Conversion of ETBE

To generate plasma-treated buffer, 5 mL of buffer were treated with the  $\mu$ APPJ for 15 min with constant stirring. Subsequently, 50 mM of ETBE were added and the reaction solution was mixed for 15 min by overhead rotation to allow for the substrate to go into solution. Then, 50 nM of rAaeUPO were added and the solution incubated at 30 °C for 15 min with constant shaking.

When the rotating bed reactor was used, 5 mL of buffer containing 50 mM of ETBE were mixed and placed into a suitable vessel with the rotating bed reactor. Plasma exposure was conducted at approx. 4 mm distance between the nozzle and the liquid surface.

## Analysis of (R)-1-Phol

Aliquots of 150  $\mu$ L were withdrawn and mixed with the same volume of ethyl acetate containing 2 mM of 1-octanol as internal standard. The organic phase was transferred to a new vial, dried with  $MgSO_4$ , and subjected to gas chromatography. Samples were analyzed using a Shimadzu 2010 system with a Hydrodex  $\beta$ -6TBDM column (Macherey-Nagel, Germany) in an isothermal program (125 °C, 10 min). Concentrations were determined by a calibration curve with racemic 1-Phol.

## Acknowledgements

We thank Marco Krewing for fruitful discussions and the workshop of the Department of Biology and Biotechnology (Bochum) for constructing the rotating bed reactor. Funding was provided by

CRC1316-1 to JEB and PA and RTG2341 to JEB. Open access funding enabled and organized by Projekt DEAL.

**Keywords:** atmospheric pressure plasma · biocatalysis · hydrogen peroxide · peroxygenase · plasma chemistry

- [1] Y. Wang, D. Lan, R. Durrani, F. Hollmann, *Curr. Opin. Chem. Biol.* **2017**, *37*, 1–9.
- [2] B. O. Burek, *ChemCatChem* **2019**.
- [3] S. Bormann, A. Gomez Baraibar, Y. Ni, D. Holtmann, F. Hollmann, *Catal. Sci. Technol.* **2015**, *5*, 2038–2052.
- [4] A. Yayci, Á. Gómez Baraibar, M. Krewing, E. Fernandez Fueyo, F. Hollmann, M. Alcalde, R. Kourist, J. E. Bandow, *ChemSusChem* **2020**, *13*, 2072–2079.
- [5] R. Brandenburg, *Plasma Sources Sci. Technol.* **2017**, *26*, 53001.
- [6] P. J. Bruggeman, *Plasma Sources Sci. Technol.* **2016**, *25*, 53002.
- [7] F. Kogelheide, B. Offerhaus, N. Bibinov, P. Krajinski, L. Schücke, J. Schulze, K. Stapelmann, P. Awakowicz, *Plasma. Process. Polym.* **2020**, *17*, 1900126.
- [8] M. Kuchenbecker, N. Bibinov, A. Kaemling, D. Wandke, P. Awakowicz, W. Viöl, *J. Phys. D* **2009**, *42*, 45212.
- [9] J. Golda et al., *J. Phys. D* **2016**, *49*, 84003.
- [10] Y. Gorbanev, J. Golda, V. S.-V. D. Gathen, A. Bogaerts, *Plasma* **2019**, *2*, 316–327.
- [11] a) J. Winter et al., *J. Phys. D* **2014**, *47*, 285401; b) Y. Gorbanev, C. C. W. Verlackt, S. Tinck, E. Tuenter, K. Foubert, P. Cos, A. Bogaerts, *Phys. Chem. Chem. Phys.* **2018**, *20*, 2797–2808;
- [12] a) M. Pesic, S. J.-P. Willot, E. Fernández-Fueyo, F. Tieves, M. Alcalde, F. Hollmann, *Z. Naturforsch. C, J. Biosci.* **2018**; b) W. Zhang et al., *Nat. Can.* **2018**, *1*, 55; c) Y. Ni, E. Fernández-Fueyo, A. Gomez Baraibar, R. Ullrich, M. Hofrichter, H. Yanase, M. Alcalde, W. J. H. van Berkel, F. Hollmann, *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 798–801;
- [13] M. Dias Gomes, J. M. Woodley, *Molecules* **2019**, *24*, 3573.
- [14] a) F. Tieves et al., *Angew. Chem. Int. Ed. Engl.* **2019**; b) W. Zhang, B. O. Burek, E. Fernández-Fueyo, M. Alcalde, J. Z. Bloh, F. Hollmann, *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 15451–15455; c) S. J.-P. Willot, E. Fernández-Fueyo, F. Tieves, M. Pesic, M. Alcalde, I. W. C. E. Arends, C. B. Park, F. Hollmann, *ACS Catal.* **2019**, *9*, 890–894;
- [15] a) J. F. Kolb, A.-A. H. Mohamed, R. O. Price, R. J. Swanson, A. Bowman, R. L. Chiavarini, M. Stacey, K. H. Schoenbach, *Appl. Phys. Lett.* **2008**, *92*, 241501; b) P. Thana, A. Wijaikhum, P. Poramapijitwat, C. Kuensaen, J. Meerak, A. Ngamjarrojana, S. Sarapirom, D. Boonyawan, *Heliyon* **2019**, *5*, e02455;
- [16] D. Śmiłowicz, F. Kogelheide, K. Stapelmann, P. Awakowicz, N. Metzler-Nolte, *Sci. Rep.* **2019**, *9*, 1–13.
- [17] D. Ellerweg, J. Benedikt, A. von Keudell, N. Knake, V. Schulz-von der Gathen, *New J. Phys.* **2010**, *12*, 13021.
- [18] P. Molina-Espeja, S. Ma, D. M. Mate, R. Ludwig, M. Alcalde, *Enzyme Microb. Technol.* **2015**, *73–74*, 29–33.

Manuscript received: July 26, 2020

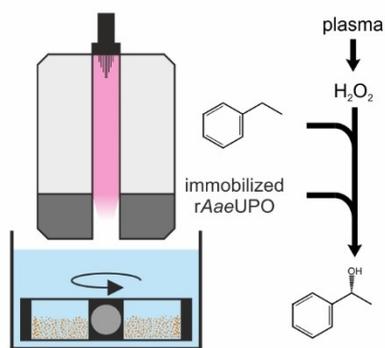
Revised manuscript received: August 26, 2020

Accepted manuscript online: August 27, 2020

Version of record online: ■■■, ■■■■

## COMMUNICATIONS

**Plasma jets and enzymes!** A microscale atmospheric pressure plasma jet was employed to generate  $H_2O_2$  for biocatalysis. Unspecific peroxygenase from *Agrocybe aegerita* was immobilized and used in a rotating bed reactor system. Conversion of ethylbenzene to (*R*)-1-Phol using plasma-generated  $H_2O_2$  was performed with high enantioselectivity and satisfactory TON.



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**Microscale Atmospheric Pressure  
Plasma Jet as a Source for Plasma-  
Driven Biocatalysis**

