Automatic Image Analysis of the Effects of Non-Thermal Plasma on Mold Growth
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J. Hrudka[1], V. Scholtz[1], J. Jiresová[1], Z. Rácová[1] , J. Khun[1], M. Klenivskyi[1] and A. Machková[1]

[1] *Department of Chemical Engineering, Department of Physics and Measurements, Faculty of Chemical Engineering, University of Chemistry and Technology Prague, Praha, Czech Republic*

Abstract: The study evaluates the impact of non-thermal plasma on mould growth using machine learning-based image analysis software. The results show that non-thermal plasma is an effective method in suppressing mould growth and highlights the potential of machine learning in evaluating mould growth and control methods. This method looks to be very effective for both the acceleration of the evaluation and decreasing of the evaluation errors for the growth monitoring.

Keywords: Automatic Image Analysis, Non-Thermal Plasma, Mold Growth, *Aspergillus brasiliensis*, Fungal Control, Material Surfaces, Human Health, Time-saving, Elimination of Bias, Machine Learning-based Software, Image Segmentation, Image Labelling, Efficacy, Chemical-based Methods, Potential, Reliable, Objective Method, Generalizability.

1. Introduction

The study of mould is a multi-disciplinary field that encompasses medicine [1], material technology, environmental quality, food contamination [2], and the impact on various material surfaces. The primary concern for this research is the harmful effects on human health and the deterioration of materials.

Each field utilizes unique techniques and approaches to determine the susceptibility of substrates to fungal growth, and different model organisms are used. Along with evaluating the sensitivity of materials to mould, researchers also focus on methods of protection. With stricter regulations on chemical use, there is a drive to find alternative methods of safeguarding material surfaces from microbial growth. One potential solution is the use of non-thermal plasma treatments as a protective measure.

2. Cultivation and NTP treatment

The current contribution focuses on the assessment of growth of fungi *Aspergillus brasiliensis* CCM8222 (ATCC 16404) in 23 °C MEA (Malt Extract Agar) mediums. Three sets of samples were prepared and treated 10 minutes later with non-thermal plasma at different stages of *A. brasiliensis* growth. The treatment was done directly after inoculation, 2 and 5 days after inoculation, using a direct bipolar corona discharge generated by a point-to-ring electrode system. The reference samples were not treated after inoculation in any way.

MEA (Malt Extract Agar) is a type of growth medium commonly used in microbiology for the cultivation of fungi and yeasts. It contains malt extract as a carbon source, yeast extract as a source of nitrogen and vitamins, and agar as a gelling agent. The pH of the medium is typically around 5.6-6.2. The combination of ingredients in MEA provides a suitable environment for the growth and reproduction of many fungi and yeasts. The agar in the medium solidifies it, allowing for the formation of colonies and making it easier to observe and study the organisms. Additionally, the malt and yeast extracts provide essential nutrients for the organisms to grow and reproduce. MEA is commonly used as a primary screening tool in fungal strain isolation and characterization.

A. brasiliensis is a species of the *Aspergillus* genus of fungi. It is a filamentous fungus, meaning it forms long, thread-like structures called hyphae, and reproduces by forming spores called conidia. *A. brasiliensis* is commonly found in the environment and is known to produce a wide variety of secondary metabolites, including mycotoxins.

In terms of biology, *A. brasiliensis* is a saprophyte, meaning it obtains nutrients from dead organic matter, and can be found in soils, plant debris, and other organic materials. It is a facultative aerobe, meaning it can grow both aerobically (in the presence of oxygen) and anaerobically (in the absence of oxygen), although it prefers anaerobic conditions.

A. brasiliensis has been the subject of much research due to its ability to produce various biologically active compounds, including enzymes, pigments, and mycotoxins. Some of these compounds have potential applications in the food, pharmaceutical, and industrial industries. However, it is also known to cause infections in immunocompromised individuals and animals, and to produce mycotoxins that can contaminate crops and food products.

Overall, *A. brasiliensis* is a biologically and chemically diverse species of fungus with both beneficial and potentially harmful properties, making it a right choose of our scientific study.

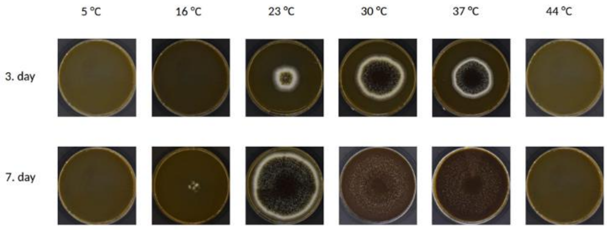

Fig. 1. The pictures of *A. brasiliensis* on MEA cultivation medium at 3rd and 7th day of cultivation for different temperatures

Figure 1. illustrates the relationship between cultivation temperature and the growth of *A. brasiliensis*. The cultivation temperature increas from 5 °C to 44 °C, representative pictures for 3. and 7. day are shown. We observed the most rapid growth at around 30 °C. This information is crucial for setting up base conditions for following steps of our experiment for accurate determining the effectiveness of treatment methods for disrupting mould growth.

The point-to-ring discharge was created using the needle-ring electrode configuration. The ring electrode was conical, made of brass, and had a diameter of 11mm at its top, 3.3mm below the needle electrode tip. The ring electrode was connected to the positive high-voltage source terminal and the needle electrode was connected to the negative one. A bipolar corona discharge was formed, with a negative corona discharge near the needle tip and a positive corona on the ring's edge. The discharge created reactive species that were carried by ions accelerated in the electric field between the electrodes. Most charged particles from the negative corona were drawn to the ring electrode, while neutral reactive particles were expelled through the ring electrode and onto the treated surface [3].

The electrode system was powered by a high-voltage power supply consisting of a 0-20V DC voltage source and a step-up DC/DC converter (up to 20 kV) with separate primary and secondary circuits. No ballast resistance was needed as the DC/DC converter had high output impedance.

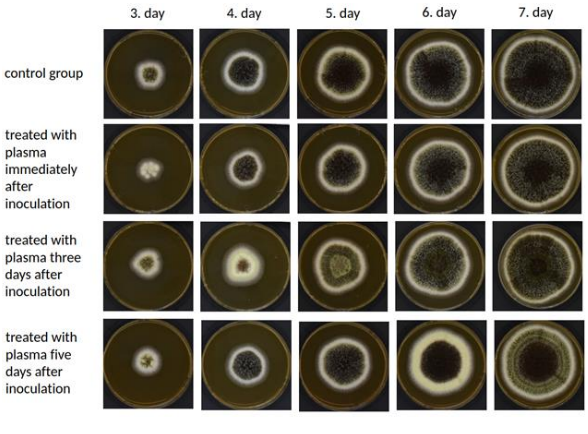

Fig. 2. *A. brasiliensis* on MEA

The cultures affected by NTP immediately and 3rd and 5th day after inoculation are shown in **Figure 1**. The different treatment groups represent the effects of NTP treatments on the growth of the mould. The change of growth over time is not that significant, but the change of colour in these areas shows that our methods can slow down the development of mould especially in case of treatment method 3rd and 5th days after incubation.

3. Image analysis evaluation

The image analysis presented is derived from the automatic assessment of photographs captured at fixed intervals during the growth of four sets of samples. This approach not only streamlines the process but also eradicates any subjective errors that may occur with human evaluators. Upon its initial implementation, multiple evaluators utilize the system, and the results are consistent, irrespective of the evaluator, unlike traditional methods of evaluating mould growth.

The software has been developed by our research group and it integrates machine learning algorithms to perform image segmentation, enabling the identification and classification of individual elements in the image, and labelling, which allows for the annotation of the elements with specific attributes, providing a valuable tool for studies like the one presented in this paper.

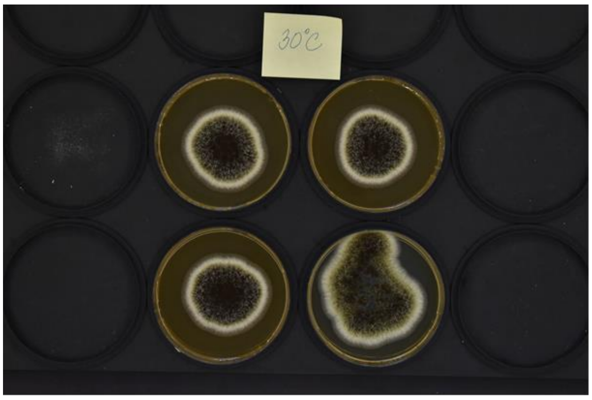

Fig. 3. *A. brasiliensis* on MEA, software raw input example

Software input is shown in **Figure 3.** Here can we see the presence artifact that are needed to by automatically remove for accurate evaluation also in one picture there is multiple samples that are needed to be labelled and separated. This is enabled by machine-learning based on previous training. Output example is shown in **Figure 4**.

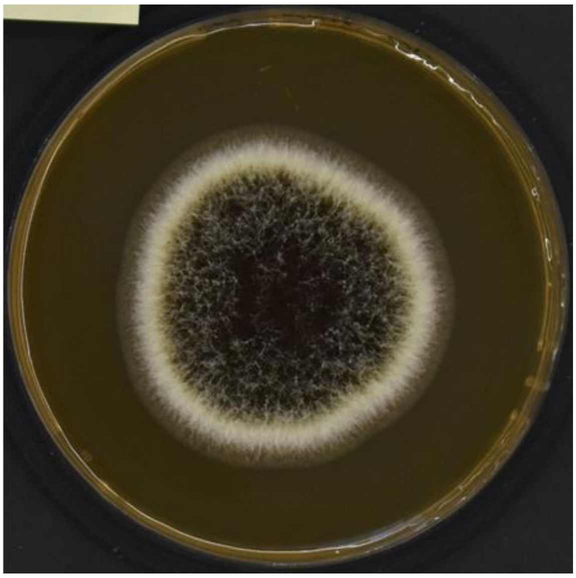

Fig. 4. *A. brasiliensis* on MEA, software preprocessed input example

After first image preprocessing there are still some artifacts that are needed to by remove such as paper note or the edge of dish. After that the program uses Neural network to transfer image to probability field represented on following **Figure 5**.

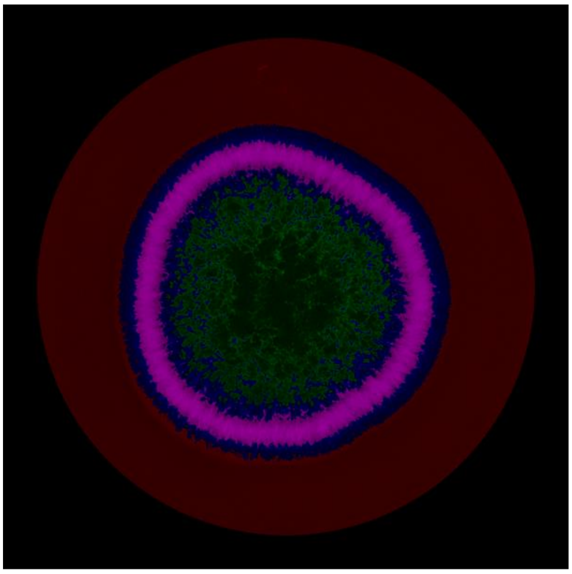

Fig. 5. *A. brasiliensis* on MEA, software proceeds output example as probability field

The outcome of neural net processing is probability field displayed in **Figure 5.**, where we can see the relative confidence of classification for each pixel from preprocessed image represented as the intensity of colour group. This can be easily transformed to final output by assigning pixels to particular grope based on highest probability as shown in **Figure 6**.

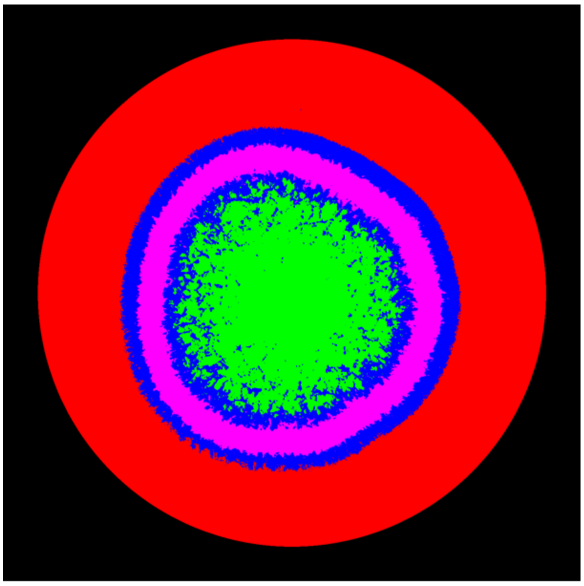

Fig. 6. *A. brasiliensis* on MEA, software final image output example

In last step of image separation, we get precise pixel count of each colour grope that represents different stages of mould grow, that is basically the relative area. We can compere relative areas between each other over time to get accurate date about effects of our treatment methods.

One of the key benefits of the automatic image analysis software is the elimination of human observer bias. The use of machine learning algorithms to perform image analysis ensures that the results are free from human error, providing a more objective evaluation of the data.

The software also offers the advantage of being able to perform the evaluation by multiple evaluators, reducing the dependence on a single evaluator and minimizing subjective errors. The ability to have multiple evaluators work on the same dataset is especially valuable in large-scale studies, as it helps ensure the reliability and consistency of the results. This also allows for multiple evaluations to be performed, providing a more robust evaluation of the data.

4. Conclusion

In conclusion, this study highlights the potential of NTP treatments as an effective alternative method for suppressing the growth of *A. brasiliensis*. The use of the machine learning-based image analysis software in combination with automatic image analysis provides a valuable tool for the study of mould growth and the assessment of control methods. The image analysis software used in this study was developed by the research group and integrated machine learning algorithms to perform image segmentation and labelling.

5. References

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