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DECONTAMINATION
SENSING

Real-time monitoring of hydrogen peroxide vapour decontamination of bacterial spores by means of UV fluorimetry

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Abstract. This study is devoted to the development of a UV fluorimetry sensor capable of real-time monitoring of the decontamination process of microbiological pathogens by hydrogen peroxide vapour (HPV) treatment. The sensor is operating on the autofluorescence signal of tryptophan. The *Bacillus atrophaeus* (*B. atrophaeus*) and *Geobacillus stearothermophilus* (*G. stearothermophilus*) spores were exposed to HPV and the resulting dynamic change in tryptophan fluorescence intensity as a function of time was recorded and analysed. It was revealed that the introduced HPV atmosphere caused a 4-time decrease in the fluorescence intensity of the tryptophan emission due to the interaction of HPV with the spores. It was shown that achieving a persistent minimal level of the autofluorescence signal due to the microorganism-bound tryptophan during the defined time period is well correlated with the efficiency of the ongoing decontamination process in the HPV treatment course. Therefore, the progress of the HPV decontamination procedure can be firmly evaluated, using fluorescence data obtained in real time and the validity of the method was demonstrated by comparing the fluorescence data with the reference information obtained by implementing classical microbiology viability tests (incl. time behaviour) for various microorganisms in the HPV atmosphere.

Key words: hydrogen peroxide vapour, decontamination, fluorimetry, sensors, *Bacillus atrophaeus*.

1. INTRODUCTION

Microbiological decontamination of enclosed spaces by using hydrogen peroxide vapour is a well-established process [1,2], which is much more effective than applying H_2O_2 aerosol [3] and, thus, being able to decontaminate every surface that has contact with the air in the room. The common procedure to check the success of the decontamination efforts is to position biological indicators in various representative locations in the room before its treatment. The typically used biological indicators are spores of different Class I bacteria, such as *Geobacillus stearothermophilus* or *Bacillus atrophaeus* (for example in MesaLabs APEX BIs [4]). At the end of the decontamination procedure, these indicators are collected and then placed into a growth culture medium for checking the viability of the spores after the treatment. When also taking into account the uncertainty caused by inherent variations in microbial counts, up to seven days (although typically 24 h–48 h) are needed to provide a confirmatory result of the bacterial growth. Obviously, for practical purposes the decontamination process monitoring would benefit from real-time feedback on the process efficiency as well as from feedback upon its

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completion. The current state of the art is to use online HPV concentration measurement [5], but using signals from proven biological simulants can be a viable alternative providing verified evidence. Such an approach can even have advantages because an optical fluorimetric sensor can in principle have a faster reaction time compared to the common capacitance-based HPV gas sensor, and the autofluorescence of an easily replaceable spore sample (or other active biomaterial) is a direct indication of the influence of HPV on pathogens rather than HPV concentration itself.

The autofluorescence signals of microorganisms have been used not only for distinguishing between different microorganisms [6] but also for monitoring the process of cell death by using cell-extracted reduced nicotinamide adenine dinucleotide (NADH) [7,8]. Liang et al. [7] have explored the experimental possibility to quantify the HPV lethal influence directly on microorganisms during the ongoing HPV decontamination process. Although it is known that fluorescence lifetime imaging of NADH inside living cells gives a good indication about the metabolism of cells [9], the intensity of NADH fluorescence (excited by 340 nm photons and monitored at 460 nm, designated below as Exc/Em) itself is approximately two orders of magnitude weaker than that of the prevalent tryptophan-related fluorescence peak under ~280 nm excitation and monitoring at ~330 nm emission. Also, the NADH signal is detected in the same spectral region as the autofluorescence signal of some B-vitamins, making it less attractive for use in sensorics [10]. Finally, the biochemical extraction of NADH before recording the fluorescence signal is required, which makes the whole process not suited for real-time monitoring.

In contrast to NADH, the well-studied fluorescent amino acid tryptophan needs no extraction and gives much higher signals, which are highly dependent on the local micro-environment (see [11] and references therein for more information on the fluorescence properties of tryptophan). In the current study, the dynamic behaviour of the tryptophan fluorescence intensity during HPV decontamination was investigated for the first time in the course of the development of a novel fluorimetric sensor for real-time monitoring of a decontamination process. The knowledge published already earlier and our original experimental results presented in this paper served as a basis for the sensor development, using a methodology not applied so far. Firstly, it is known that hydrogen peroxide treatment results in the quenching of tryptophan fluorescence [12]. Secondly, it has been demonstrated that the local-environment-caused variations of the tryptophan emission spectra related to microorganisms enable the distinction between surfaces contaminated by bacteria from non-contaminated areas on solid surfaces [13]. Previous studies have shown that the spores are biologically dormant and there is not much NADH-related metabolism activity which could be revealed spectrally [14]. For the above-mentioned reasons, it is the tryptophan-related fluorescence that was studied by the authors to be implemented in the fluorimetric sensor for real-time decontamination monitoring.

The reported research is focused on two areas:

(1) reporting on the study of the long-term static effect, aimed at measuring the sample autofluorescence before and after the HPV treatment;
(2) the investigation of the short-term dynamic effect, the goal of which is to monitor the behaviour of the autofluorescence signal during the HPV decontamination process in real time. In order to study the long-term static effect, it was essential to compare the performance of several spectral devices with different detection capabilities as well as to understand the autofluorescence response of samples. This first study pointed out the relevance of a real-time approach and conditions necessary for such a sensor. Therefore, it is an integral part of the sensor development reported in this paper.

During this study further development of the H2BM fluorimetry device was accomplished. It is not designed for precise determination of pathogen concentrations on surfaces at the low-value sanitary limits, but the authors demonstrate that it can be applied in real-time monitoring of the HPV decontamination process by using the autofluorescence signal of model pathogens.

2. MATERIALS AND METHODS

The HPV device VCS-100 [15] developed by Cleamix Oy was used in the present decontamination studies (Fig. 1a). It is known that high concentration of HPV can be applied to sterilize litre-sized volumes in seconds [16], low concentration of HPV is applicable for house-sized volumes on a day scale [17] and HPV treatment can be used even to sterilize Class 3 pathogen experimental areas [18]. But in the current research the HPV device was opted to be most efficient on the time scale of minutes to hours, applied for room-sized test enclosures. The VCS-100 device vaporizes the concentrated H_2O_2 solution into the experimental enclosure up to a pre-set concentration and for a pre-set decontamination time. The device has a pump, a vaporizer and multifunctional sensors for real-time temperature, relative humidity and H_2O_2-concentration measurement. The device has also a combined saturation sensor, showing how far the room air is from actual condensation during the HPV decontamination procedure. The VCS-100 can be remotely controlled over WiFi, using a tablet PC outside the room, so that no hazardous materials suit is needed for the

controller who starts, monitors and stops the HPV decontamination process.

The hand-held bio-detector H2B [19] developed by the authors was specifically modified for monitoring the spores' fluorescence in the HPV decontamination experiments by continuous measurements with a pre-set time interval. The biodetector H2B (see Fig. 1b and Fig. 1c) is a lightweight hand-held fluorimeter device weighing approximately 0.5 kg. In the initial phase of planning it was developed to locate the surface areas characterized with high microbial contamination in the industrial environment as well as for guiding the decontamination procedures. It could also be applied to the random check of bio-contamination of equipment and facilities by first responders responsible for bio-decontamination activities.

In the H2B device the sample surface under monitoring is installed at a fixed distance of less than 5 millimeters from the device's optical window, so that the air in the room can flow between the sensor and the sample surface, without most of the background light entering the detection region. The device synchronously excites the sample with 280 nm UV radiation and collects the fluorescence emission at ~330 nm, automatically subtracting the background light in the room during each measurement. Both the fluorescence excitation radiation and the fluorescence emission light to be detected are spectrally selected by high-quality OD6 optical bandpass filters. The sample surface is optically excited within an area of ~2-mm diameter by the UV-LED. The mobile device is fully wireless, containing a battery that enables unplugged operation for several hours, and the recorded data is transferred over WiFi to a computer in real time. The microwatt-level 280 nm excitation LED operates at minimum power and it performs one 1-millisecond exposure every 10 seconds to minimize the possible influence of the UV radiation's own harmful effect on the viability of the microorganism sample, which may interfere with the effect to be monitored during the HPV decontamination process. The device also has a LCD screen showing the most recent measurement result. The low-level fluorescence light signal detection is achieved by using a temperature-compensated highly sensitive silicon photomultiplier (SiPM) detector.

To be characterized for the monitoring application in the given conditions, the H2B sensor was adapted for continuous real-time fluorescence measurements of the bio-layer on a sample disk under the influence of HPV. The modifications included the integration of the device in the experimental laboratory setup to maintain the H2B sensor at a fixed distance from the spore sample and enable air flow over the spores. Additionally, a change in the H2B device firmware was made to measure the fluorescence continuously (rather than triggered by button press in the hand-held mode). Finally, the continuously

(a)

(b)

(c)

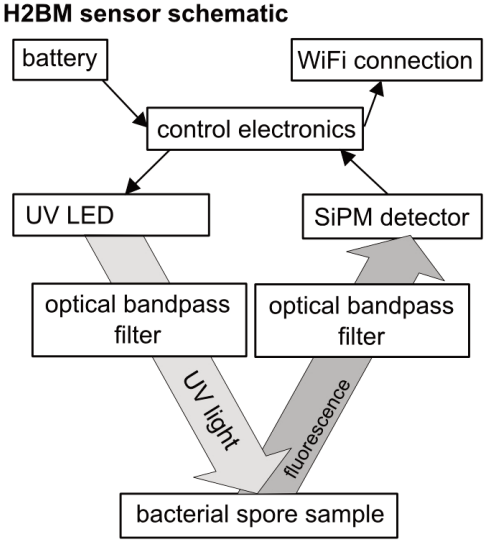

Fig. 1. Main devices used in the research: (a) hydrogen peroxide vapour generation device VCS-100; (b) modified hand-held fluorometer H2BM device for online monitoring of the decontamination process; (c) block scheme of the H2BM device and its operation principle.

operating fluorimetric sensor H2BM (H2B modified) was developed and integrated into the same laboratory experimental setup.

The sensing (excitation) and emission wavelength ranges of the H2BM were finally selected based on the initial long-term experiments on the pathogen-simulating samples by implementing the spectrofluorometer SFS-Cube [20]. The SFS-Cube device is a compact scanning spectrofluorometer designed for field measurements of solid, powder and liquid objects. It records signals from the samples under investigation and performs analysis of the Spectral Fluorescence Signatures (SFSs) represented as a matrix of the fluorescence intensity in the coordinates of the excitation and emission wavelengths with the filtered out Rayleigh scattering signal [13]. The composite shape of the detected SFS undergoes the spectral deconvolution and the identified patterns are compared with the reference SFS library. The device is based on two fast scanning monochromators with a 20 W Xe flashlamp excitation and the photomultiplier tube (PMT) detection system. The SFS of the studied objects is obtained by using the excitation wavelength range from 230 nm to 350 nm and analysing emissions in the wavelength range from 250 nm to 565 nm with 5nm step. During the initial experiments the device was used to study fluorescence and analyse the difference of SFSs before and after the HPV exposure to pathogen-simulating spore samples dried on a stainless steel plate. It allowed identifying the optimal excitation/emission wavelengths and ranges for the H2BM design. Compared to the H2BM sensor, the SFS Cube spectrofluorometer is more versatile for the spectral analysis but its response time of up to 3 minutes is much longer than the instant detection speed (< 1 second) by the H2BM. Due to the much higher measurement speed, the H2BM device was further used in the online monitoring of HPV decontamination experiments.

The pathogen-simulating samples used during the decontamination process were either *G. stearothermophilus* or *B. atrophaeus* spores at around 2×10^6 CFU/cm^2 surface concentration on thin stainless steel plates. Both of these bacterial spores are known to be relatively resilient to various decontamination procedures and, therefore, can be used to reveal the worst-case scenario in the case of real contamination. The performed measurements demonstrated that the studied spores were sufficiently resilient to show growth after almost a 10-minute exposure to the 400 ppm HPV environment.

The decontamination experiments were carried out either in an enclosure with one-cubic-meter volume or in a big indoor tent (Fig. 2), where the HPV device created the 400 to 600 ppm H_2O_2 atmosphere for different exposures with durations from 30 minutes to 4 hours. The samples were exposed in these experimental volumes and the autofluorescence was monitored before, during and after the decontamination process by the H2BM sensor. After the decontamination procedure, the remaining HPV containing atmosphere was completely evacuated from the enclosure, so that it would be safe for humans to open the door or enter.

(a)

(b)

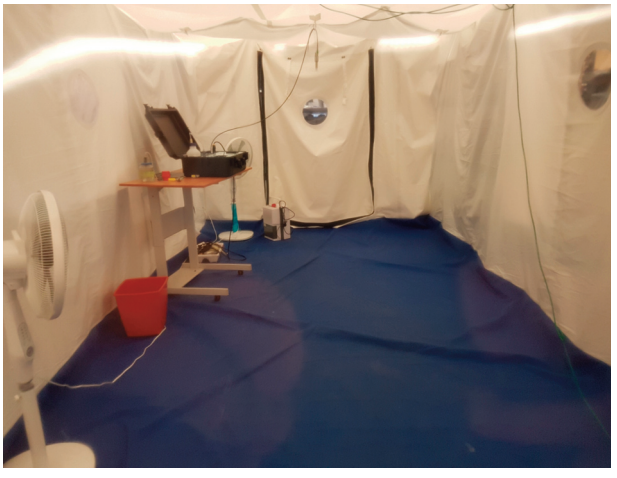

Fig. 2. The experimental enclosures used in the experiment: (a) one-cubic-meter stainless steel enclosure for online device testing; (b) the internal view of the larger 36 m^3 tent used on Cleamix Oy premises for long-exposure decontamination tests.

The fluorescence measurements by the SFS-Cube and the H2BM were conducted in the front-face mode by placing the sample disk of stainless steel with the controlled surface concentration of spores into the excitation light beam of the (spectro)fluorometer and recording the emitted autofluorescence light level. All fluorescence measure-

ments were performed at room temperature. The background fluorescence signal intensity of the stainless steel substrate was recorded before making a new series of measurements from the sample. To perform measurements in the given conditions, this background signal intensity was kept at the level of less than 5% of the expected sample fluorescence signal, which was achieved by re-cleaning the sample substrate, if needed.

3. RESULTS

As stated above and discussed in [13], the autofluorescence signal of the mainly tryptophan-related spectral region (Exc/Em 280/330 nm) is used in this study because the fluorescence emission intensity (caused by the conjugated π-system of the indole ring) in tryptophan is largely dependent on the micro-environment around the tryptophan molecule [21] and can (among other things) be used to monitor protein folding and unfolding. The intensity of the tryptophan-emission region (Exc/Em 280/330 nm) of pathogen fluorescence showed two major effects during decontamination with HPV – the long-term static decrease and the short-term dynamic change in the signal (see the graph in Fig. 3 – the dashed red line shows the long-term static effect and the black line with blue points illustrates the short-term dynamic effect). The first one is characterized by a persistent decrease (i.e. fall-off) of the signal intensity, which indicates a permanent oxidative damage of the spores. However, the recorded signal intensity falls much slower than the death rate of the bacterial spores determined by a classical microbiology method. Table 1 shows the corresponding data from our reference study with the 400 ppm HPV decontamination process, where the survival rate was evaluated by using the classical microbiological growth method. At 600 ppm HPV concentration, which was used in the short-term effect experiments reported, the growth is even more prohibited. Comparison of the data in Table 1 and Fig. 3 shows that the decontamination process was

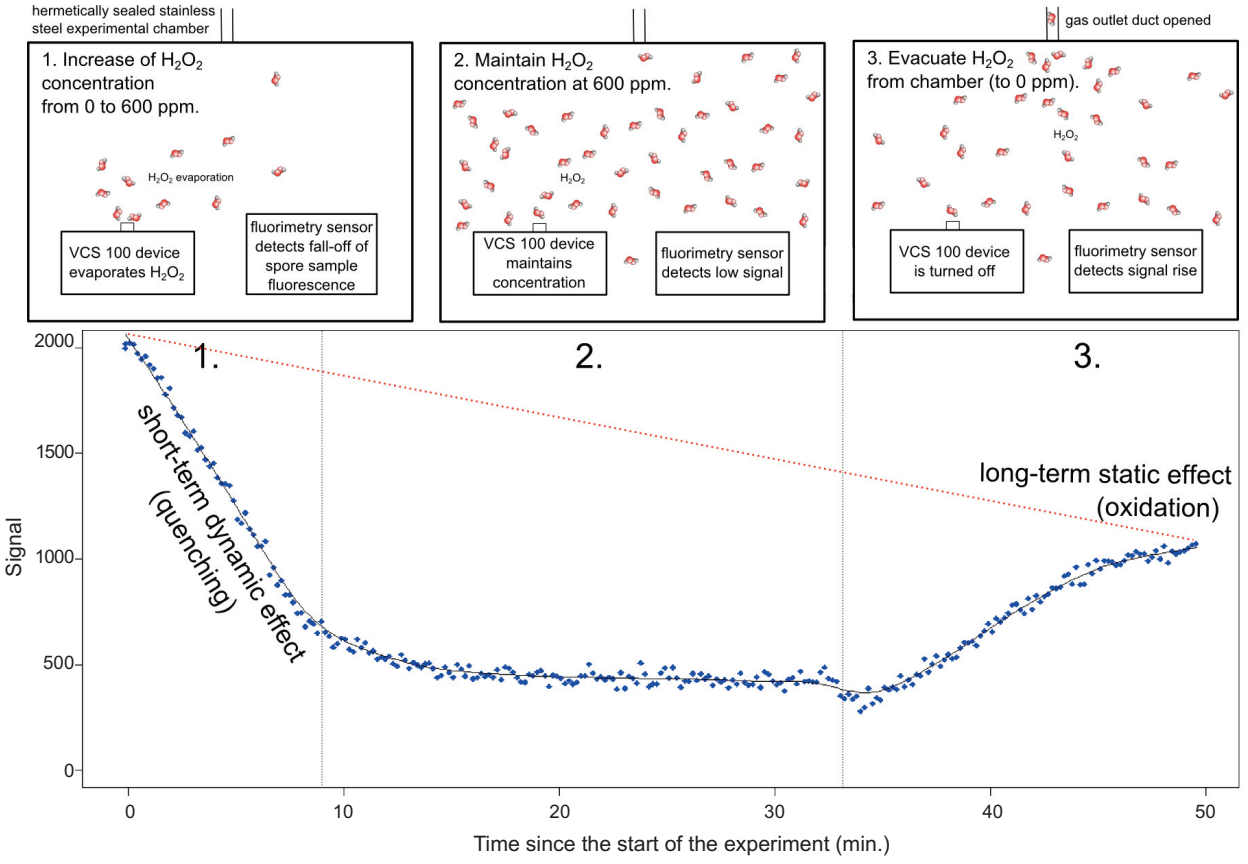

Fig. 3. Three stages of the HPV treatment are shown with relation to the short-term dynamic effect and long-term static effect. At the 1st stage the HPV concentration is increased by the VCS 100 device and the fluorescence signal from bacterial spores falls rapidly (the short-term dynamic effect). At the 2nd stage the HPV concentration stays constant and one can see a slower decrease in the fluorescence signal. At the 3rd stage the HPV concentration returns to zero and the fluorescence signal from spores rises again, but not to the same level at which it was before the experiment. This remaining difference between fluorescence signal before and after the experiment is the long-term static effect.

Table 1. Survival rate of *G. stearothermophilus* exposed to 400 ppm HPV in the tent (Fig. 2.b) as a function of time, using the classical microbiological growth test as a reference study

Exposure time (min.)	Growth indicator	Cell count (CFU/mL)
1	Colour change	3290
2	Colour change	3690
3	Colour change	275
4	Colour change	115
5	Colour change	10
6	Colour change	Not detected
7	Colour change	Not detected
8	Colour change	Not detected
9	Almost yellow	Not detected
10	Slight change	Not detected
11	Slight change	Not detected
12	Slight change	Not detected

completed within ~5–10 minutes (300–600 s), whereas a long-term intensity change, as indicated in Fig. 4, becomes bigger than the standard deviation of different samples measured only after ~1 hour has passed from the beginning of the decontamination process. This implied that a more detailed study is needed during the decontamination process, which would lead to the discovery of the second effect which has a short-term dynamic nature.

3.1. Long-term static effect of the decontamination process

The long-term decontamination experiments were carried out in an indoor tent on Cleamix Oy premises (see Fig 2b). Along with fluorimetry experiments the classical microbiological growth tests were performed. There was no indication of survival after 10 minutes of HPV exposure at the relatively high concentrations used in these experiments and, therefore, no numerical results on spore survival in such experimental conditions are reported in this paper. The main focus was on the behaviour of fluorescence of the spore samples, which was monitored in parallel by using both the SFS-Cube spectrofluorometer [20] and the single-channel H2BM fluorometer before and

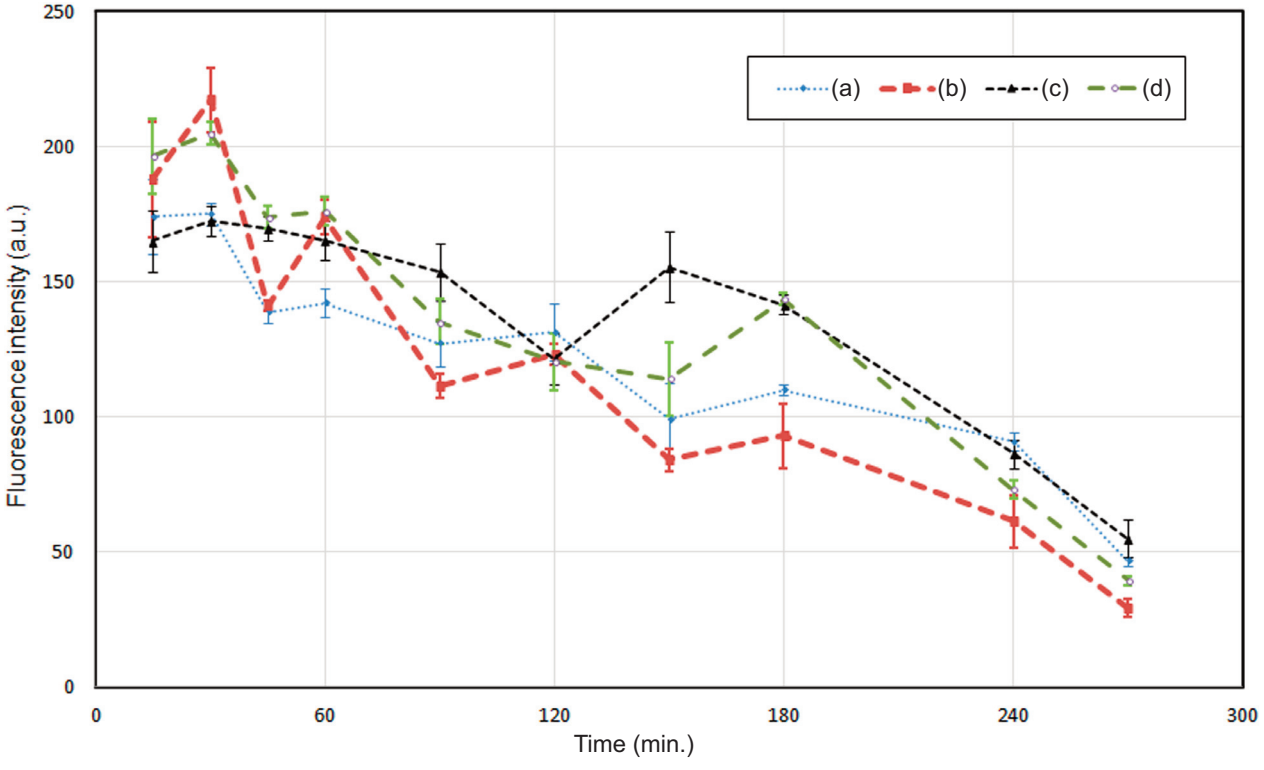

Fig. 4. Persistent fall-off of tryptophan fluorescence peak at ~280 nm / 330 nm after the long-term HPV decontamination procedure studied by using the response of *G. stearothermophilus* spores: (a) spectrofluorometer measurement result for unwrapped sample; (b) H2BM fluorometer result for unwrapped sample; (c) spectrofluorometer result for unopened sample; (d) H2BM fluorometer result for unopened sample. Wrapping in Tyvek pouch does not hinder the effect of HPV.

after the HPV decontamination procedure. The response of the spores was characterized by a slow spectral fall-off of the fluorescence intensity of tryptophan analogously to a smaller test volume. By the time all model pathogens are supposed to be dead (5–10 min. after the start at 400 ppm according to the classical microbiological study, see Table 1), there was no discernible (above noise level) decrease in the tryptophan fluorescence peak intensity (see Fig. 4). A much longer timeframe, of almost one hour, was needed to achieve the easily identifiable fluorescence signal fall-off, thus making this approach at first glance less attractive to monitor a typical (time-limited) decontamination process before and after HPV exposure.

In the static effect experiments the response of *G. stearothermophilus* spores was studied inside the standard Tyvek pouch, which is used by the manufacturer when shipping indicator disks [4], as well as outside the pouch to ensure that it transmits HPV comparably well to the uncovered substrate plates (Fig. 4). To make sure that there is no device-related measurement bias, two different device types were used to record the persistent spectral fall-off. Namely, the SFS-Cube spectrofluorometer and the modified H2BM single-channel fluorometer were used in monitoring tryptophan fluorescence intensity before decontamination and then at various time moments during the decontamination process, taking them out of the HPV atmosphere for measurement. Preliminary research indicated that there was a rather large variability of surface concentration between different unopened samples and to exclude such effects, three samples were measured at each time point. Such studies have revealed that the samples do not quite behave uniformly and are variable from one sample to another. Therefore, the signal levels recorded show rather large standard deviations but the overall signal fall-off is still clearly observable, independently of the spectral device used and the sample preparation (in the Tyvek pouch or bare substrate). Although experiments established that tryptophan fluorescence intensity was observed to be falling at a slower rate in comparison to the microbiological reference data, it was expected that even such fall-off could be detected with a sufficiently sensitive fluorosensor and implemented in a real-time online monitoring device.

3.2. Short-term dynamic effect

Further experiments were carried out inside a specially designed experimental chamber consisting of one-cubic-meter stainless steel box (Fig. 2a). These experiments revealed a remarkably well-defined result – the fluorescence intensity started to fall immediately after turning on the hydrogen peroxide vapour generator. The fluorescence intensity signal typically decreased up to ~3 times in 10 minutes (600 s) and ~5 times in 30 minutes (1800 s) at the exposure to 600 ppm HPV. Such an effect could be easily used to monitor a decontamination process, since the initially almost perfect exponential fall-off allows estimating the viable vs dead spore concentration. The experimental curve is reasonably well approximated to an exponential decay function with the time coefficient ~200 s (Fig. 5b), which is the time span characterizing spore death during the decontamination process in the given conditions.

In order to establish the repeatability in the monitoring of the decontamination process, extensive experimental research was carried out. During a series of experiments (some selected ones are shown in Fig. 5a) it was detected that under the predetermined conditions: the given surface concentration of the spores (~10^6 CFU/cm^2), HPV concentration and treatment duration (~30 min. in this case), properly conditioned substrate (ultrasonically cleaned stainless steel), etc., the process monitoring was highly repeatable. The fluorescence fall-off rate was observed to be correlated with the concentration of the hydrogen peroxide vapour applied in the decontamination process.

As expected from the results of the initial persistent fall-off experiments, the fast-falling fluorescence signals began to rise immediately after the concentration of HPV was reduced, when the experimental chamber was refilled with clean air (~ from 600 ppm to 0 ppm in 10 min.). It is noticeable that the fluorescence intensity never recovers to the initial level but remains at the lower value (Fig. 6).

4. DISCUSSION

The initial quick decrease in tryptophan fluorescence intensity was observed to have a timescale similar to the decrease of pathogen viability (~10 min.) determined by classical microbiological methods (see Table 1 and e.g. [4]). Although the discovered dynamic recovery of fluorescence in short-term experiments does not reduce the usability of the observed effect in real-life monitoring, further investigation is required to estimate the living-to-dead ratio of pathogens. Also, Cavatorta et al. have detected [12] that the fluorescence of tryptophan itself becomes quenched in the presence of hydrogen peroxide. For estimating the role of such quenching contribution, pure L-tryptophan was purchased from Sigma-Aldrich and it was studied under the exact same conditions as were the spore samples. The surface concentration of tryptophan was chosen close to 5 micrograms per square centimetre to match the signal intensity with the spore samples in order to make it comparable with the results of our previous studies.

As can be seen in Fig. 7, the fluorescence intensity of micro-organism-bound tryptophan falls much quicker

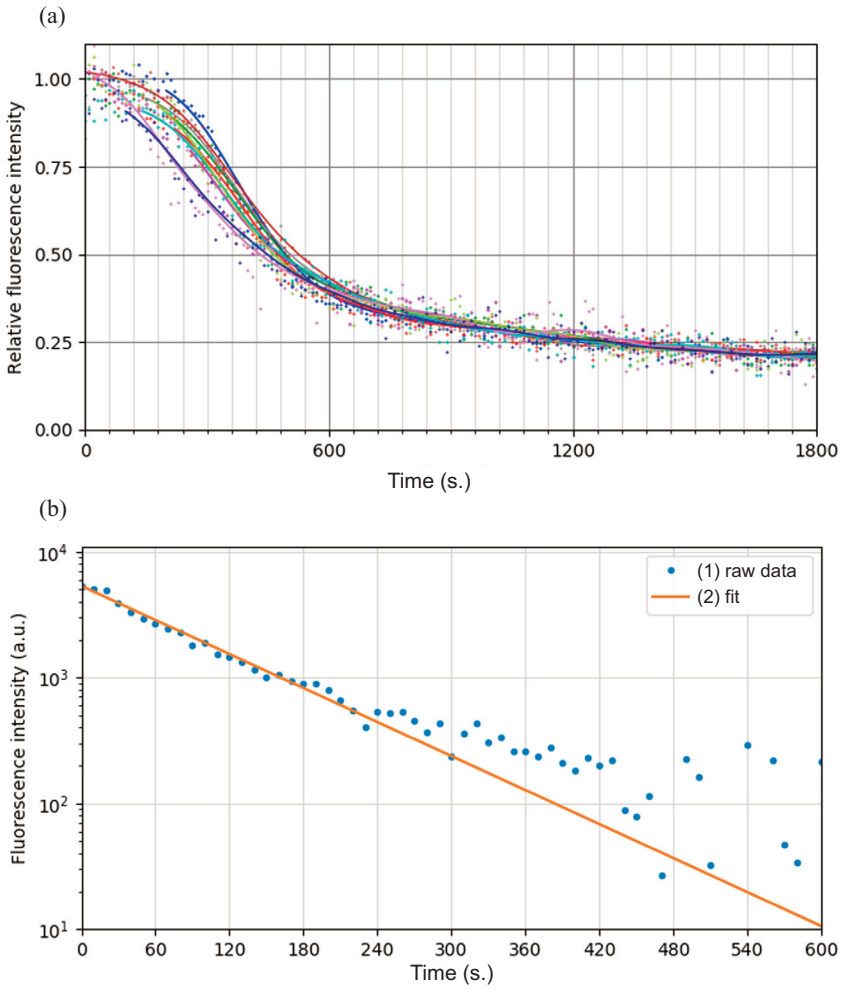

Fig. 5. Online monitoring result of tryptophan fluorescence peak intensity of *B. atrophaeus* spores, using the H2BM fluorometer is illustrated: (a) repeatability of HPV treatment at 600 ppm; (b) initial decrease in fluorescence peak intensity can be approximated by a single exponential decay function (to a plateau level).

compared to pure tryptophan. This indicates that the decrease in tryptophan fluorescence in the spores studied is caused by the influence of hydrogen peroxide on the living cells, thus likely indicating the changes in the cell structure, functioning and viability. The exact mechanism of HPV-induced killing and the resulting fluorescence signal change in *B. atrophaeus* spores are still to be clarified. A possible hypothesis suggests that treatment by high HPV concentration can lead to the damage of cell walls, thereby modifying the walls' protein composition or even inducing a leakage of cellular components, including tryptophan. Such a process can be similar to the influence of acids on the spores, as shown in [22]. The increase of tryptophan fluorescence intensity after HPV termination can be caused by renaturation of the native protein structure. Another explanation for the increase may lie in the fact that tryptophan is hydrophobic by itself and the hydrophilic H_2O_2 molecule can be adsorbed or diffused more easily into the adjacent more hydrophilic parts (e.g. other amino acids building a protein) of the spores. As a result, the hydrogen peroxide molecules position themselves close to the tryptophan molecules, allowing energy transfer [23] between the species and facilitating the quenching process. It cannot be excluded that some other unknown effect on micro-organisms may increase the fall-off rate of tryptophan fluorescence. The increase in tryptophan fluorescence after HPV treatment requires further studies.

5. CONCLUSION

In this study the sensor prototype – a modified H2B fluorometer developed by LDI Innovation – was used in

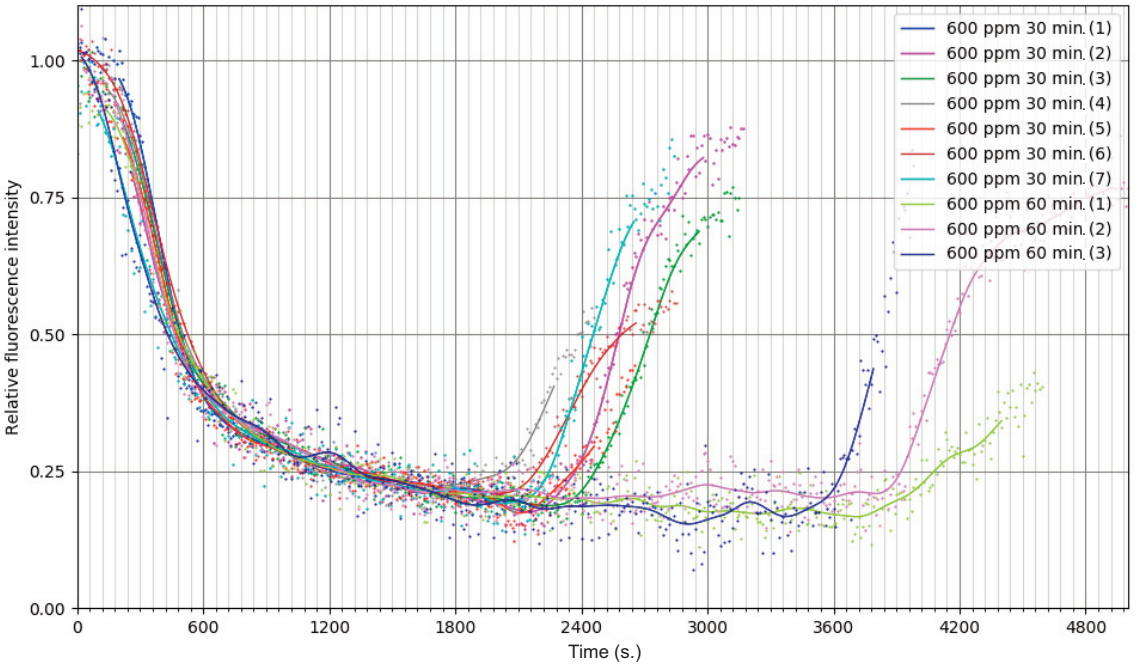

Fig. 6. Online measurement results of tryptophan fluorescence signal intensity of *B. atrophaeus* spore samples, which show an immediate increase in the signal level after lowering the concentration of HPV in a test volume.

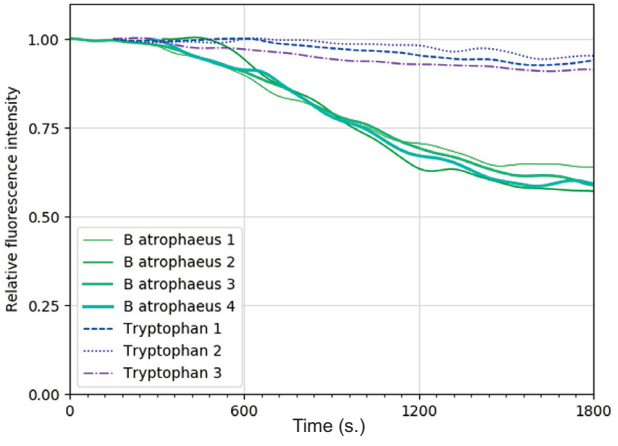

Fig. 7. Fluorescence signal fall-off in pure L-tryptophan versus *B. atrophaeus* spores under equal experimental conditions (partial pressure of HPV) and at comparable signal levels.

integrated operation with the Cleamix VCS-100 decontamination device to monitor the quick fall-off of the fluor-escence signal of the micro-organism bound tryptophan, enabling a path for photonic sensors to analyse the efficiency of HPV decontamination processes via measuring spore autofluorescence. More spectral information on the behaviour of living cells is still to be collected in further studies and additional correlations as well as HPV concentration dependence have to be identified to reveal the death of micro-organisms. This is a prerequisite for the creation of a reliable quantitative online indicator device to monitor the HPV decontamination process. According to the present knowledge obtained in this research, tryptophan fluorescence surely provides a quick response to the HPV treatment influencing the pathogens and can, therefore, be used as a reliable single channel sensing technique to monitor the decontamination procedure in real time. Our study has revealed that the recorded experimental data, where the autofluorescence signal reached a persistent minimal level during a defined time period, and its comparison in real time with the obtained reference information on the survival behaviour of micro-organisms in the HPV atmosphere provides reliable identification of the fully completed decontamination process.

DATA AVAILABILITY

The raw data is available at: https://drive.google.com/file/d/1cg9x_PstejmRoI7yU27ARupe1KHaiKmW/view?usp=sharing

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Vesinikperoksiidaurul põhineva bakterispooride puhastusprotseduuri seire reaalajas UV-fluoromeetri abil

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Käesolevas uurimistöös kasutati modifitseeritud H2B fluoromeetri prototüüpi (LDI Innovation OÜ) koos VCS-100 vesinikperoksiidauru generaatoriga (Cleamix Oy), et seirata patogeensete mikroorganismide sisese trüptofaani fluorestsentssignaali intensiivsuse kiiret vähenemist vesinikperoksiidi mõjul. See nähtus võimaldab luua fotoonika sensorid, millega oleks vesinikperoksiidaurul põhineva puhastusprotseduuri efektiivsust võimalik reaalajas mõõta. Uurimistöö eksperimentaalosas paigutati *B. atrophaeus*'e ja *G. stearothermophilus*'e bakterispoorid vesinikperoksiidauru sisaldavasse keskkonda ning mõõdeti nende autofluorestsentssignaali muutust aja jooksul. Leiti, et vesinikperoksiidi (~400 ppm) lisamine atmosfääri, milles bakterispoorid asusid, vähendas lühikese aja jooksul nende fluorestsentssignaali ligi neli korda. Fluorestsentsi kustumise jälgimine koos protseduuri kestuse infoga võimaldab ühe uue meetodina hinnata patogeenide hävitamise vesinikperoksiidpuhastusprotsessi efektiivsust.