Vapourised hydrogen peroxide for disinfecting historical objects from the Auschwitz-Birkenau State Museum in Oświęcim

The collections of the Auschwitz-Birkenau State Museum in Oświęcim, Poland (A-BSM) have over 200 000 objects including 3.8 thousands of suitcases dated to the Second World War. Some of the suitcases are stored in the storage warehouses and the rest are presented on the exhibition available to visitors. Protection and conservation of such objects cause major both methodological and technological problems. One of the stages of conservation treatments is the disinfection of objects. Due to the need to replace the previously used method with ethylene oxide (EtO), which is on the list of carcinogenic substances, new and safe method of disinfection is being sought, which will be applicable to mass quantities of museum objects. Vapourised hydrogen peroxide (VHP) in doses adapted to the historical material providing biocidal effectiveness and limiting the adverse impact on historical material, may be an alternative disinfection method. There are no scientific reports on the possibility of using VHP in museology and research on the impact of VHP on porous historical materials taking into account particularly historical cellulosic materials, therefore new research, that is the subject of this work, has been started.

The aims of the study were to assess the microbiological contamination of historical suitcases from the Auschwitz-Birkenau State Museum in Oświęcim, to assess the impact of VHP disinfection on microorganisms colonising historical objects and on the condition and also optical, structural, morphological and chemical properties of new technical materials and historical objects taking into account writing media. The goal was to establish the parameters of VHP disinfection that will be effective and safe for the historical suitcases and can be used for mass disinfection of A-BSM collections.

The materials used in the work were both historical suitcases and their replicas, as well as new technical materials corresponding to the materials of which historical suitcases are made (fabric, vulcanised fibre, cardboard, wood, paper, leather, leather-like material and metal) and writing media (printing inks, pencils, crayons, stamp ink, inks and paints). In order to observe changes in condition and colour that may occur in disinfected objects after a longer time of storage, an accelerated ageing process was applied. Microorganisms isolated from the surfaces of historical suitcases stored in the A-BSM storage warehouses and strains from the ŁOCK 105 (Łódź Center of Pure Cultures) Pure Culture Collection were the biological material for model tests. VHP disinfection was carried out in a model chamber and in real conditions in one of the buildings in the A-BSM. The tests were also performed after the application of EtO fumigation, to compare the results. The microbiological contamination of the suitcases was determined by the culture-dependent and metagenomic methods, whereas the effectiveness of VHP disinfection by the culture-dependent method. The impact of VHP and EtO disinfections on the condition and also optical, structural, morphological and chemical properties were assessed with visual analysis, spectrophotometric colour measurements, scanning
electron microscopy (SEM), infrared spectroscopy (FTIR), size-exclusion chromatography (SEC), X-ray photoelectron spectroscopy (XPS) and X-ray fluorescence (XRF).

The microbiological contamination of the 50 historical suitcases ranged from $10^2$ to $10^6$ CFU/100 cm$^2$, it was $4.6 \times 10^4$ CFU/100 cm$^2$ (bacteria) and $7.5 \times 10^3$ CFU/100 cm$^2$ (fungi) on average. Most of the tested suitcases (64%) showed medium level of microbial contamination and 14% high level. On the surfaces of historic suitcases and their replicas mainly gram-positive sporulating bacteria belonging to the genera Bacillus (*B. atrophaeus, B. licheniformis, B. simplex*) and *Lysinibacillus fusiformis, Paenibacillus provencensis* and *Psychrobacillus psychrodurans* were identified. The most frequently isolated filamentous fungi included the genera: *Alternaria, Aspergillus, Cladosporium, Epicoccum, Fusarium* and *Penicillium*. The metagenomic analysis allowed detection of additional bacteria belonging to the genus *Janthinobacterium* and fungi belonging to the genera: *Acremonium, Aureobasidium, Boeremia, Coprinellus, Cosmospora, Debaryomyces, Mycosphaerella, Naganishia* and *Wallemia*.

It was found that the diversity of microorganisms colonising historical objects depends on the type of material from which they were isolated, the highest biodiversity was shown on the cardboard. Among the microorganisms isolated from the A-BSM suitcases there were cellulolytic and pathogenic species.

The following optimal parameters of effective VHP disinfection were established: concentration 300 ppm and time 20 minutes. VHP disinfection under optimal conditions caused a reduction in the number of microorganisms by 1.5-8.0 log (1.5-3.0 log for bacteria and 3.5-8.0 log for fungi), what corresponds to a reduction of 97-100%. The group of microorganisms that were the most sensitive (R=99.92-100.00%) to the VHP disinfection (300 ppm, 20 min) included all of the following bacteria and filamentous fungi: *Bacillus licheniformis, B. subtilis NCAIM B.01644, Cladosporium cladosporioides, Fusarium poae* and *Penicillium chrysogenum*. The least sensitive (R=97.17-99.97%) to the VHP were: *Micrococcus luteus, Alternaria alternata* and *Aspergillus niger*. It has been shown that in the case of monocultures, the effectiveness of VHP disinfection in optimal parameters (300 ppm, 20 min) is comparable to that of EtO fumigation, while the VHP method was characterised by lower than EtO efficacy against multispecies cultures. Both VHP in optimal parameters and EtO disinfection showed greater effectiveness against fungi compared to bacteria originating from historical suitcases stored in A-BSM. It was found that the results obtained for VHP are satisfactory in the case of disinfection of historical objects.

Disinfection with VHP (300 ppm, 20 min) do not affect the condition of samples of historical materials from the replica of the suitcase and writing media and also do not change the colour visible to the naked eye of the new and historical materials (ΔE*<2.0 CIELab units). A higher dose of VHP (700 ppm, 25 min), tested to reveal the possible direction of the changes only changed the colour visible to the naked eye of the new vegetable-tanned leather and new cardboard, but both values did not exceed ΔE*=2.6. SEM photographs and FTIR spectra confirmed that VHP disinfection (300 ppm, 20 min and 700 ppm, 25 min) do not affect the surface morphology and structure of the samples of both new and historical materials. There are no damages of fibres in the form of micro-cavities, micro-damages and cracks, and also no new bands associated with new functional groups that could modify the molecular structure of materials due to VHP disinfection. No significant changes occurred in the cellulose structure of new materials after VHP disinfection (300 ppm, 20 min) in the model chamber and also increasing the concentration of VHP to 700 ppm and an exposure time of up to 20 min did not result in statistically significant changes in the degree
of cellulose polymerization (DP) except the vulcanised fibre. In the case of historical materials VHP disinfection (300 ppm, 20 min) carried out in real conditions did not cause statistically significant changes of the degree of cellulose polymerisation.

Studies on the degree of cellulose polymerisation showed that control samples of historical materials, i.e. non-disinfected, were characterised by much lower DP than control samples of new materials. It is related to the natural degradation processes of cellulosic materials over time. XPS analysis showed that, on the surface of all tested historical materials, contamination, protective substances or dyes are present, forming a kind of a barrier to the biocide. Therefore VHP has an oxidising effect primarily on the surficial layers of historical material and does not interact directly with the cellulose structure. There were no significant changes in the percentage of particular elements in metal samples after VHP disinfection.

Accelerated ageing process did not cause differences in the condition of the majority of materials including writing media between samples disinfected with VHP and non-disinfected. None of the writing media blanched and smudged. Only lines of 2 pencils and 1 crayon, notes made with black ink and two oil paints changed colour after ageing. These changes has appeared both in non-disinfected samples as well as after VHP disinfection. After ageing, no differences were observed in the appearance of non-disinfected samples and samples after VHP disinfection, which means that disinfection does not affect the condition of samples after a longer period of time.

EtO disinfection resulted in an impact on the condition, colour, surface morphology, molecular structure of materials and also percentage of elements in metals similar to that of VHP. Both methods of disinfection have an oxidising effect on the surface layers of the material, but the force of interactions depends on the type of material. EtO disinfection in contrast to VHP caused a statistically significant change of degree of cellulose polymerisation of new cotton fabric, cardboard and historical wood. No differences between the condition, colour and percentage of elements of non-disinfected samples and samples after VHP and EtO disinfection were observed, both aged and not subjected to accelerated ageing.

It was found that the VHP disinfection method in a dose of 300 ppm and 20 minutes, which was analysed in this dissertation, may be an alternative to the used hitherto EtO fumigation in the case of surficial disinfection of new materials and historical objects, especially before conservation treatments, i.e. removing protective and undesirable substances. Application of this tested technique for historical objects should be preceded by an assessment of the condition of objects and an exclusion of the presence of materials other than those tested. Moreover, in doubtful situations, protection against direct action of VHP of areas with prints and inscriptions should be considered.