 DOI: 10.1002/masy.200650608

The Effects of UV Laser Radiation as Sterilizer for Cultural Heritage

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Summary: This work reports on a novel sterilization technique for bacteria which may be involved in deterioration of cellulose-based antiquities (paper, textile and wood). The bacterium we processed, a *Nonomuraea* sp. strain, was a mycelial microorganism belonging to the Actinomycetes group. This group includes many microorganisms that are able to attack cellulose and/or lignin. *Escherichia coli* was used as a reference microorganism. The UV source used as sterilizer was an excimer laser operating at 308 nm, 1 Hz. We demonstrated that, under different experimental conditions, the sterilization process was effective just after few seconds. For laser irradiation at 80 mJ/cm^2 per pulse, survival rate decreased of three orders of magnitude just after 75 s. The measurement of the absorption cross section for 308 nm photons on *Nonomuraea* sp. cells was also carried out.

Keywords: biodegradation; cellulose; cultural heritage; sterilization; UV laser irradiation

Introduction

Cultural heritage artefacts are exposed to the weather and submitted to the influence of several environmental factors. Physical, chemical and biological factors interact with constitutive materials inducing changes both in their compositional and structural characteristics. Important transformations are due to the metabolic activity connected with the growth of living organisms. In fact, several kinds of micro- and macro-organisms can find a suitable habitat for their growth either on monumental buildings, archaeological remains or other works of arts made with wood, textiles, paper, etc.

Strategies aimed at conserving and protecting artworks and monuments are rapidly adopting molecular techniques for the detection of microorganisms involved in the biodeterioration of cultural assets. Molecular biology techniques have been successfully used for detection of microorganisms in their environments. Currently, the detection of microorganisms from natural samples is mainly based on the sequences of the small subunit (16 S for prokaryotes and 18 S for eukaryotes) ribosomal RNA genes.[1]

The most harmful microorganisms for paper are bacteria, micro-fungi and Actinomycetes (both cellulolytic strains, damaging the chemical structure of paper, and non cellulolytic ones with non-specific action). Fungi are the most common because they show a great tolerance to environmental conditions and can live with lower water content than bacteria and Actinomycetes.

Paper samples are good sources of nourishment for heterotrophic microorganisms and organisms. The indispensable condition for a microbial attack is a high water content, and the hygroscopicity of paper makes it more sensible to the biodegradation. The chemical, enzymatic

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and molecular aspects of the bacterial attack of cellulose–based material represent the key step of deterioration.[2,3]

Paper is primarily composed of cellulose and other substances related to the origin of the raw materials used in its manufacture: lignin, hemicelluloses, pectins, waxes, tannins, proteins and mineral constituents.[4] Different oxidative enzymes (oxidoreductases) are involved in lignin degradation. These include laccases, high redox potential ligninolytic peroxidases (lignin peroxidase, manganese peroxidase, and versatile peroxidase), and oxidases.[5] During metabolism, many microorganisms produce different organic acids (oxalic, fumaric, succinic, citric, etc.), which reduce the pH of paper, conditioning the dynamics of bacterial and fungal growth in secondary attacks. Frequently, bacterial and fungal attack makes the paper felted and fragile.[2]

The present work is aimed at studying the feasibility of a novel sterilization technique for cultural heritage, by means of an excimer laser beam at 308 nm wavelength. Germicidal lamps, emitting at 254 nm wavelength, are usually employed for sterilization by irradiation. The choice to utilise a laser source has been suggested by the well-known laser beam "ballistic" properties, i.e. natural collimation, low divergence, high irradiance, and easiness in beam deflection and spot dimensioning. The region of the UV spectrum with germicidal activity ranges roughly between 300 and 200 nm, with a maximum of activity between 275 and 230 nm. Nevertheless, 308 nm radiation can realize the best compromise between sterilizing effectiveness and preservation of delicate materials, minimizing the modification of some compounds (e.g. cellulose, proteins) and the decolouration of surfaces.

Irradiation experiments have been carried out on *Nonomuraea* sp. cells. *Nonomuraea* sp. is a mycelial microorganism belonging to the Actinomycetes group, able to attack cellulose and/or lignin. Survival data have been compared with the results obtained from irradiation of *Escherichia coli*, chosen as a reference microorganism.

Materials and Methods

Nonomuraea sp. spores (Actinomycetes group) were grown in yeast-starch (YS) broth at 28 °C for 15 days and, after dilution, plated in mini-plates (3 cm in diameter) on yeast-starch-agar (YSA) solid culture medium. *Escherichia coli* was used as a reference microorganism for the survival rate. The *E. coli* strain MG1048 (wild type) was grown to late logarithmic phase in Luria-Bertani (LB) broth at 37 °C under vigorous shaking. Diluted cells were plated in mini-plates on LB agar.

A XeCl excimer laser was used for cell irradiation. It provided pulsed UV radiation at 308 nm wavelength, with a pulse duration of 20 ns (FWHM). A joulemeter was used to measure the pulse energy, which resulted about 100 mJ. The laser spot (1.1×1.1 cm^2) struck the surface of the mini-plates by the use of a suitable optical system, see Figure 1. The laser fluence was fixed at 80 mJ/cm^2 and the repetition rate at 1 Hz. The exposure dose ranged from 0 to 6000 mJ/cm^2 and it was evaluated as the

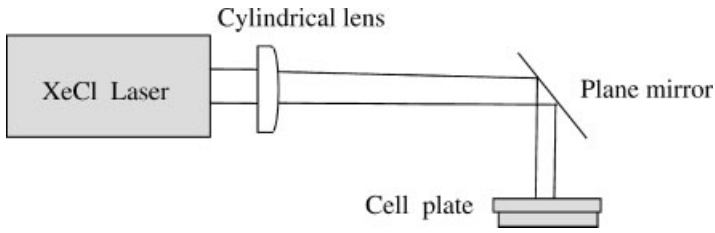

Figure 1.
Scheme of the experimental set-up for cell irradiation.

product of the pulse fluence times the number of laser shots.

Five samples for each dose were irradiated at room temperature, in presence of daylight illumination. Four dose values were imparted for each cell line. Survived cells were quantified following over-night incubation at 37 °C, by counting the number of colony forming units (cfu) at different dilutions. Data were normalized to the survival of the control sample.

The measurement of the absorption cross section for 308 nm photons on *Nonomuraea* sp. cells was also carried out. Absorbance measurements were performed through a cell dispersion (LB broth, 1.6×10^5 cells/mm^3) contained in quartz cuvettes of different thickness, by using the XeCl laser beam, suitably diaphragmed. The Lambert-Beer law was applied to determine the absorption cross section by a fit of the absorbance curve, according to the following equation:

$$A(x) = (\sigma_a c + \mu)x \quad (1)$$

where A is the absorbance, x is the cuvette thickness, c is the cell density, σ_a is the cell absorption cross section and μ is the broth absorption coefficient at 308 nm wavelength. In parallel measurements we found $\mu = 0.36 \pm 0.04$ mm^{-1}.

Results and Discussion

Figure 2 shows the survival data and the fitting curves for *E. coli* and *Nonomuraea* strains as a function of the exposure dose. The data points are the mean of five independent experiments; error bars are reported as standard deviation and are generally negligible. The curves are the best fit to the surviving fractions, S, according to the linear-quadratic equation:

$$S(D) = \exp(-\alpha D - \beta D^2) \quad (2)$$

where D is the exposure dose and α, β are the parameters. Values of the α and β

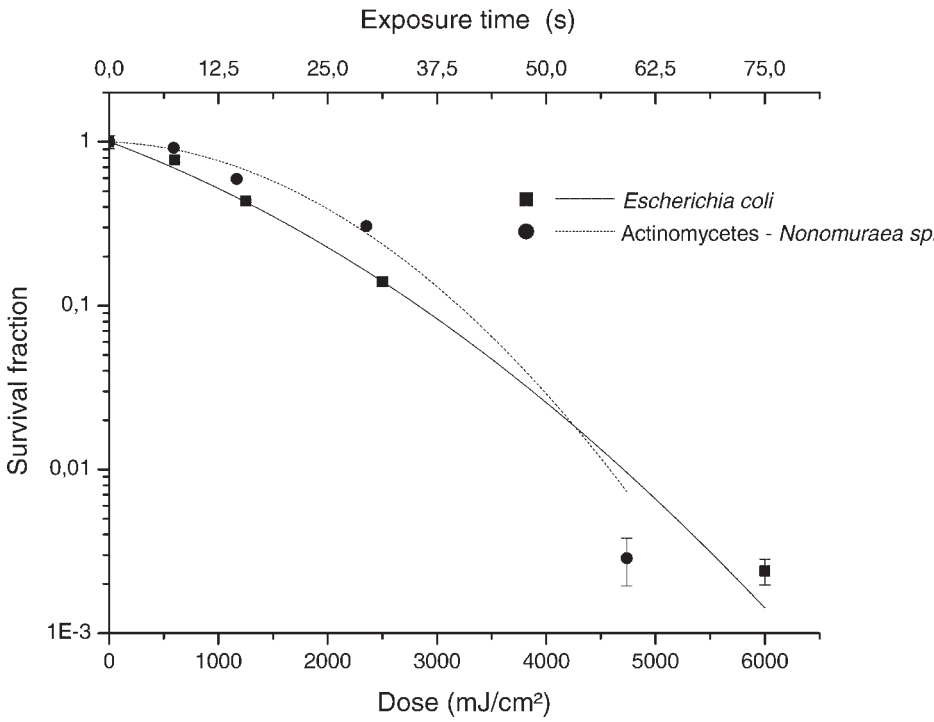

Figure 2.
Comparison of the survival rate of *E. coli* and *Nonomuraea* sp. as a function of the exposure dose.

parameters are $\alpha = (0.60 \pm 0.02) \times 10^{-4}$ (mJ/cm^2)$^{-1}$ and $\beta = (21 \pm 1) \times 10^{-8}$ (mJ/cm^2)$^{-2}$ for *Nonomuraea* sp. and $\alpha = (5.7 \pm 0.2) \times 10^{-4}$ (mJ/cm^2)$^{-1}$ and $\beta = (8.8 \pm 0.8) \times 10^{-8}$ (mJ/cm^2)$^{-2}$ for the *E. coli* strain.

It is evident that the survival rate decreases of three orders of magnitude for both microorganisms just after roughly 75 s, working at a laser fluence of 80 mJ/cm^2 and a repetition rate of 1 Hz. The two survival curves are substantially similar in the dose range $0 \div 4500$ mJ/cm^2; for higher doses, *Nonomuraea* sp. seems to be much more sensitive than *E. coli*. By comparison with the *E. coli* survival curve, Actinomycetes cells enable probably the same DNA repair systems: photo reactivation process (phr) for doses below 1000 mJ/cm^2; repair by recombination in the range $1000 \div 3000$ mJ/cm^2; error-prone synthesis system for doses higher than 3000 mJ/cm^2.

The absorption cross section at 308 nm of the *Nonomuraea* sp. cell was derived from the slope of the absorbance curve, shown in Figure 3, as described in ''Materials and methods''. We found $\sigma_a = 0.84 \pm 0.06$ μm^2. Such a value has to be compared with the geometrical cross section of a cell, elliptic in shape with typical length of the minor and major axes of 1 μm and 2 μm, respectively.[6] A rough value of 1.6 μm^2 can therefore be estimated for the geometrical cross section of a cell, σ_g. It can be said that approximately one half of the cross section surface of a cell is involved in the absorption (or diffusion) process of 308 nm radiation. In terms of photon absorption probability per cell, p, calculable as

$$p = \frac{\sigma_a}{\sigma_g} \approx 0.5 \tag{3}$$

one can state that 308 nm radiation absorption in *Nonomuraea* sp. bacteria is a high efficiency process. Moreover, it should be pointed out that the transmittance of the bacterial patina in actual growth conditions is much lower than 0.5, owing to the cell pile up. This aspect is important when irradiation of artefacts, e.g. cellulose-based materials, is considered. It results in a moderate radiation exposure for the substrate zone under the bacterial patina.

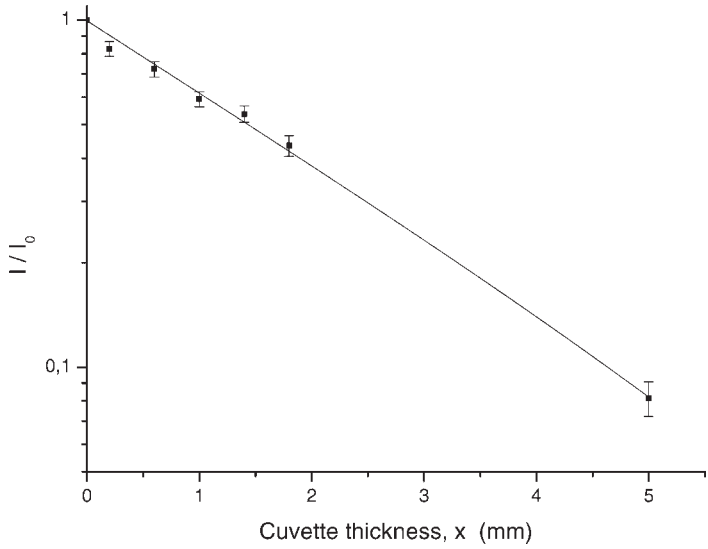

Figure 3.
Intensity attenuation (I / I_0) for 308 nm radiation in measurements of absorption through the *Nonomuraea* sp. cell dispersion, as a function of the cuvette thickness.

Conclusion

We found great sensitiveness of the investigated bacteria to 308 nm pulsed laser radiation. In particular, a slightly higher resistance of *Nonomuraea* sp. with respect to the reference bacterium, *E. coli*, was found for exposure doses up to about 4500 mJ/cm^2. Sterilization by 308 nm radiation seems to be particularly suitable for cultural heritage preservation, on account of the negligible radiation dose transmitted to the substrate through the bacterial patina.

The present study provides clear indications on the possibility to generalize these results also to other similar bacteria. Irradiation experiments on substrates of interest for cultural heritage (paper, textiles, wood) will be performed in the near future.

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