Short Communication

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.52.008192

Action Of UV-C Radiation for Biomolecules Inactivation, With Application in Diagnostics

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ARTICLE INFO

Received: July 21, 2023

Published: August 04, 2023

Citation: Munteanu Ion. Action Of UV-C Radiation for Biomolecules Inactivation, With Application in Diagnostics. Biomed J Sci & Tech Res 52(1)-2023. BJSTR. MS.ID.008192.

ABSTRACT

The action of UV-C radiation for the decontamination of pathogens is a very actual subject to be studied, as a result of the recent pandemic (Covid-19), in finding new effective methods of inactivating pathogens (viruses, bacteria, etc.) with their application in diagnostics. The effects of UV radiation with a 254 nm wavelength on the DNA elements of yeast fungi are observed and analysed in this communication. Quick information about the dose of radiation applied and the level of inactivation of pathogens is necessary, and spectroscopy methods can be of great help in this regard. By using spectroscopy analysis methods, the strong correlation between the decontamination rate and absorption spectra of yeast solution was find. This relationship between the spectrum and the pathogen decontamination rate can be successfully used in diagnostics of pathogens such as Candida Albinus, which is more resistant than a class of viruses and bacteria, including COVID-19.

Keywords: UV-C Radiation; Biomolecules; Absorbance Spectrum; Ultraviolet Germicidal Irradiation; Diagnostics

Introduction

Following the recent Sars-Cov-2 pandemic situation, the problem of finding new solutions and effective methods of inactivating viruses has arisen among scientists. Recently, numerous studies were dedicated to the application under an appropriate distribution of UV-C radiation for the decontamination of fluids and surfaces in order to destroy pathogens (microbes and viruses), such as SARS-Cov2 [1-3]. UV-C radiation, which has the shortest wavelength and the highest energy of ultraviolet range (100-280 nm), is the most effective at killing or inactivating microorganisms, making it a popular choice for disinfection and decontamination Ref [4,5]. The interaction of UV-C radiation with viruses and bacteria has been extensively studied, and research in this field conclusively demonstrates the direct action of DNA denaturation induced by UV-C photons on nucleotides, therefore causing pathogen inactivation [6]. The fact that the experiments on viruses required special authorizations to be carried out in our laboratory, it was decided that in the given work to use baker's yeast solution for the study which are not contagious and due to their eukaryotic cellular constructions provide a better resistance to UV-C radiation, compared to pathogenic prokaryotic colonies. The aim of this study was to investigate the effect of UV- irradiation (254 nm) on the molecular sizes of yeast fungi and on their surface properties. The changes in chemical structure were studied using UV-VIS spectroscopy. The sample morphology was observed in optical microscopy.

Materials and Methods

UV-C Radiation for Inactivation of Pathogens

The first research demonstrating ultraviolet (UV) radiation as a germicidal agent was studied since the 1877s [7]. The UV disinfection

technology is known for the disinfection of pathogens from surfaces, air and water, for several decades. Ultraviolet radiation range are especially damaging to cells because they are absorbed by nucleic acids. But the germicidal effectiveness of UVC peaks at about 260– 265 nm, which corresponds to the peak of UV absorption by bacterial DNA. The ultraviolet germicidal irradiation (UVGI) damages the DNA of viruses and bacteria, rendering it incapable of replicating. The most damage in ultraviolet (UV)–irradiated DNA is the cyclobutane pyrimidine dimer (Figure 1) that is formed between adjacent thymine bases [6].

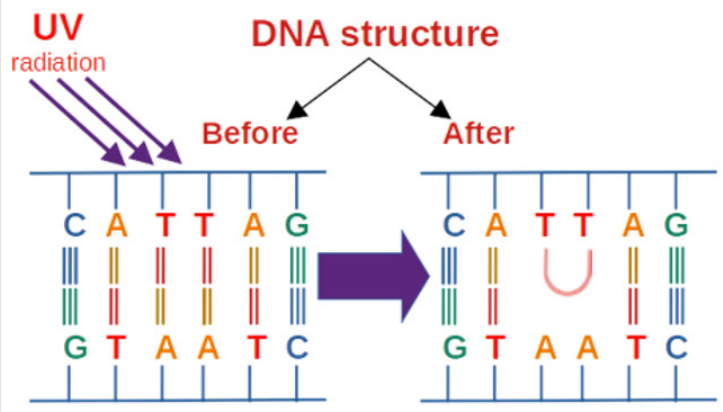

Figure 1: Guanine (G) is associated with cytosine (C) by three hydrogen bonds, and adenine (A) is associated with thymine (T) by two hydrogen bonds. Thymine dimers are caused by UV absorption in adjacent nucleotides (thymine doublets).

When UVC photons are absorbed by thymine dimers, critical damage is caused to the genomic system of microorganisms (nucleic acid and microorganism proteins), preventing them from replicating and surviving, and the adenine-thymine bond is broken. A covalent bond, the pyrimidine dimer, is generated between two adenines, resulting in the cell's inability to replicate. That is why the effect of UV irradiation on microorganisms is called "inactivation" but not "killing".

UV-VIS Spectroscopy Study of 254 nm Wavelength Irradiation on Pathogens

For determining the structure of an organic compound may be possible through the basis of information obtained by help of analysis methods. Physical analysis methods, generally non-destructive, assume the interaction of the substance with radiation of a certain energy. The optical properties of organic molecules can be examined using stationary techniques such as spectroscopy that is a method based on the property of substances to selectively absorb electromagnetic radiation, therefore in the last several years, it has become possible to directly observe the dynamics of excited electronic states in DNA model compounds by femtosecond spectroscopy [8]. In the case of the interaction of radiation with the substance, transitions are present due to the absorption of energy by the molecules. That is, once they are subjected to irradiation, the molecules absorb energy and pass from the "normal" state (basic - fundamental energy level) to the "excited state" (higher levels) excited states [9] (Figure 2).

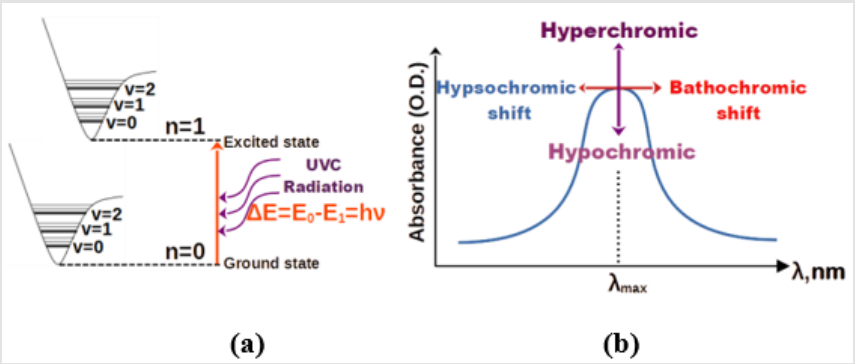

Figure 2:
(a) The transition of the analyte molecule from the ground state to an excited state as a result of the absorption of the photon of energy hv. To each electronic level correspond several vibrational levels, and to each vibrational level correspond several rotational levels.
(b) Terminology for absorption shifts, absorption resonance located at the wavelength of the absorbed photon.

Therefore, the changes produced in molecules as a result of electronic transitions induced by radiation from the ultraviolet and visible domains are studied by UV-VIS electronic spectroscopy, which this will be demonstrated in the experimental part of this manuscript by studying the absorption spectra of the yeast solution at different time intervals under the action of UVC radiation.

Experimental Results

Decontamination Rate Dependance of UVC Pulsed Radiation as a Function of Time Using UV-vis Spectroscopy Method

The main part of our measurement equipment, shown in (Figure 4b), consists of the ultraviolet C radiation source that emits short, pulsed radiation of 254 nm to the sample placed in a cuvette holder with four light ports perpendicular to each other. As seen in (Figure 4), one port serves for the UVC radiation to penetrate the sample containing the solution to be analysed, and two other ports serve for the connections to the spectrometer. The fact that experiments on bacteria or viruses are not allowed in the laboratory, the Baker's yeast solutions (has been a key ingredient in baking, winemaking, and brewing for millennia) will serve as the sample for the measurements, which can be prepared very quickly in laboratory conditions, a mixture of 10 grams of yeast with 5 grams of sugar and 200 grams of water (Figure 4a). In fact, yeast has stronger resistance to UVC radiation in comparison with many viruses or bacteria, according to existing investigation [10], and does not cause negative health effects.

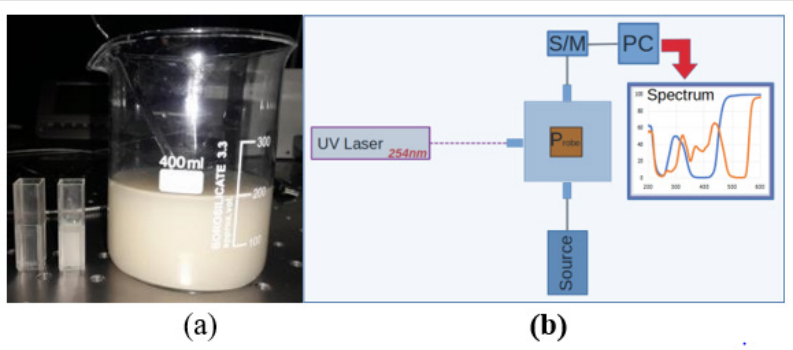

Figure 4:
(a) Solutions of baker's yeast prepared for the experiment, the cuvettes, serve as a sample.
(b) The experimental design of laboratory tests.

Every five minutes, the ultraviolet radiation is stopped to make spectral measurements using the UV-VIS spectrometer also in these time intervals pictures will be taken, using the electron microscope. To observe changes in the dynamics of the yeast solution decontamination, the experiment will be repeated 5 times for detailed analysis of the absorption spectra. The basic elements in the DNA of pathogens absorb ultraviolet radiation very well in the 220280 nm range [11,12], therefore in this experiment we will study the spectral changes specifically in the given range (Figure 5a). Based on the spectral graph data from this experiment, a new graph is obtained showing the decontamination rate as a function of time duration (Figure 5b). After decontamination, a decrease in the number of yeast bacteria is observed with the increase in the time interval of their exposure under the action of UVC radiation.

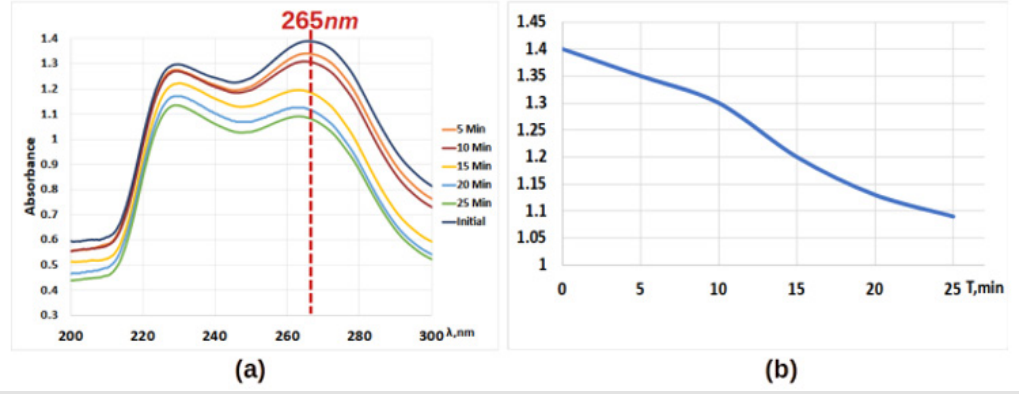

Figure 5: The decontamination rate of the Baker's yeast solutions under the action of short pulses of UVC radiation as a function of time.

Decontamination Rate Dependance of UVC as a Function of time Using Optical Microscopy

Once the spectral analysis gives us clear information about the decontamination rate of the yeast bacteria as a function of time, it is interesting whether this will also be observed following the microscopic analysis? UVC radiation is absorbed by yeast, as mentioned above. To observe some effects, at five minutes intervals, pictures will be taken using the electron microscope to monitoring the evolution of the number of yeast colonies during the decontamination procedure. The process of inactivating the yeast colonies following the decontamination procedure can be observed in the microscopic images shown in (Figure 6).

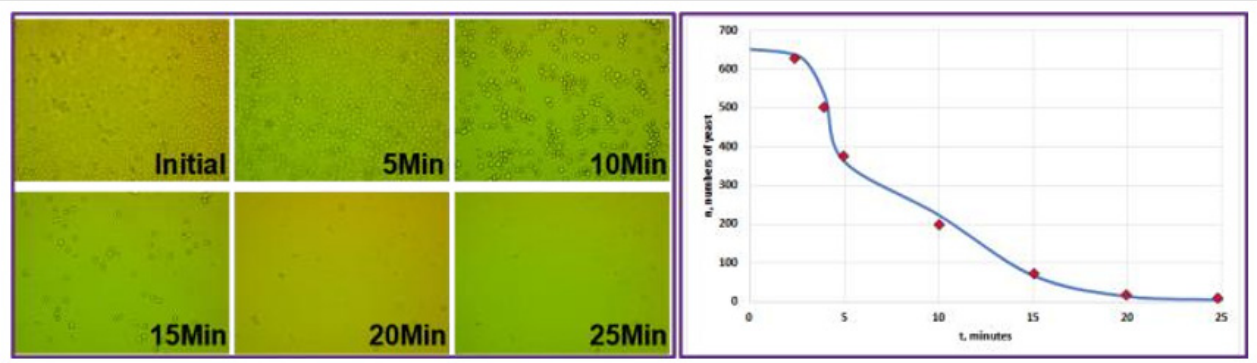

Figure 6:
(a) The yeast solution samples represent in evidence an aleatory distribution of colony size in each sample (Initial and 5-25 Min intervals after decontamination), seen under microscope.
(b) Monitoring the number of colonies and decontamination rate as a function of time duration of UVC radiation application.

Typical optical images of the initial yeast fungus colonies prior to decontamination are displayed in (Figure 6a). The number of colonies per sample varied from 2 to 14, see (Figure 6a) (10Min). The size distribution seems to be aleatory but the number of colonies decreases with dimension. For the number (n) and diameter (d) of the yeast colonies from the sets of experimental samples we obtained, it is proposed to use the Gaussian distribution for the statistical description [13], which is defined as:

$$W(n,d)=\frac{1}{\sqrt{2\pi\sigma_n^2}}\exp\left[-\frac{(n-n_0)^2}{2\sigma_n^2}\right]\times\frac{1}{\sqrt{2\pi\sigma_{d_n}^2}}\exp\left[-\frac{(d_n-d_{n_0})^2}{2\sigma_{d_n}^2}\right] \quad (1)$$

here σ_{d_n} is the size variance, and σ_n - the variance of yeast colonies number for the same diameters d_n. For an analysis of the experimental results which we obtained, we will estimate the average values of the number (n) and the size of the diameter (d) of the yeast colonies present in the samples prepared for decontamination. The values before decontamination are $n_0 \approx 7\,\sigma_n = 2$; other parameters are: $d_{n_0}/d_{s_p} = 0.05/n_0$ and $\sigma_{d_n} = 0.1/d_{sp}$, where d_{sp} is visualized diameter of the microscope image (Figure 6a).

Results and Discussion

As follows from the experimental results, by action of UVC radiation on biomolecules the inactivation of yeast colonies increases in a strongly nonlinear. Therefore, from these observations, we conclude that the number of yeast colonies decreases non-linearly with increasing duration of time under the action of UVC radiation up to a given value, after that, a smooth decrease prevails (Figure 6b). At the same time, (Figure 5) shows a decrease of the absorbance value, which means that the number of pathogens also decreases, in this case the DNA chain is destroyed and solution is disinfected. Once the DNA elements of the biomolecules absorb ultraviolet radiation C, it is important to pay attention to (Figure 7a), where the existence of 2 peaks approximately 230 nm and 265 nm can be observed in each spectrum. Special attention is paid to the peak with the values 265 nm, because according to Kowalski's manual [6] page 26, the dependence of wavelength on absorption for the four main nucleotides shows that Thymine peak is around 265 nm (Figure 7b), which correspond with the peak obtained in our experiment, means that thymine from ADN Baker's yeast bacteria absorbs very well this UVC radiation. Once this UVC radiation is absorbed by the yeast colonies, the formation of T=T dimers takes place in the DNA structure, and these biomolecules become inactive [14], this is observed following the study of spectral analysis. We proposed to investigate the effect of UV irradiation and obtaining the absorption spectrum in order to highlight the degree of inactivation of the pathogen, and the correlation between the spectrum and decontamination rate may be used in diagnostics.

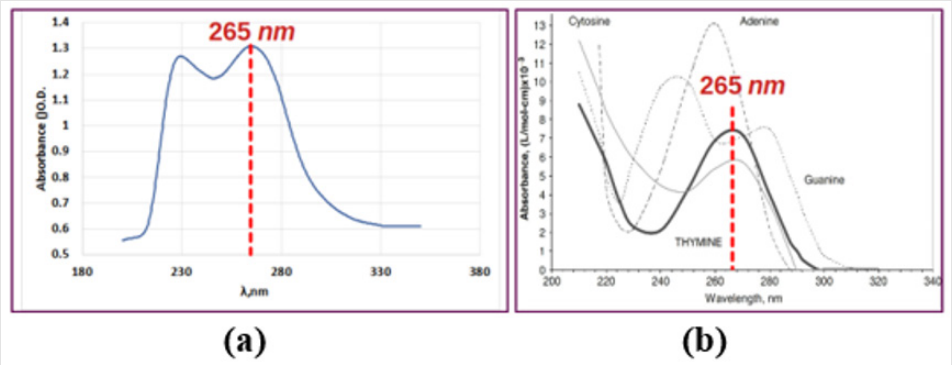

Figure 7:
(a) Absorption spectra of Baker's yeast bacteria in UV range.
(b) Comparison of response spectra for the four main nucleotides (figure taken from [6]).

The results show that the relationship between theory and experiment has a common effect observed in the figure above, and this clearly says that the action of UVC radiation on biomolecules can successfully inactivate them. Therefore, we must treat this radiation with care, because it can affect the eyes, the skin or other parts of the body.

Conclusion

In summary, in this manuscript, we have studied both theoretically and experimentally the dependence of the inactivation rate of yeast colonies on the duration of time of the applied UVC radiation. The correlation between the theoretical and the experimental part was found in the study of the absorption spectrum of yeast colonies where the existence of the same peak was observed with wavelength 265 nm. Finally, based on the theoretical and experimental part of this manuscript, the construction of a new device concept is proposed to be used in the diagnosis of different forms of pathogens. This device will contain the experimental design of the equipment with the help of which the experiment in the given work was carried out (Figure 4b), only that all the parts will be in one device. Of course, similar experimental studies can be studied as well to the case of SARS-CoV-2 virus, but this type of experiment in our laboratory is impossible to carry out due to the absence of the conditions and protocols for the manipulation of biological viruses, that why we used baker's yeast bacteria.

Conflict of interest

The authors don't have the financial, commercial, legal, or professional relationship with other organizations, or with the people working with them, that could influence their research.

Acknowledgment

This paper is supported by the projects: No. 20.80009.5007.01 and NATO EAP SFPP 984890

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.52.008192

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