SURVIVAL OF *CANDIDA MYCODERMA* CELLS UNDER INTENSE LIGHT PULSES TREATMENT ON GLASS USED AS PACKAGING MATERIAL

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Abstract: Cells of *Candida mycoderma* were inoculated on glass and subjected to intense light pulses (ILP) treatment for up to 1.27 J/cm^2 energetic density and different time at 7 cm distance from the flash lamp. Population reductions higher than 2 $\log_{10}$ colony-forming units per square centimetre (CFU/cm^2) were achieved. Calculation revealed a D-value ranging from $0.797*10^{-3}$ s to 2.826*lO^{-3} s and a Z-value of 0.69 J/cm^2. The study has demonstrated that ILP is a promising treatment for packaging material decontamination and/or sterilisation.

Keywords: decontamination, *Candida mycoderma,* flash lamp, intense light pulses (ILP), packaging material, glass, aseptic packaging.

INTRODUCTION

Sooner or later, every practicing food technologist and scientist will become involved in food packaging, since it is almost impossible to think of more than a handful of foods which are sold in an unpacked state. The concerns related to a prolonged shelf life of food lead to an increase of the importance of food packaging. Moreover, the demands of food as free as possible of micro-organisms lead to a huge development of aseptic packaging (Reuter 1989; Robertson 1993) (Moruzzi 2000).
Aseptic packaging involves the absence or exclusion of any unwanted microorganisms from the product, package or other specific areas (packaging machinery and environmental, closures). Therefore the product has to be commercially sterile, the containers and closures have to be sterilised just before filling, the filling operation has to take place under aseptic conditions and the containers have to be hermetically (air-tight) sealed so that reinfection is prevented.
Heat has been the preferred method for killing microorganisms in food or on surfaces, including packaging materials surface, even before Nicolas Appert

and 1ater Louis Pasteur developed the process named appertisation / pasteurization (Burton, 1988) (Durance, 1997) (Ionescu-Boeru, 1999).
At present, several methods achieve similar results using chemical treatments, irradiation, ultrasound treatment or combined procedures of heat (saturated steam, hot air) and new techniques.
Chemical treatments are based on the lethal effect of hydrogen peroxide or peracetic acid on microorganisms, including heat-resistant spores (Robertson, 1993).
Irradiation involves the action of ultraviolet radiation, infrared rays or ionizing radiation to reduce the number of microorganisms for the sterilization of the food contact packaging material surface (Robertson, 1993) (Reuter, 1989) (Riganakos et al., 1999) (Fengmei, 2000) (Sharma and Demirci, 2003).
The formal discovery that specific monochromatic wavelengths of UV light are bactericidal was made as early as 1928 (***, 2003) and developed with laser welding (Brown et al., 2000), gamma-irradiation and electron-beam (Mittendorfer et al., 2002) (Woo and Sandford, 2002) applied on food packaging material surfaces.
Similar to ultraviolet radiation is a quite new technique called intense light pulse (ILP) treatment. That treatment involves the use of very intense and short duration pulses of broad-spectrum "white light", including wavelengths in the ultraviolet to the near infrared region. The difference is that the very intense light is about 20,000 times the intensity of sunlight at sea level. Therefore it is a very intense flash of light but very brief. The material to be treated is exposed to at least 1 pulse o f light (typically 1 to 20 flashes per s) having an energy density in the range of about 0.01 to 50 J/cm^2 at the surface (Dunn 1995) (**, 2002).
The intense light pulse technology is applicable mainly in sterilizing or reducing the microbial population on the surface of packaging materials, transparent pharmaceutical products, or other surfaces.
Usually, the packaging material used in aseptic processing is sterilized with hydrogen peroxide, which may leave highly undesirable residues in the food or package. Light pulses could be used to reduce or eliminate the need of chemical disinfectants and preservatives, and to extend the shelf life or improve the quality of packaged food.
The aim of this work is to study the influence of ILP treatment on survival of yeast cells of *Candida mycoderma* on glass used as packaging material. It continues the work previously done to study the decontamination / sterilisation of paper-polyethylene packaging material inoculated with

mould spores *of Aspergillus cynnamomeus* (Turtoi *et al.* 2004a) and *Cladosporium herbarum* (Turtoi *et al.* 2004b).

MATERIALS AND METHODS

Materials

Packaging material used to perform the research was glass, the material used for packing wines, juices and soft drinks.
Due to equipment design it was necessary to work on plane surfaces and Petri dishes were chosen to perform the experiments. The exposed surface to ILP treatment was the interior of glass Petri dishes.
Candida mycoderma is part of the epiphyte microbiota of fruits and vegetables, sometimes being present also on cereal and oleaginous kernels. It is one o f the frequently occurring spoilage agent in low-alcoholic weak-sulphytated wines, fruit and vegetable juices, soft beverages and lactic fermented products (Dan, 2000).
The 3 days *Candida mycoderma* colonies developed on MEA (malt extract agar) are 2.5-5.8 mm, cream white, with margins irregularly lobate or fimbriate and rough mate surface. Cells on MEA at 3 days vary from short ellipsoids to long cylinders of (2-4)x(6-13) μm and occurs single or in chains as result of the multipolar budding (Dan et al., 1999) (Pitt and Hocking, 1985).

Experimental set-up
Pulsed 1ight has been produced using engineering technologies that multiply power many folds. Power was magnified by electrical energy accumulation in an energy storage capacitor over a fraction of a second and releasing this storage energy to do work in a much shorter time (10^{-3} of a second).
The experimental set-up is shown in fig. 1. The IFP-800 flash lamp (2) is fixed above a plate of wire sieve (6) on which the sample (5) has to be treated. The lamp is fed by an impulse generator (7). The flash lamp used was IFP type (IFP-800), with discharge through gas (xenon). The spectral distribution of flash lamp was similar to that of sunlight, with peak emissions between 200 - 500 nm. The emission characteristics of the flash lamp were electromagnetic field, X = 200 - 1000 nm, impulse regime, x = $10^{-1} – 10^{-4}$ s, light intensity, E = 1000 - 8000 J.

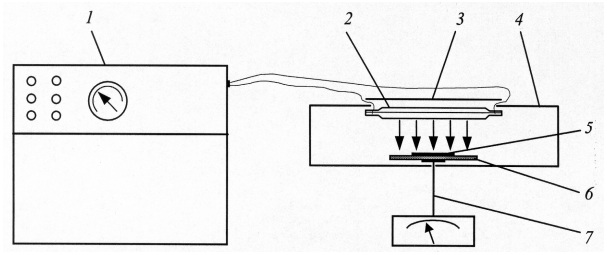

Figure 1. Experimental set-up of ILP treatment
1 - impulse generator; *2* - ILP-800 flash lamp; *3* - lid; *4* - treatment space; 5 - Petri glass dish inoculated with *Candida mycoderma; 6* - plate of wire sieve; 7 - support system.

Methods

Sample preparation

Glass Petri dishes with a diameter of 86.5 mm (a surface of 58.765 cm^2) have been sterilised to be free of any microorganism.
A cell inoculum of *Candida mycoderma* was prepared in order to contain about 100-150 cells per milliliter. This concentration was chosen to allow the growth of a reasonable number of colonies in a Petri dish.

Inoculation of samples

Each glass Petri dish was inoculated with cells of *Candida mycoderma* in aseptic conditions.

Treatment with ILP

Inoculated samples were set on the wire sieve at a distance of 7 cm from the flash lamp and treated with ILP, excepting the control sample.
The effect of the energetic density on the mould survival was determined with 0.317, 0.497, 0.715 and 0.974 J/cm^2 for the same number of pulses (the same time of ILP action).
To study the effect of the treatment length, the number of pulses was varied for the same energetic density: 2, 4, 6 and 8 pulses, meaning $2*10^{-3}$, $4*10^{-3}$, $6*lO^{-3}$ and $8*l0^{-3}$ s.

Microbiological analysis

One milliliter of a cell suspension containing about 100-150 *Candida mycoderma* cells was poured in several Petri dishes. The dishes were

submitted to different ILP treatments, excepting a pair of dishes that were left untreated in order to allow the determination of the initial number of cells. The treated and untreated inoculates were then covered with MEA.
The colonies of *Candida mycoderma* were enumerated after incubating the Petri dishes at 27°C for 3 to 5 days.

Calculations
All treatments were replicated 2 times. The results were analysed using TableCurve 2D and MS Office Excel to determine the significant and non-significant differences in 1 g CFU/cm^2 of *Candida mycoderma* cells on samples subjected to each treatment.
The same programs were used to create the linear regression plots for calculating the D and Z values of each treatment.

RESULTS AND DISCUSSION

Pulsed light generated at different energetic density (different input voltage) was used to treat glass samples inoculated with *Candida mycoderma* cells. The treatments had the same duration.
The number of viable colonies was determined after the incubation of cells (figure 2), and used to plot the variation of CFU/cm^2 against the energetic density (figure 3).

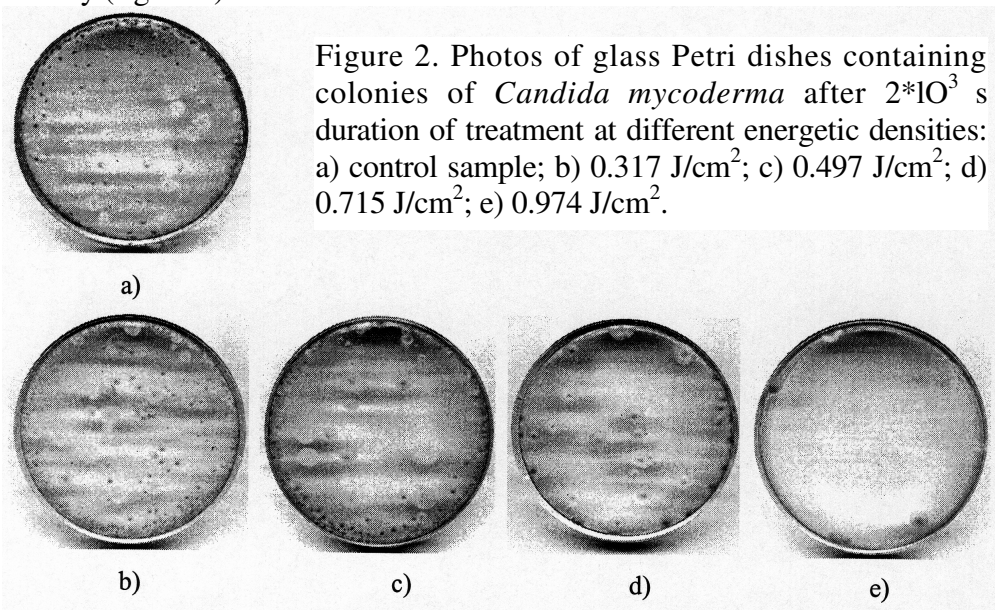

Figure 2. Photos of glass Petri dishes containing colonies of *Candida mycoderma* after 2*lO^3 s duration of treatment at different energetic densities: a) control sample; b) 0.317 J/cm^2; c) 0.497 J/cm^2; d) 0.715 J/cm^2; e) 0.974 J/cm^2.

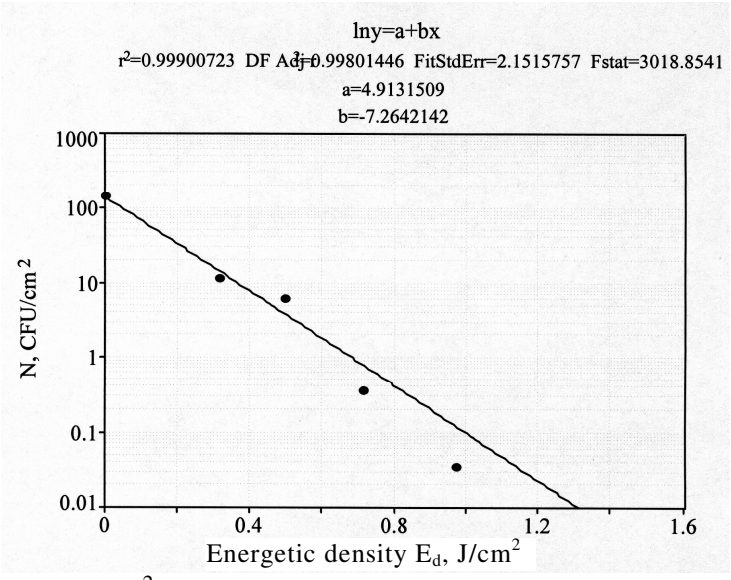

Figure 3. CFU/cm^2 variation against energetic density for $\tau = 2*10^{-3}$ s.

From figures 2 and 3 could be observed that there was a significant increase in the reduction of population along with an increase of energetic density for the same duration of the ILP treatment.
The reduction in population for the various time treatments at the same energetic density is shown in figure 4.

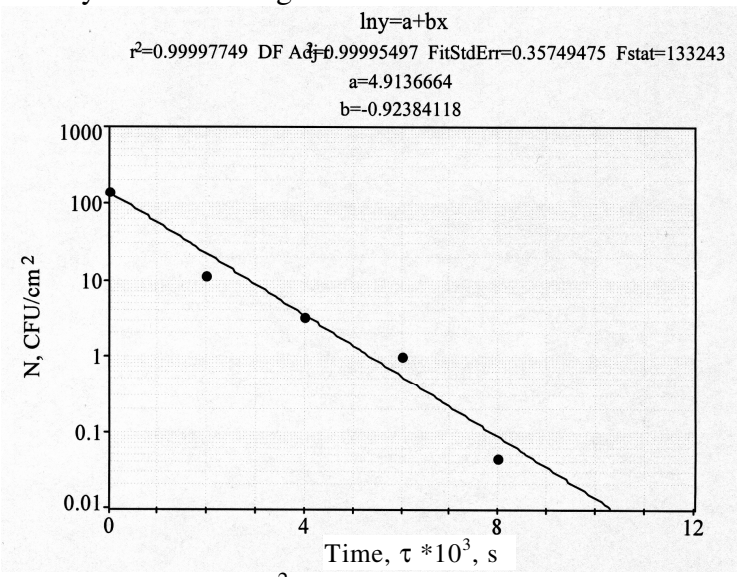

Figure 4. Example of CFU/cm^2 variation against time for E_d = 0.317 J/cm .

If in figure 4 an example of CFU/cm variation against time for one energetic density value is presented, all the experiments result for different energetic densities are illustrated in figure 5.

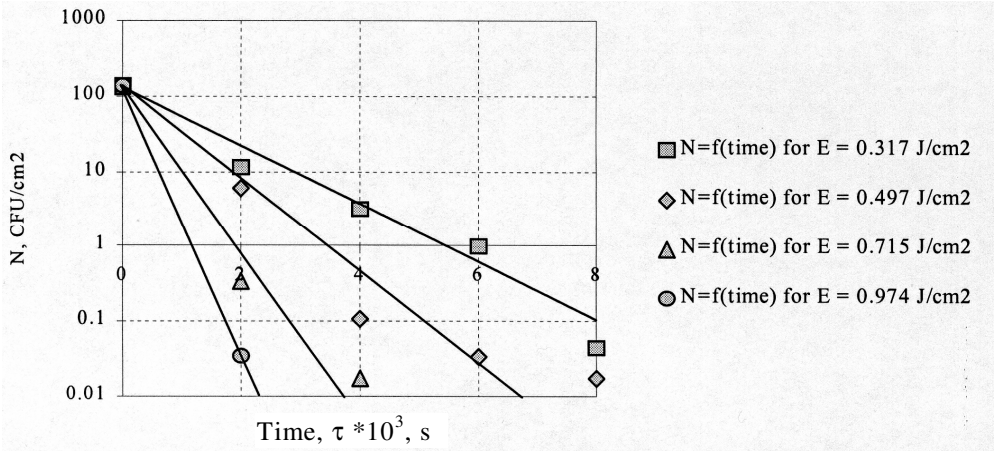

Figure 5. Variation of CFU/cm^2 against time for all E_d values used for ILP treatment.

From figure 5 can be observed that the higher is the energetic density, the lower is the number of survival cells of *Candida mycoderma* on glass samples.

From each plot $lgN = f(\tau)$, the time required to destroy 90% of the microorganisms, namely D value, was calculated and a reduction from $2.826*10^{-3}$ s for 0.317 J/cm^2 to $0.797*10^{-3}$ s for 0.974 J/cm^2 was achieved.
Afterwards, D values were used to calculate the Z value as the energetic density of which the destruction curve passes a logarithmic cycle (figure 6): Z = 0.69 J/cm^2.
It has to be mentioned that the initial number of yeast cells (determined for the control samples) was higher than in normal conditions, meaning that in such conditions the reduction in population will be also higher, being possible to achieve a complete decontamination of the packaging material surface.

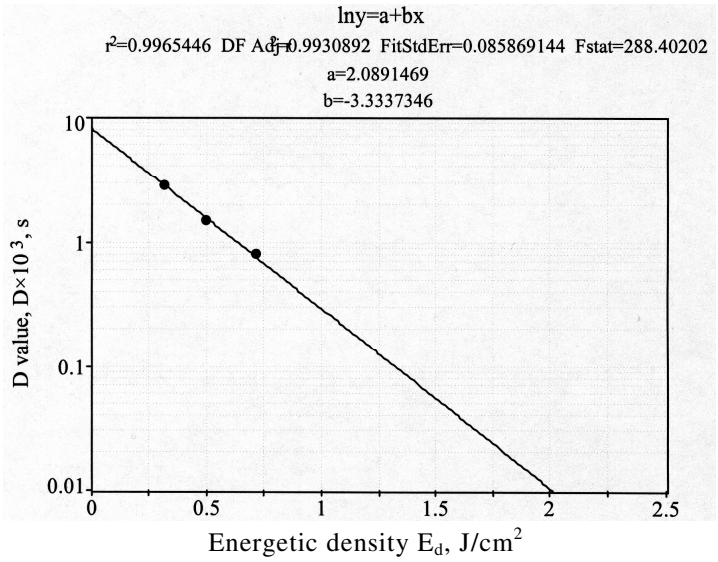

Figure 6. Variation of D value against energetic density.

Comparing the above mentioned results and discussion to those published in previous (Turtoi et al., 2004a, 2004b) one could see the same evolution of microorganisms behavior under intense light pulses treatment. Thus, Z value calculated for each type of microorganism is:

- for *Cladosporium herbarum* spores subjected to ILP treatment Z = 0.978 J/cm^2;
- for *Aspergillus cinnamomeus* spores subjected to ILP treatment Z = 0.96 J/cm^2, and
- for *Candida mycoderma* cells subjected to ILP treatment Z = 0.69 J/cm^2.

The lower Z-value obtained for the ILP treatment of *Candida mycoderma* cells is due to the state of the microorganism, cells being less resistant than spores.

CONCLUSIONS

The researches demonstrate that intense light pulses treatment holds promise for eliminating the microorganisms from glass packaging material.
In order to assert that ILP treatment is a proven technology for packaging material decontamination/sterilisation, to study the influence of other parameters, such as the distance of the sample from the flash lamp, and to validate the mathematical models obtained, further researches are necessary.

Abbreviations
CFU - Colony-Forming Units
D value - Decimal reduction time
ILP - Intense Light Pulses

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