EFFECT OF OZONE AND VUV LIGHT IN THE PRESENCE OF FOOD COMPONENTS

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ABSTRACT

The aim of the current work is the comparison of the ozone, ozone/UV, UV and UV/VUV induced destruction of a spore-former (*Bacillus megaterium)*, a Gram positive (*Micrococcus luteus)* and a Gram negative (*Serratia marcesens*) bacterium in the presence of a model food component carbohydrate lactose, and carbohydrate and protein containing solution. In "clear" solutions both method was sufficient to achieve 1 or in most cases min. 2 order of magnitudes decrease of microorganisms. The Gram negative Serratia marcesens appeared to be the most sensitive, while the Bacillus megaterium and Micrococcus luteus were more resistant to the treatments. The presence of organic matter had affected to disinfecting efficiency of all methods in the case of all microorganism, but the effect depends on the type of the substrate. In the presence of protein and carbohydrate containing solution the situation is markedly different. It was found that lactose inhibits the disinfection, while protein increase the disinfection efficiency which can be explained by the formation of toxic degradation products derived from proteins.

1. INTRODUCTION

Advanced Oxidation Processes, e.g., ozonation, UV-light and their combination has been used to disinfect drinking water for some times. Both ozone and UV light are powerful antimicrobial agents. Ozone (O_3) is a highly unstable triatomic oxygen molecule. It rapidly decomposes to produce molecular oxygen and atomic oxygen which can oxidise the compounds present. The antimicrobial effect of ozone is based on the fact that ozone directly destroys the cell wall of microorganisms by oxidising the cellular constituents including proteins, unsaturated lipids, and membrane-bound enzymes (Scott and Lesher, 1963).

UV light can destroy bacteria, viruses and parasites that are dispersed in either liquid or air or those deposited on surfaces. UV light offers several advantages over other agents because it leaves no residues, does not affect moisture and temperature and is economical, thus its use in food industry has great potential. Nevertheless the efficiency of photooxidation affected by several factors, e.g. in liquids the nature of the matrix (colour, molar aborbance at the wavelength of the radiation, the quantum yield, etc.

Gaseous ozone absorbs short-UV wavelength with a maximum absorption at 253.7 nm with 3000±30 $dm^3mol^{-1}cm^{-1}$ absorption coefficient (Gordon and Grunnwell, 1983). The absorption of the light may induce the destruction of ozone thus enhance its oxidising efficiency. VUV light (λ<190 nm) decomposes the H_2O molecule to H· and ·OH radicals. ·OH radical is a stronger oxidising agent than ozone thus it can be expected that the VUV light also enhance the antimicrobial efficiency of ozone.

Applications of ozone in the food industry have been suggested in areas like food surface hygiene (Rice et al. 1982), sanitation of food plant equipment (Greene et al. 1993), etc. While the bactericidal action of ozone and UV light have been documented on a wide variety of organisms (Restaino et al. 1995), relatively little is known on the effect of various food components on the bactericidal activity of ozone and UV light (Güzel-Seydim et al. 2004).
The aim of the current work is the comparison of the ozone, ozone/UV, UV and UV/VUV induced destruction of a spore-former, a Gram positive and a Gram negative bacterium in the presence of a model food component carbohydrate lactose, and carbohydrate and protein containing solutions.

2. METHODS

Ozone was produced from oxygen by a flow-type ozone-generator (OZOMATIC Modular 4, Germany) operating by silent electric discharge. The ozone containing gas continuously was bubbling throughout the reactor (V=1000 cm^3). The flow rate was 1.0 dm^3 min^{-1}. The ozone concentration of bubbling gas was monitored by an UV spectrophotometer (WPA Lightwave S2000) at 254 nm. The concentration of ozone in the bubbling gas was 30 mg/l.
The UV sources were low-pressure mercury lamps, one of them radiate mainly at 254 nm, while the other have 185 nm light component. The intensity of the 185 nm component is about 8% of the 254 nm component. The geometry and power of the lamps were the same: the surface of the lamp is 0,025 m^2, the power is 3 W. The lamp was immersed to the centre line of the reactor.
The microorganisms investigated were the spore forming *Bacillus megaterium,* the Gram positive *Micrococcus luteus,* and the Gram negative *Serratia marcesens.* The microorganisms were grown in TGE nutrient (0,3% triptone, 0,5% caseine peptone, 0,1% glucose) then centrifuged and washed to separate the microorganism suspension from the organic matter of the nutrient. The microbe suspensions were prepared from the centrifuged cleaned suspensions, physiological salt solution and occasionally it contained 1% lactose. In that cases when the organic matter was the TGE solution, the samples were not centrifuged. The CFU (colony forming unit) were after 10, 20 and 40 min during the treatment. The bacterial counts were enumerated by plating in TGE agar and incubating at 37°C for 48 h. All samples were plated triplicate. The initial CFU varied between 10^4 and 10^6 CFU/ml but typically it was 10^5 CFU/ml.

3. RESULTS AND DISCUSSION

Antimicrobial effect of ozone, ozone/UV, UV and VUV treatment in absence of organic matter

In the first series of experiments the antimicrobial efficiency of ozone, O_3/UV, UV light and UV/VUV light were compared in absence of any organic substrate. The changes of microbial counts are shown in Fig. 1.1-1.3. in logarithmic scale.

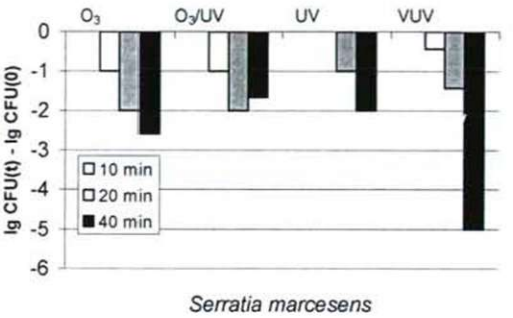

Figure 1.1. Variation in microbial counts of *Serratia marcesens* after 10, 20, 40 min treatment in absence of organic substrate

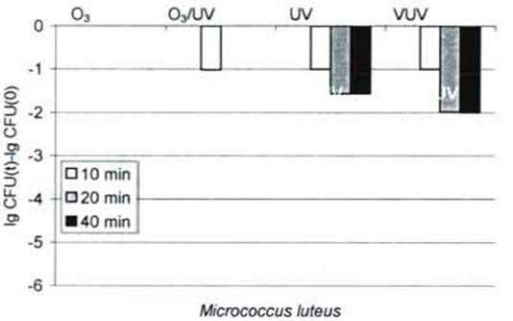

Figure 1.2. Variation in microbial counts of *Micrococcus luteus* after 10, 20, 40 min treatment in absence of organic substrate

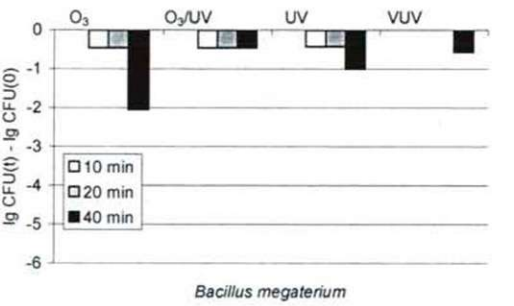

Figure 1.3. Variation in microbial counts of *Bacillus megaterium* after 10, 20, 40 min treatment in absence of organic substrate

Figures 1.1-1.3 show that the most responsive microorganism was the Gram negative *Serratia marcesens*, as its microbial count was found to decrease about 2 order of magnitude by the treatments. The *Bacillus megaterium* and the *Micrococcus luteus* were more resistant, in the most cases the decrease of the CFU were less than 1 order of magnuitude. It was observed that the antimicrobial efficiency increased with treating time in most cases.

Comparing the efficacy of different type of treatments it was observed that the *Serratia marcesens* was killed by ozone and VUV treatment very efficiantly, while the UV and O_3/UV dereased the bacterial count by a maximum of 2 orders of magnitude. Ozone and the combination of ozone/UV were more effective against *Micrococcus luteus*. The VUV/UV treatment proved to be the most effective method against the Gram positive microorganisms (*Micrococcus luteus*). At the same time the spore forming *Bacillus megaterium* was destroyed by ozone most effectively.

Antimicrobial effect of ozone, ozone/UV, UV and VUV treatment in presence of organic matter

In the second series of experiments the effect of presence of organic matter on antimicrobial efficiency were investigated. In order to achieve comparable data the efficiency of the treatment was calculated by Equation 1:

$$\Delta\eta = \lg\frac{CFU_{S,0}}{CFU_{S,t}} - \lg\frac{CFU_{0,0}}{CFU_{0,t}} \qquad \text{Eq. 1}$$

where $CFU_{S,0}$ is the initial bacterial count in substrate containing untreated solution, $CFU_{S,t}$ is the bacterial count in substrate containing, treated solution, where t is the treating time, $CFU_{0,0}$ is the bacterial count in untreated solutions in absence of substrate, $CFU_{0,t}$ is the bacterial count in absence of substrate treated solutions, the treating time is t.

The results obtained in solutions contained 1% lactose, and with a treatment time of 40 min are shown on Figures 1.4-1.6.

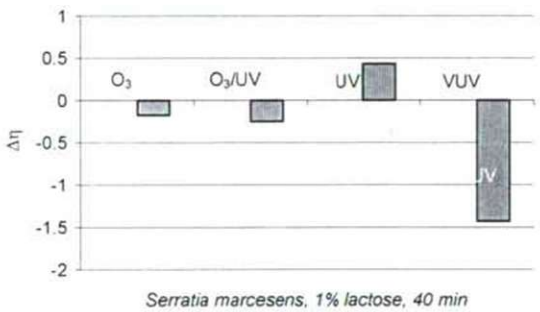

Figure 1.4. Variation in treating efficiency of *Serratia mercesens* after 40 min treatment in the presence of 1% lactose

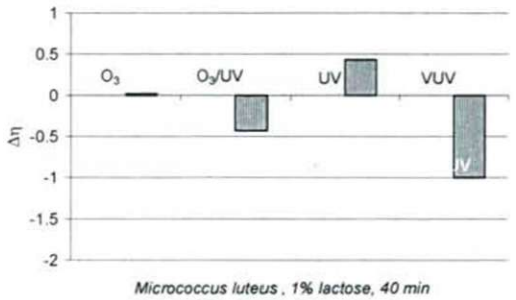

Figure 1.5. Variation in treating efficiency of *Micrococcus luteus* after 40 min treatment in the presence of 1% lactose

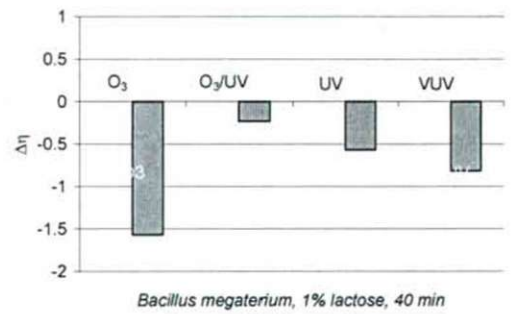

Figure 1.6. Variation in treating efficiency of *Bacillus megaterium* after 40 min treatment in the presence of 1% lactose

The inhibition efficiency of the lactose was the most expressed in the case of ozone treatment of *Bacillus megaterium* containing suspensions, while for non-spore forming microorganisms the inhibition effect of the organic substance was not considerable. The presence of lactose did not significantly affect the efficiency of O_3/UV and UV treatment. At the same time it was observed that the antimicrobial efficiency of VUV/UV treatment in all cases decreased in the presence of the organic substance. This can be explained by considering that the radicals originated from the homolysis of water by VUV light may also significantly affect the bactericid activity. These radicals react very rapidly in solutions containing organic matter, thus their bactericid impact may well decrease.

Comparing the antimicrobial efficiency in the lactose or TGE containing solutions of *Micrococcus luteus* it was observed that the presence of TGE decresed the efficiency of ozone treatment, but in the case of O_3/UV, UV and VUV/UV treatments the presence of TGE an opposite effect was observed (Figure 1.7.).

The phenomena that the presence of TGE enhances the antimicrobial efficiency of UV treatments can be explained by the presence of (presumably) toxic intermediers formed from proteins by UV photolysis. These intermediers may not be accumulated in ozone treated solutions, thus TGE also have protecting effect on the microorganisms.

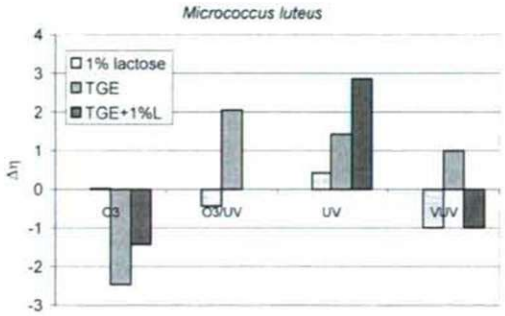

Figure 1.6. Variation in treating efficiency of *Micrococcus luteus* after 40 min treatment in the presence of 1% lactose, TGE or TGE+1% lactose

4. CONCLUSIONS

The aim of the our work was the comparison of the ozone, ozone/UV, UV and UV/VUV induced destruction of a spore-former (*Bacillus megaterium*), a Gram positive (*Micrococcus luteus*) and a Gram negative (*Serratia marcesens*) bacteria in the presence of a model food component carbohydrate lactose, and carbohydrate and protein containing solution. In absence of organic matter the Gram negative *Serratia marcesens* appeared to be the most sensitive to the treatment, while the *Bacillus megaterium* and *Micrococcus luteus* were more resistant to all of the treatments. The presence of organic matter affected the disinfecting efficiency of all methods studied in the case of all micro organism, but the effect seemed to depend on the type of the substrate. In the presence of protein and carbohydrate containing TGE solution the results obtained are remarkably different. The presence of lactose inhibits the disinfection, while the presence of protein may increase the disinfection efficiency which can be explained by the formation of proteinaceous toxic degradation products.

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