Bacterial Inactivation & Study of Damages in Subcellular Level during Disinfection of Aqueous Samples

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Abstract

The aims of the study were i) to investigate ozonation and UVA/TiO_2 photocatalysis as water disinfection techniches & ii) to study damages in subcellular level, in terms of disinfection effects on cellular components (lipids, membrane and proteins). Disinfection experiments were conducted with the bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Ozonation proved to be more effective for the disinfection of aqueous samples compared with UV-A photocatalysis, as for the Gram (-) bacteria a complete reduction was achieved within 15 min, whereas for the Gram (+) bacteria the same reduction was obtained within 30 min.

Regarding the subcellular level the lipid peroxidation progressed at an exponential rate in the course of treatment. The ONPG hydrolysis assay showed negligible alterations in teh course of treatment, indicating that the cell membrane may act as an effective barrier between the cytoplasm and the outer solution in each case.

Keywords: Photocatalysis, Ozonation, Membrane permeability, Lipid peroxidation, SDS-PAGE

1. Introduction

Water contamination is a severe problem worldwide and has escalated due to growing population, anthropogenic use and improper management. Thus, there is an urgent need to develop efficient and cost effective means to eliminate pathogenic microorganisms. Several water disinfection methods such as chlorination, ozonation or UV radiation have been extensively used. Efficient inactivation of bacteria often involves interruption or complete destruction of their essential physiological functions, including cell membrane, cytoplasm, and nucleic acids. Thus, different disinfection processes may have varying influences on these subcellular structures of bacteria. Disorders in the cell membrane (membrane permeability, lipid peroxidation) and proteins could potentially lead to the inactivation of pathogenic bacteria, which are considered important for public health (Long et al., 2015).

2. Materials & Methods

2.1. Bacterial strains

The bacterial indicators used in this study were *E. coli* & *P. aeruginosa* (Gram -) and *B. cereus* (Gram +).

2.2 Disinfection experiments

Photocatalytic experiments were conducted in a batch type, laboratory scale photoreactor. UV-A irradiation was provided by 9 W lamps (Radium Ralutec, 9W/78, 350–400 nm) and the catalyst used was the commercially available TiO_2 in the concentration of 25, 50 and 100 mg/L. Ozone was produced by supplying dry air to an Aqua-Flo CD1B ozone generator (air flowrate: 140 L/h). The impact of ozone and TiO_2 photocatalyst on bacteria survival was investigated by monitoring cell cultivability.

2.3 Subcellular components

2.3.1 Lipid peroxidation

Lipid peroxidation level was measured by the formation of malondialdehyde (MDA), analyzed by the thiobarbituric acid (TBA) method (Maness et al., 1999).

2.3.2 Membrane permeability

The membrane permeability was monitored through the ortho-Nitrophenyl-β-galactoside (ONPG) assay (Long et al. 2015).

2.3.3 Proteins

The total protein content was determined using the Quick start Bradford protein assay kit.

The extracted membrane protein samples were analyzed by SDS-PAGE electrophoresis (Laemmli 1970).

3. Results

Ozonation proved to be more effective for the disinfection of aqueous samples compared with UV-A photocatalysis (Fig. 1), as an almost 10 Log reduction was achieved within 15 min of treatment in all cases (Fig. 2).

However, the higher inactivation rates were recorded for Gram (-) bacteria (*E. coli*, *P. aeruginosa*), whose complete elimination was achieved after 15 min of treatment. The same bacterial reduction (8 Log) was obtained when B. cereus was tested (Gram +) but in longer period of time, i.e. 30 min.

Regarding the subcellular level the lipid peroxidation and MDA destruction progressed at an exponential rate in the course of treatment, while ONPG hydrolysis assay showed negligible alterations (Table 1). The latter indicates that the cell membrane may act as an effective barrier between the cytoplasm and the outer solution in each case.

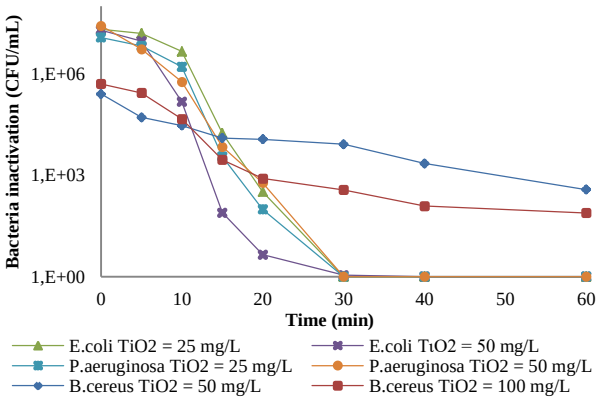

Figure 1. Bacterial inactivation during UVA/ TiO_2 photocatalysis.

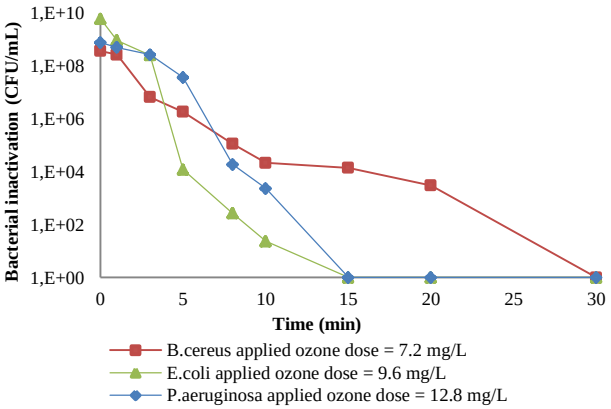

Figure 2. Bacterial inactivation during ozonation. Applied ozone dose: [(Inlet ozone concentration – Outlet ozone concentration) * Flowrate * Contact time]/ Vsample.

Table 1. Indicators of alterations in bacterial subcellular components during disinfection processes.

		UVA/TiO_2 (50 mg/L)		Ozonation	
		0 min	**20 min**	**0 min**	**3 min**
MDA (nmol/ mg cell dry wt)	*E. coli*	7.76	6.61	2.88	3.45
	P. aeruginosa	5.61	12.45	6.90	7.76
	B. cereus	3.79	10.64	13.80	25.87
ONPG (µmole/ (min*mg cell dry wt)	*E. coli*	0	0.020	0	0.080
	P. aeruginosa	0	0.013	0	0.087
	B. cereus	0	0.019	0	0.088
PROTEINS (µg/ mL)	*E. coli*	72.36	72.43	78.58	77.43
	P. aeruginosa	69.90	69.66	72.86	71.67
	B. cereus	68.57	68.56	71.48	68.05

Figure 3 shows representative results regarding the analysis of proteins profiles of the tested bacteria, generated with SDS-PAGE electrophoresis, during each disinfection process. According to cluster analysis the proteins patterns did not alter significantly in the course of treatment.

Nevertheless, not all bacteria exhibited comparable levels of damages in their structure and cellular format. The actual comparison which should be under consideration is between the thick wall of Gram (+) and the outer membrane of Gram (-), as each one represents the first line of defense. Cell wall complexity still remains a nebulous parameter and conclusions regarding its role in resistance during disinfection are still difficult to be definitive.

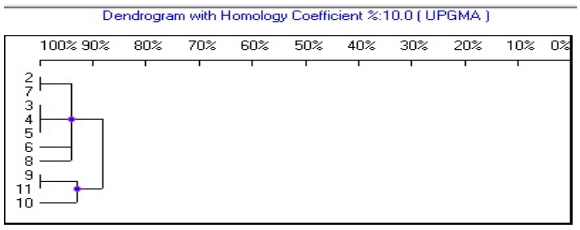

Figure 3. Cluster analysis of the proteins profiles (generated with SDS-PAGE) of E. coli after disinfection. Numbers correspond to the isolates during treatment as follows. 2: intact bacterial strain; 3, 4, 5: UVA/ TiO_2 (25 mg/L) after 10, 15 & 20 min, respectively; 6, 7, 8: UVA/ TiO_2 (50 mg/L) after 5, 10, 15 min, respectively; 9, 10, 11: ozonation after 3, 8, 15 min, respectively.

4. Conclusions

Photocatalysis results confirmed efficient disinfection capability of TiO_2 catalyst under UVA irradiation. A higher tolerance of Gram (+) bacteria was observed compared to Gram (-) bacteria. Antibacterial photocatalytic and ozone activity was accompanied by lipid peroxidation and MDA destruction, which progressed at an exponential rate in the course of time. ONPG hydrolysis assay showed negligible alterations, which showed that the cell membrane could act as an effective barrier between the cytoplasm and the outer solution. Regarding the total proteins, their leakage increased in the course of bacterial inactivation.

References

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