Surfactants Toxicity Assessment in *Tetrahymena pyriformis* Using Fluorescent Probes

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Ciliated protozoa have a wide distribution in aquatic environment and play an essential role in the purification process of aerobic and anaerobic biological wastewater treatment systems. In these systems, surfactants are one of the major anthropogenic organic components. They are included in detergent formulation and are widely used in domestic and industrial cleaning processes, as well as in pharmaceutical, cosmetic and food industry. Due to their strongly hydrophobic and hydrophilic moieties, surfactants may affect cell membrane integrity of wastewater biological communities. This fact leads to a decrease of the treatment system performance increasing sludge handling and management costs.

Concerns regarding the environmental impacts of surfactants led us to evaluate their exposure effects on *Tetrahymena pyriformis*, a ciliated protozoon used as a model organism for the assessment of protozoa cytotoxicity. By their ionic proprieties in water surfactants are classified into four classes: non-ionic, anionic, cationic and amphoteric. In this work two surfactants of each ionic class, in a total of eight toxicants commonly used in household and industrial laundry and cleaning operations, were tested. Evaluation of cell viability was performed using fluorescent markers, Calcein/AM and EthD-1, taken from the LIVE/DEAD® Viability/Cytotoxicity Assay Kit (Molecular Probes Europe BV, The Netherlands). Viable green cells versus red dead cells were observed by fluorescence microscopy.

In order to confirm the results obtained with fluorescence assay, cell viability was determined by direct counting under light microscopy. The results obtained clearly showed that in earlier time exposure, less than one hour of contact with the surfactants at sub-lethal concentrations, the fluorescent method is more sensitive and provides more accurate results than direct counting under light microscopy. With increasing time exposure, no significant differences were obtained with both methods. On the other hand, different surfactants showed different concentration range toxicity patterns. These results will be presented and discussed.