Recognition of protozoa and metazoa using image analysis tools, discriminant analysis and neural network

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A mixed culture of microorganisms is usually present in biological wastewater treatment processes such as the activated sludge system in aeration tanks. These microorganisms are capable of reducing the organic matter and other pollutants in the sewage. Protozoa and metazoa play an important role in this system because they maintain the density of bacterial populations by predation and contribute to the flocculation process, being responsible for an improvement in the quality of the effluent. Moreover, protozoa and metazoa are considered to be important bioindicators of the activated sludge process due to their association with physical, chemical and operational parameters of the treatment plant. Furthermore, the analysis of the number and classes of the predominant groups of these organisms is used to predict the effectiveness of the aeration, extent of the nitrification process, sludge age and final effluent conditions[1,2]. Classical microfauna analysis is frequently done by microscopic observation and assessment of the different protozoa and metazoa species present. However, this task is not only time-consuming and labour intensive but also requires the expertise of a zoologist or protozoologist. Therefore, digital image analysis can be seen as a useful tool to achieve taxonomic classification and organism's quantification in an automatic, non subjective manner. Some studies have already been carried out using this technique combined with statistic multivariable analysis such as Neural Networks, Discriminant Analysis, and Principal Components Analysis to perform the recognition of protozoa and metazoa commonly present in the aeration tank of wastewater treatment plants activated sludge, including the works of Amaral *et al.* (2004)[3].

In this work an image analysis programme was developed in MATLAB code for the semi-automatic recognition of several groups of protozoa and metazoa commonly present in wastewater treatment plants. The protozoa and metazoa were characterized by different morphological parameters of Euclidean and fractal geometry, with or without their external structures (peduncles, cirri, tentacles). Finally, the morphological parameters (around 40) of the above-mentioned geometries were analysed using the multivariable statistical techniques *Discriminant Analysis* and *Neural Network* to identify and classify each protozoan or metazoan image.

The procedure obtained was adequate for distinguishing between amoebas, sessile ciliates, crawling ciliates, large flagellates and free swimming ciliates in terms of the protozoa classes and also for the metazoa. Furthermore, with the exception of some sessile species, the value of overall species recognition was high. In terms of the wastewater conditions assessment such as aeration, nitrification, sludge age and effluent quality the obtained results were found to be suitable for the prediction of these conditions.

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