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Recognition of protozoa and metazoa using image analysis tools, discriminant analysis, neural networks and decision trees

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Abstract

Protozoa and metazoa are considered good indicators of the treatment quality in activated sludge systems due to the fact that these organisms are fairly sensitive to physical, chemical and operational processes. Therefore, it is possible to establish close relationships between the predominance of certain species or groups of species and several operational parameters of the plant, such as the biotic indices, namely the Sludge Biotic Index (SBI). This procedure requires the identification, classification and enumeration of the different species, which is usually achieved manually implying both time and expertise availability. Digital image analysis combined with multivariate statistical techniques has proved to be a useful tool to classify and quantify organisms in an automatic and not subjective way.

This work presents a semi-automatic image analysis procedure for protozoa and metazoa recognition developed in *Matlab* language. The obtained morphological descriptors were analyzed using discriminant analysis, neural network and decision trees multivariable statistical techniques to identify and classify each protozoan or metazoan. The obtained procedure was quite adequate for distinguishing between the non-sessile protozoa classes and also for the metazoa classes, with high values for the overall species recognition with the exception of sessile protozoa. In terms of the wastewater conditions assessment the obtained results were found to be suitable for the prediction of these conditions. Finally, the discriminant analysis and neural networks results were found to be quite similar whereas the decision trees technique was less appropriate.

Keywords: Discriminant analysis; Decision trees; Neural networks; Protozoa; Metazoa; Image analysis

1. Introduction

Activated sludge process can be briefly defined as a controlled aerobic biological treatment of wastewaters. A biomass, consisting of micro-organisms (mainly bacteria and protozoa, but also fungi and small metazoans) and some other solid particles, is aerated and maintained in a constant state of suspension to ensure the removal of organic matter and nutrients that are used by decomposers [1]. Protozoa and small metazoa predominate among eukaryotes in well-performing plants. Protozoa feed either by grazing on suspended particulate material including bacteria cells or by predation upon other protozoa, and have been shown to attain densities higher than 10^6 micro-organisms mL^{-1} in activated sludge systems [2]. Representatives of all the major taxa have been reported in several plant samples around the world [3–11]. Protozoan taxa in wastewater treatment plants (WWTP) can be classified in terms of flagellates, amoeba and, in particular high numbers, ciliates. The last group is normally divided into free swimming, crawling, carnivorous and stalked ciliates, according to their feeding and motion behavior [2].

Moreover, most samples of activated sludge biomass reveal the presence of larger organisms—small metazoan, like nematodes, rotifers and oligochaete worms, although limited to some simple forms with generation times shorter than the sludge age.

Since the operation in 1922 of the first industrial-scale wastewater treatment plant by activated sludge, a few studies have focused on the significance of protozoa and metazoa in biological wastewater treatment plants. Most of the literature support the view that protozoa play a vital direct role in reducing the numbers of freely suspended and loosely attached bacte-

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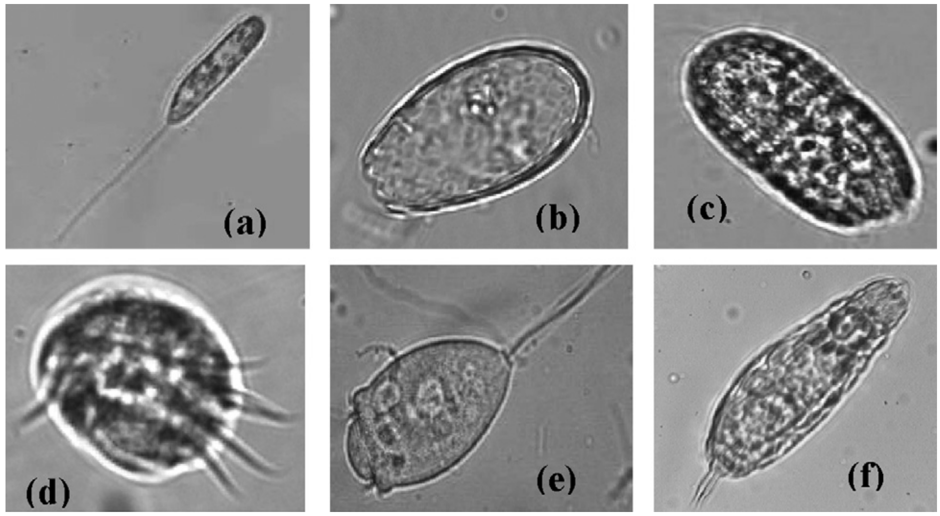

Fig. 1. Examples of protozoan and metazoan present in a WWTP. (a) *Peranema*, (b) *Euglypha*, (c) *Coleps*, (d) *A. cicada*, (e) *Vorticella*, and (f) Rotifer.

rial cells in the bulk liquid by the process of grazing, and thus improve the quality of the final effluent leaving the plant [2]. In the same way, metazoa play an important role in the plant operation as predators, consuming bacterial cells and protozoa. Metazoa seem to contribute to the flocculation process by excreting mucus in which new filamentous and flocculating bacteria may adhere and by breaking up bigger flocs when these organisms move in the medium. Some species representative of the main groups of protozoa and metazoa present in WWTP (flagellates, sarcodines, ciliates, sessile and metazoa) are illustrated in Fig. 1.

Furthermore, protozoa have proved to be excellent tools in assessing and predicting the performance of WWTP and of the final effluent quality [12–21].

The relationship between the clarity and quality of the liquid effluent from the clarifiers and the occurrence of these protozoa and metazoa has led to the suggestion that the presence of particular classes may be used to indicate the overall plant performance [22,23,5,24]. The community structure of protozoa and metazoa rapidly changes as a response to different operating conditions [14], and each plant has a particular faunal structure, so regular monitoring of the plants is important for accurately predicting day-to-day performance. The optimal efficiency occurs when a correct balance between crawling and sessile ciliates and metazoa is achieved. An overpopulation of flagellates, sarcodines or free-swimming ciliates reflects high organic loads (high F:M ratio), whereas the dominance of sessile (stalked) ciliates and metazoa reveals the opposite [25].

Frequent microscopic examination of the biomass may provide a fast, simple and convenient method for indicating sudden changes in the plant performance. Methods based on protozoan population structure have been used to assess activated sludge plant performance. Among them, the Sludge Biotic Index, (SBI), developed by Madoni [2] and inspired in the "Extended Biotic Index" of Woodiwis [26], is the most common method to accomplish the diagnosis of the WWTP operation state using the activated sludge microfauna as biological indicator. This method is based on the abundance and specific diversity of the microfauna community and in the different sensibilities revealed by some protozoan classes to the prevalent physiochemical factors in the system. However, up to date, the identification and micro-organisms enumeration to estimate the SBI have been determined manually, through microscopic observations of activated sludge samples which are highly time-consuming and needs considerable skills in protozoology.

Therefore, automatic image analysis could be seen as a useful tool for performing the taxonomic classification and quantification of organisms in an automatic and non-subjective way. Some studies have already been done using these technique combined with multivariable statistical analysis through techniques such as neural network, discriminant analysis, and principal components analysis in order to perform the recognition of protozoa and metazoa commonly present in the aeration basins of WWTP such as the works of Amaral et al. [27,28] and da Motta et al. [29]. The results of these studies have shown to be promissory for the classification and identification of these classes in a semi-automatic way.

In this work, an image analysis procedure was developed in *Matlab* for the recognition of protozoa and metazoa. Discriminant analysis, neural network and decision trees techniques were employed to allow the recognition of the main protozoa and metazoa found in the WWTP activated sludge.

2. Materials and methods

The protozoa and metazoa classes studied in this work were collected from aeration tanks of WWTPs of Nancy (France) and Portugal treating domestic and industrial effluents.

A total of 22 classes of protozoa and metazoa belonging to several species, genera, orders and sub-classes were included in the study and are presented in Table 1. In all cases the maximum period between the samples collection and the images acquisition did not exceed 3 h, and aeration was provided to the sludge samples during this period.

After the mixed liqueur collection, a drop of the samples was deposited carefully in a slide and covered with a cover