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Development of an image analysis procedure for identifying protozoa and metazoa typical of activated sludge system

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ABSTRACT

A procedure for the semi-automatic identification of the main protozoa and metazoa species present in the activated sludge of wastewater treatment plants was developed. This procedure was based on both image processing and multivariable statistical methodologies, leading to the use of the image analysis morphological descriptors by discriminant analysis and neural network techniques. The image analysis program written in Matlab has proved to be adequate in terms of protozoa and metazoa recognition, as well as for the operating conditions assessment.

1. Introduction

A biological wastewater treatment plant (WWTP) can be considered an artificial ecosystem (Fried et al., 2000), consisting of abiotic and biotic components interacting. The abiotic components are represented by the plant and the sewage, whereas the biotic components comprise the decomposers (bacteria and fungi) that take energy for their growth from the dissolved organic matter and the oxygen in incoming wastewater and by protozoa and metazoa microfauna grazing on the decomposers (Madoni et al., 1993). The microfauna present in the aeration tank of an activated-sludge plant includes protozoa (flagellates, sarcodines and ciliates) and metazoa (rotifers, nematodes, tardigrades, gastrotrichs and oligochaetes) specimens.

Although the bacteria are generally prevalent in the aeration tank, high concentrations of protozoa in the tank normally indicates a good performance. The faunal species distribution and abundance have been pointed out as indicators of the water quality of the effluent issuing from an activated-sludge plant, providing a useful mechanism to evaluate and assess its performance. Several authors have already investigated the importance and role of the protozoa and metazoa community in the purification process of activated-sludge plants (Curds, 1973, 1975, 1982; Curds and Cockburn, 1970a, b; Curds and Vandyke, 1966). The correlation between the plant performance or operational conditions and the abundance of certain species has also been an object of study (Al-Shahwani and Horan, 1991; Esteban et al., 1991; Fried et al., 2000; Madoni 1984, 1994a, b, 2000; Madoni et al.,

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1981, 1993, 1996; Poole, 1984; Poole and Fry, 1980; Salvadó and Gracia, 1993; Curds and Cockburn, 1970b; Nicolau et al., 2001, 2005), which led to the development of a number of methodologies based on protozoa populations structure to assess the activated sludge plant performance, being the sludge biotic index (SBI) of Madoni (1994b) the most known method to accomplish this propose.

Species belonging to the genus *Opercularia* and *Trachelophyllum*, and to the species *Vorticella microstoma* are usually considered indicators of low effluent quality whereas *Aelosoma* sp., *Arcella* sp., *Carchesium* sp., *Epistylis* sp., *Euglypha* sp., *Euplotes* sp., order Monogononta, *Peranema* sp., *Trithigmostoma* sp. *Trochilia* sp., *Vorticella aquadulcis* and *Zoothamnium* sp., are regarded as indicators of a high quality of the treated effluent. Moreover, the organisms belonging to the Nematoda sub-class, *Opercularia* sp. and *V. microstoma* are believed to dominate under poor aeration conditions (below 0.2–0.5 mg $O_2\,L^{-1}$), while *Aelosoma* sp., *Carchesium* sp., *Euglypha* sp., *Arcella* sp., Monogononta order, *Trochilia* sp., *V. aquadulcis* and *Zoothamnium* sp. are indicators of a satisfactory aeration (above 1–2 mg $O_2\,L^{-1}$). The nitrification process can be inferred by the occurrence in high densities of *Aelosoma* sp., *Arcella* sp., *Carchesium* sp., *Coleps* sp., *Epistylis* sp., *Euplotes* sp., *Trochilia* sp. and the Monogononta order. Furthermore, the presence of *Peranema* sp. and *V. microstoma* can be an indication of fresh sludge (few days), while *Aelosoma* sp., *Arcella* sp., *Euglypha* sp., and the Digononta and Monogononta orders have been pointed out as indicators of old sludge (20 days or more).

Usually, the identification and quantification of each protozoa and metazoa species is achieved by microscopic inspection and manual counting, requiring both time and high technical expertise. However, the technological advances and the decrease of computation costs gave the opportunity for new techniques such as image analysis to be used in routine classification and quantification of micro-organisms in an automated and non subjective manner overcoming some of the drawbacks of manual techniques. In image analysis, the inherent accuracy and precision of microscopy techniques and the speed of the hardware computation are combined thus reducing the human error factor.

Image analysis, in a wide sense, can be referred to both the strictly image analysis processes as well as to the overall processes of image capture, processing and analysis (Dougherty, 1994). This technique allows images improvement as well as automatic recognition and identification of patterns, such as arrangements or groups of elements that follow certain characteristics, resulting in a reduction of time and work. Indeed, image analysis has already proved to be a potentially alternative tool to overcome the drawbacks associated to the micro-organisms visual identification and quantification through the studies carried out by Amaral et al. (1999, 2004) and da Motta et al. (2001). In these studies, image analysis was used for identifying protozoa and metazoa commonly present in activate sludge treatment plants, and used straightforward to monitor the plant operational conditions in a way similar to the SBI developed by Madoni (1994a, b).

In the present work, an image analysis procedure, previously developed in *Visilog* by Amaral et al. (2004), was extended to the recognition of other protozoa and metazoa species and adapted to the *Matlab* language. The morphological descriptors obtained after the image analysis were then processed using the discriminant analysis (DA) and neural network (NN) statistical multivariate techniques in order to identify the main protozoa and metazoa found in the WWTP activated sludge.

2. Materials and methods

2.1. Experimental survey

The protozoa and metazoa species studied in this work were collected from aeration basins of the urban section of Nancy (France) and Braga (Portugal) wastewater plants.

A total of 22 groups 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 during this period.

After the mixed liqueur collection, a drop of the samples was carefully deposited in a slide and covered with a cover slip (with addition of methyl-cellulose for the acquisitions between 2002 and 2006 in Portugal) for visualization and image acquisition using the bright field microscopic technique. The total magnification for visualizing and acquiring each protozoa and metazoa micro-organism was dependent on its size as follows: *Aelosoma* sp (25 and 100 times); Nematoda (100 and 250 times); Digononta, Monogonta, *Arcella* sp. and *Euglypha* sp. (250 and 400 times); *Aspidisca cicada*, *Carchesium* sp., *Epistylis* sp., *Euplotes* sp. *Litonotus* sp., *Coleps* sp., *Opercularia* sp., *Peranema* sp., Suctoria, *Trachellophyllum* sp., *Trithigmostoma* sp., *Trochilia* sp. *V. aquadulcis*, *V. microstoma*, *Vorticella* sp. and *Zoothamnium* sp. (400 times). The dimensions of metric units (μm) were correlated with the corresponding pixel units using a micrometric slide.

Among the evaluated groups two different species of *Epistylis*, and *Trachellophylum* were additionally analysed. Moreover, a group of micro-organisms with similar morphological characteristics of *Epystilis* sp. and *Opercularia* sp. was included due to the fact that when these organisms occur with the buccal apparel closed it is quite difficult to distinguish one group from the other. Finally, the frontal and lateral views of *Arcella* sp., *Aspidisca cicada* and *Trithigmostoma* sp. were also analysed, on cause of their axial lack of similitude.

2.2. Image acquisition

The image acquisition system used in Nancy was composed by a *Leitz Dialux 20* optic microscope (Leitz, Wetzlar) coupled to a grey scale video camera *Hitachi CCTV HV-720E(F)* (Hitachi, Tokyo). The images were grabbed to the computer in 768 × 576 pixels and 8-bit format (256 grey levels) by a *Matrox Meteor* frame grabber (Matrox, Montreal) using the *Visilog* 5 commercial software (Noesis, S.A., les Ulis). In Braga, the acquisition system was composed by an optic microscope *Zeiss Axioscop* (Zeiss, Oberkochen) coupled to a *Sony CCD ACV D5CE* grey