SURVEY OF A WASTEWATER TREATMENT PLANT MICROFAUNA BY IMAGE ANALYSIS

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Abstract

The microfauna present in the activated sludge of a wastewater treatment plant is mainly composed by bacteria, protozoa and metazoa. The protozoan species are quite sensitive to physical, chemical and operational processes making them, thus, precious indicators of the state of the plant. Several authors already established relationships between the predominance of certain species or group and some parameters of the plant, such as the biotic indices namely the Sludge Biotic Index. All the above-mentioned procedures demand the identification, classification and quantification of the different species. Normally this is done manually, which implies both time and expertise. In the present work a semi-automatic protozoan recognition procedure by means of image analysis is attempted. The program built for this purpose (ProtoRec v.3) was also used to study the evolution of the microfauna during transient operation times (stoppage and re-run). The results were rather satisfactory in terms of protozoa recognition and the survey of the transient phase allowed verifying the aging and degradation of the microfauna by means of the different predominant species.

1. Introduction

In the aeration tank of a wastewater treatment plant bacteria agglomerate generally in flocs. Attached to these flocs and swimming in the surrounding liquid one can find also bacteria, protozoa and metazoa may also be found. Interactions between these groups include predation (I), competition (II) and cannibalism (III) relationships, as seen in figure 1.

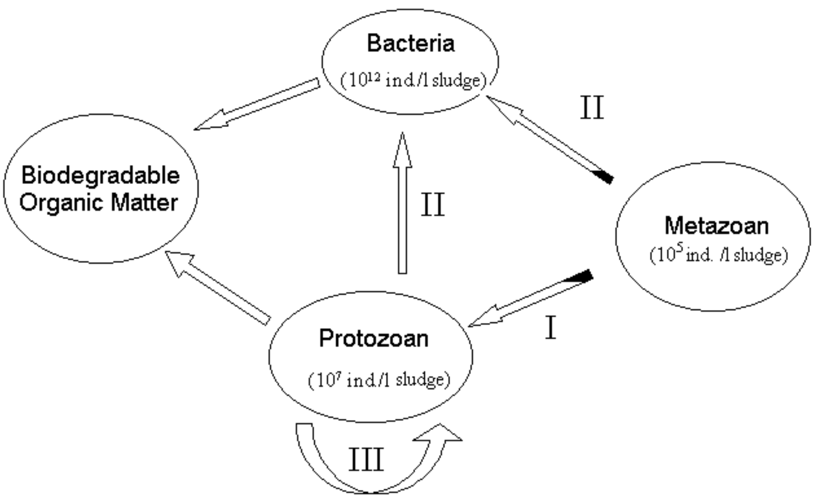

Figure 1 – Relationships between the different microorganism species in the aeration tank (Canler *et al.*, 1999)

The protozoan presence is fundamental for the good performance of the plant. As well as decreasing the turbidity of the final effluent they also stimulate the bacterial growth, promote the flocculation of the sludge and some species are responsible for the elimination of the pollution (Gerardi *et al.*, 1995; Curds *et al.*, 1968).

After bacteria, protozoa are the most important microorganisms in wastewater treatment. Their high sensibility to the variations of their environment (substrate and dissolved oxygen concentration, toxics presence, etc) makes them excellent indicators of the plant state (Degrémont, 1989).

The importance of the protozoan species, mainly bacteriophages, can be summarized as follows: by grazing the free bacteria they decrease the turbidity of the effluent as well as the Biochemical Oxygen Demand (BOD), the dry mass percentage and the pathogenic bacteria (95% and 50% respectively with and without their presence, accordingly to Gerardi *et al.*, 1995); their bacteriophage regime stimulates the bacterial growth and the development of new flocs. One final remark, although subject to controversy is that the protozoa can eliminate pollution in terms of organic matter.

Protozoan species can be classified in terms of flagellates, ciliates and amoebae. The ciliates, present in higher concentrations, can be also classified accordingly to their feeding regime into bacteriophages and carnivores. They are also classified accordingly to their mobility in free swimming, crawling and stalked ciliates.

Several authors studied the different ciliate species present in an aeration tank in order to identify them (Curds and Cockburn, 1970a; Sasahara and Ogawa, 1983), as well as the correlation between the plants performance and the abundance of certain species (Curds and Cockburn, 1970b; Madoni, 1994a; Nicolau *et al.*, 1997). Table I summarizes the relationships between each predominant protozoan group and plant efficiency, as well as probable problem causes.

Table 1 – Relationships between protozoa and plant efficiency (Madoni, 1994a)

Predominance	Efficiency	Probable cause
Small flagellates	Bad	Low oxygenated sludge; high organic load; presence of fermentescible substances.
Small swimming ciliates (< 50 µm)	Mediocre	Low contact time, low oxygenated sludge.
Large swimming ciliates (> 50 µm)	Mediocre	High organic load.
Crawling ciliates	Good	
Stalked + crawling ciliates	Good	
Stalked ciliates	Decreasing	Transient phenomena (discontinuous load; recent sludge removal).
Small amoebae and flagellates	Bad	High organic load with low biodegradability.
Testate Amoebae	Good	Low organic load; good nitrification.

The sludge colonization starts with the bacteria, flagellates and free-swimming ciliates that are normally present in the incoming effluent. As the sludge grows old the crawling and the stalked ciliates begin to settle. Contrarily to the first species these two last ones return by the recirculation channel. The reduction of the bacteria is due primarily to the bacteriophage regime of the protozoa species. Figure 2 summarizes the evolution of the different microorganisms as function of the sludge age.

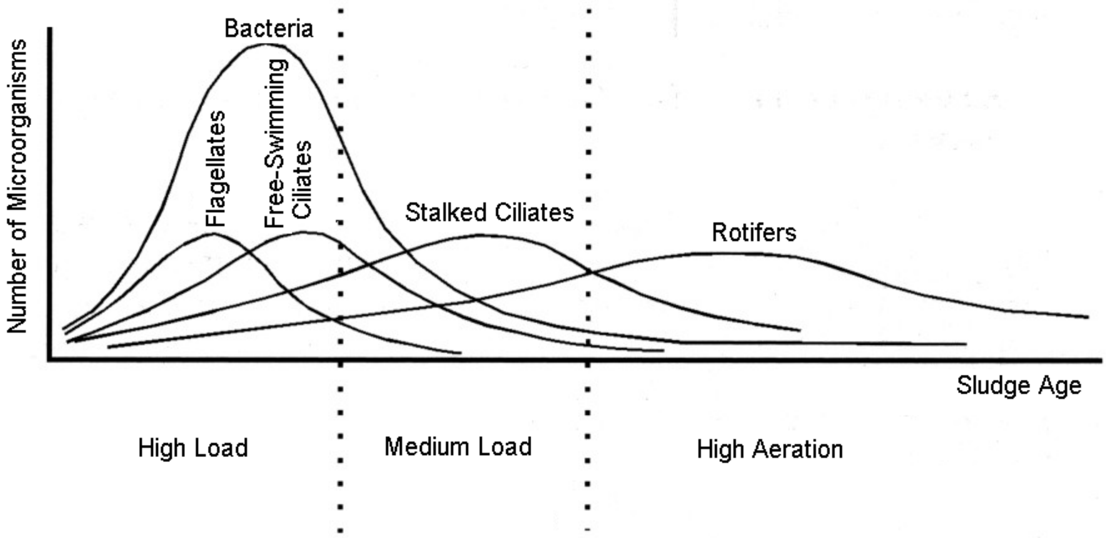

Figure 2 – Evolution of each microorganisms group as a function of sludge age (Canler *et al.*, 1999).

The Sludge Biotic Index (SBI) proposed by Madoni (1994b) uses the plant ecosystem sensibility on the physical, chemical and operational conditions and is based on the different sensitivities of each protozoan group to the above-mentioned parameters. In order to quantify the different protozoan species identification and classification steps, are needed in the first place.

Normally the identification and quantification of each species is done by visual inspection and manual counting, which requires both time and expertise. In the present work, a semi-automatic program (ProtoRec) was developed aiming to identify by means of morphological descriptors the different protozoan species (Amaral *et al.*, 1999; Da Motta *et al.*, 2001).

2. Materials and Methods

Activated sludge samples were gathered from an aeration tank of a wastewater treatment plant in Braga. A drop of each sample was mounted on a microscope slide and the image acquisition was done by direct illumination without the addition of any dyes. From each sample images of 50 individuals were acquired.
The image acquisition system consists of an Axioskop epi-fluorescence microscope (Zeiss, Oberkochen), connected to a CCD AVC D5CE camera (Sony, Tokyo). The signal is then treated by a CMA-D5CE adaptor (Sony, Tokyo) and is finally digitized by a Meteor 2 frame grabber (Matrox, Dorval).

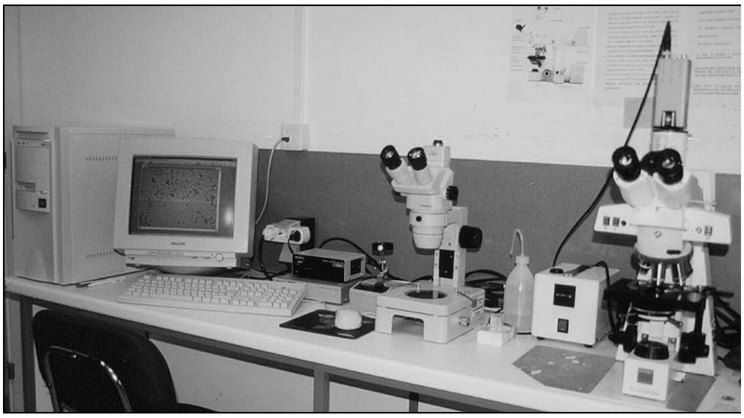

Figure 3 – Image acquisition system

Figure 4 shows some of the main steps of the ProtoRec v.3 program. First, the original image (256 gray scales) is pre-treated to enhance the protozoon contour. Secondly the regions of interest are traced by the user and the resulting image is consequently thresholded. Small debris are then removed and the protozoa are labeled. Finally the morphological parameters of each protozoan are determined. The identification step is obtained by comparison of these values to a protozoan database.

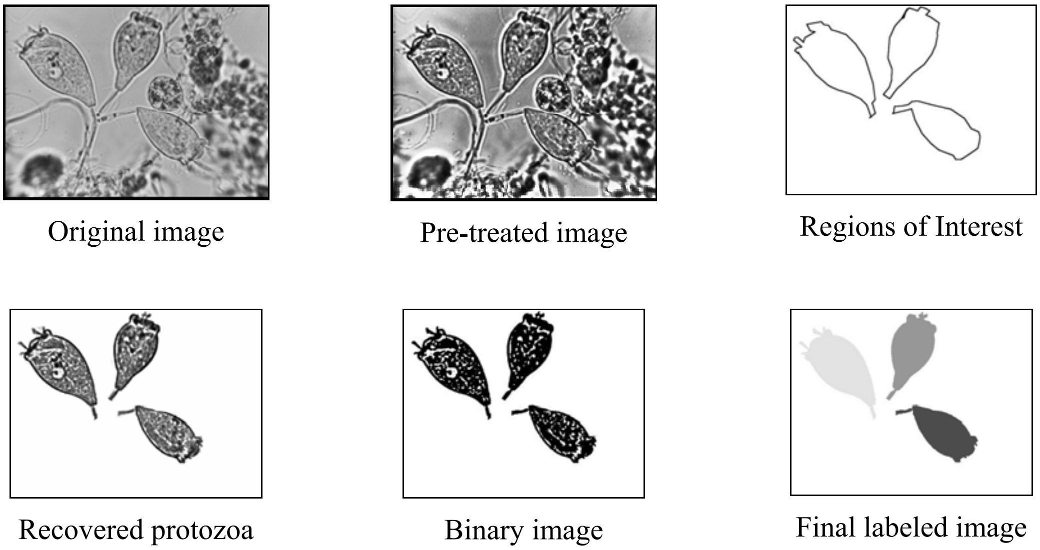

Figure 4 – Main steps of ProtoRec v 3

The morphological parameters determined by ProtoRec v.3 are the following: Area, Equivalent Diameter, Perimeter and Feret Shape; Eccentricity (Glasbey, 1994); Length, Shape factor, Width, Compacity, Convexity and Solidity (Russ, 1994); Robustness, Largest Concavity Index, Concavity Ratio and Euclidean Distance Fractal Dimension (Pons, 1999); Mean Width and Mean Width / Width. These parameters are determined for both the entire protozoan and the protozoan body. The following parameters are also determined: Body Perimeter / Perimeter, Stalk Mean Width, Stalk Mean Width / Body Mean Width.

3. Results

During the Winter of 2000/01, Braga went trough heavy rain falls, which gave rise to several problems in the wastewater treatment plant, caused by the high loading rates and sand build up leading to a cleaning stoppage. During this time the microfauna of the plant was surveyed starting from 19/03/01 when the plant was yet functioning normally. At 20/03/01 the incoming effluent was suspended (both recirculation and aeration were kept running), at 26/03/01 (last day of the survey) the recirculation was stopped and in the next day the aeration was also stopped.

The results obtained from this survey allowed for the manual analysis of the microfauna of the aeration tanks in a transient phase of the plant.

3.1. Manual Analysis of the Microfauna

As suggested by figure 2, the aging process of the sludge can be monitored by the evolution of the protozoan and metazoan groups present. First, a reduction of the swimming ciliates (*Trachelophyllum*) was observed due to their removal in the settling tanks. This phase was followed by the increase in the crawling ciliates (feeding on floc surface bacteria). As this substrate decreased the number of the crawling ciliates was also reduced. Stalked ciliates that initially had decreased their number increased again, mainly by the presence of O*percularia*, which is resistant to stress conditions such as oxygen or nutrient limitation. Analyzing table I it is clear that the plant suffered a breakdown in its efficiency. Given these results it might be concluded that up to 3 days of stopping influent feeding the incoming effluent suspension (keeping both recirculation and aeration running) the plant could be easily re-started.

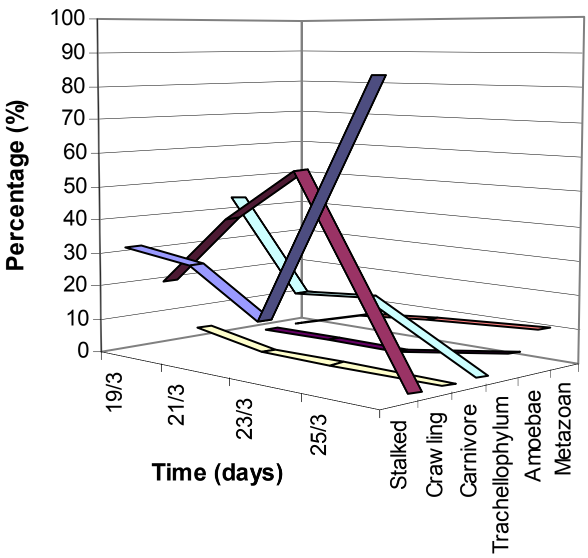

Figure 5 – Evolution of the different protozoan and metazoan groups as a function of time.

Figure 6 allows registering the reduction of the microfauna diversity: from 11 different species observed in day 1 to only 5 at the end of the survey.

Initially there is a strong predominance of the swimming ciliate *Trachelophyllum* with 45 % of the total protozoan and metazoan species. This ciliate feeds on bacteria and small flagellates and is present at any loading rate, but according to Canler *et al.* (1999) its concentration increases with higher loading rates. Its predominance is related with a mediocre depurative efficiency and is an indicator of transient phenomena such as overloads.

At day 3 *Trochilia* predominated, which is an indicator of a good effluent quality and is usually present in plants in steady state operation (Canler *et al.*, 1999). Its presence may indicate the catabolic process where bacteria consume their energetic reserves, producing mineral salts such as the nitrates.

Finally a strong predominance of *Opercularia* was encountered. This protozoan is resistant to stress conditions such as the presence of certain toxics (mineral salts, heavy metals, etc), acidity and lack of aeration, which reflects mediocre to sufficient efficiencies and high concentrations of NH_4^+ in the treated effluent.

This survey allows confirming the reduction in the depurative efficiency after 6 days without fresh effluent. The organic matters catabolism accounts for the probable presence of NH_4^+ in the final effluent.

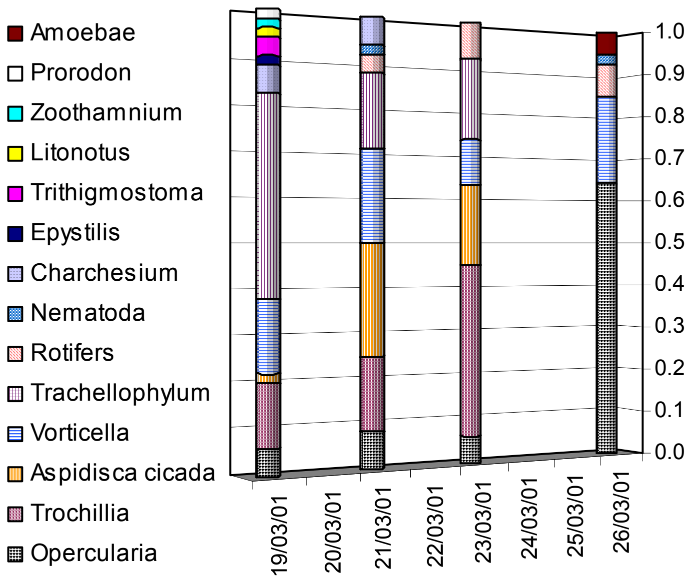

Figure 6 – Evolution of the protozoan and metazoan species present as a function of time.

3.2. Species Recognition with ProtoRec v.3

The program ProtoRec v.3 was used to determine the above-mentioned morphological parameters. These parameters were furthermore treated with the multivariate statistical technique Discriminant Analysis (Einax *et al.*, 1997). This technique has two major advantages: it decreases the number of relevant parameters (calculated as a linear combination of the initial parameters) and allows for a better separation of different groups of objects.
A total number of 50 individuals of each protozoon species was used in the discriminant analysis (except for *Trochilia* with only 37 individuals) and further 50 (of each specie) were used for the validation process. In the latter process, however, several species did not possess this number of individuals, which was the case of *Carchesium* (35), *Digononta* (17), *Nematoda* (7), *Aelosoma* (16) and *Suctoria* and *Trochilia* for which an estimate from the original values had to be made.

Although *Nematoda* (Class), *Suctoria* (Sub-class), *Monogononta* (Order) and *Digononta* (Order) are not species they are referred as species in table II for simplification reasons.

The results obtained for the validation are represented in table II.

Table II – Recognition percentages for each species.

<table>
<tr><th></th><th>% REC.</th><th></th></tr>
<tr><td>NEMATODA</td><td>100 %</td><td rowspan="4">Very Good</td></tr>
<tr><td>SUCTORIA</td><td>98 % *</td></tr>
<tr><td>TROCHILIA</td><td>95 % *</td></tr>
<tr><td>LITONOTUS</td><td>90 %</td></tr>
<tr><td>PERANEMA</td><td>88 %</td><td rowspan="9">Good</td></tr>
<tr><td>ARCELLA</td><td>86 %</td></tr>
<tr><td>TRACHELLOPHYLLUM</td><td>86 %</td></tr>
<tr><td>EUPLOTES</td><td>84 %</td></tr>
<tr><td>AELOSOMA</td><td>81 %</td></tr>
<tr><td>EUGLYPHA</td><td>80 %</td></tr>
<tr><td>ASPIDISCA CICADA</td><td>78 %</td></tr>
<tr><td>VORTICELLA AQUADULCIS</td><td>78 %</td></tr>
<tr><td>MONOGONONTA</td><td>76 %</td></tr>
<tr><td>TRITHIGMOSTOMA</td><td>74 %</td><td rowspan="6">Reasonble</td></tr>
<tr><td>DIGONONTA</td><td>71 %</td></tr>
<tr><td>VORTICELLA MICROSTOMA</td><td>70 %</td></tr>
<tr><td>VORTICELLA CONVALLARIA</td><td>66 %</td></tr>
<tr><td>EPISTYLIS</td><td>56 %</td></tr>
<tr><td>ZOOTHAMNIUM</td><td>52 %</td></tr>
<tr><td>CARCHESIUM</td><td>43 %</td><td rowspan="2">Poor</td></tr>
<tr><td>OPERCULARIA</td><td>42 %</td></tr>
</table>

* Estimates

The average recognition value for each species was 75%.

The results obtained for the classification in the main protozoan and metazoan groups are in table III. Within the ciliate group it is also useful to perform another classification (table IV).

Table III – Recognition percentages for each group.

	% REC
CILIATES	94 %
FLAGELATES	88 %
METAZOAN	86 %
TESTATE AMOEBAE	83 %

Table IV – Recognition percentages for each ciliate class.

	% REC
CARNIVORES	94 %
CRAWLING	92 %
STALKED	90 %
FREE SWIMMING	86 %

The average recognition value for each group was 92% and for each class within ciliates 91%.

4. Conclusions

The results obtained with ProtoRec can be regarded as quite promising. The average recognition value for each species was 75%, for each group 92% and for each class within ciliates 91%.

The wastewater treatment plant survey allowed for the identification of initially free swimming ciliates (*Trachelophyllum*), followed by crawling ciliates (*Trochillia*) and finally stalked ciliates (*Opercularia*) as well as some metazoan species.

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