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Effects of influent composition on activated sludge protozoa

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ABSTRACT

In the present study the characteristics of activated sludge protistan community in two sequential batch reactors operating with activated sludge (SBR) and with activated sludge and plastic biofilm carriers (SBBR) was assessed in terms of species present, biovolumes of the species and their statistical importance to the treatment efficiency. The study identified two important factors affecting the protistan community. These factors were the influent composition and the presence of biofilm carriers. Statistical analysis revealed that protozoan species observations may be used as indicators for the determination of trophic relations in the activated sludge and thus comprise precursors for the prediction of effluent quality.

Keywords: Activated sludge; Factor analysis; Municipal wastewater; Protistan microfauna; Synthetic wastewater

1. Introduction

Activated sludge is a widely used process, based on the development of appropriate bacterial aggregates and other associated organisms in an aeration process. The use of ciliated protozoa found in activated sludge biocenosis, as indicators of operational efficiency has been widely advocated [1–3]. Microfauna play an essential role in the whole process by removing dispersed bacteria, through grazing, which otherwise may result in high turbidity effluents [4,5]. Moreover microfauna reported to reduce sludge production [6,7], have an effect on floc size distribution through predation activity [8] and increase settleability in the secondary sedimentation tank by increasing effluent quality [9]. Protozoa have been used for the assessment of operation problems in wastewater treatment plants [10,11]. Several studies have been reported towards the correlation of protozoan populations to the treatment parameters and the effluent quality; these studies were based on monitoring the protozoan microfauna in municipal activated sludge plants [10–13]. In the last decade, Madoni [1,14] exploited the potential use of protozoa as indicators of wastewater treatment efficiency. The inter and intra species relationships have been categorized and quantified in order to comprise an index, the sludge biotic index (SBI) that can be used to evaluate the sludge quality and consequently the potential efficiency of the activated sludge in degrading influent pollutants. However, influent composition has an important role in the formation of microfauna. Particularly successful settling has been reported in good colonized activated sludge [15,16]. These characteristics are in turn dependent on factors such as retention time, oxygen concentration, predation/grazing effects, levels of filamentous bacteria and floc size [17].

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An alternative to conventional activated sludge systems, sequential batch reactors (SBR) have gained great attention due to their improved treatment performance and their minimal space requirements, however, this operational scheme pose stressful conditions to the activated sludge microorganisms, which have to acclimate to the intermittent feeding mode, thus the dominant organisms will be those that are the most adapted to such conditions [18]. Additionally the mixing bed biofilm processes promote attached growth biological treatment [19] and thus the dominance of sessile species is favoured. This process is based on the use of suspended polymeric carriers where biomass grows as a biofilm. Comparing this method to other types of biological treatment processes, such as suspended growth activated sludge the main advantages are [20]:

1. higher biomass concentrations in the aeration tank, which correspond to lower wastage of biomass
2. minimization of long sludge settling periods
3. aerobic and anoxic metabolic activity within the same biomass ecosystem
4. up – grading of existing wastewater treatment plants at a minimum cost
5. lower sensitivity to toxic effects, as well as to other adverse environmental conditions and
6. head loss through the reactor is insignificant.

Studies have investigated the use of activated sludge ciliated protistan species as indicator species for the treatment efficiency and/or possible operational problems in both activated sludge and biofilm operations. It has been stated the number of ciliated protozoans living in a normally functioning activated-sludge plant is about 10^6 individuals/L. When the numbers fall below 104/L, it indicates insufficient purification [21]. In this case, there is a proliferation of dispersed bacteria which renders the effluent turbid and consequently causes a greatly increased biological oxygen demand (BOD) in the output water. Some authors were able to demonstrate that the number and diversity of ciliate communities change according to the quality of the influent settled-sewage and operating conditions of the plant [1,22]. Nevertheless, due to the large variability in size among ciliate species in wastewater treatment processes, the counts of ciliate numbers may often result only in approximate estimates of the mass of these organisms in both activated sludge and biofilm. The colonisation dynamics in a full-scale activated-sludge plant and in an RBC pilot-rotating biological contactors plant were studied [1,22]. Although these investigations have provided useful information on various aspects of activated-sludge biota, they were based on continuous processes having a typical municipal wastewater influent. The aim of this study was to correlate the activated sludge microfauna to the treatment efficiency in two activated sludge reactors, one supplied with biofilm carriers, operating under intermittent feeding, with two influent types of wastewater, synthetic and municipal wastewater.

2. Materials and methods

2.1. Bench scale reactors

Both reactors were constructed from Plexiglas, having a total volume of 7 L. The content of the reactors was mixed with a magnetic stirrer, while sufficient aeration was provided by suitable ceramic diffusers located near the bottom of the reactor and producing medium size bubbles. Peristaltic pumps were used for the feeding and withdraw of the treated effluent (Fig. 1). In one of the reactors (SBBR) 50% of the working volume (5 L) was covered by plastic biofilm carriers by Anoxkaldnes™. The biofilm carriers (model K1) were of 7 mm length and 9 mm diameter having a total surface area of 800 m^2/m^3 and 500 m^2/m^3 of protected surface.

2.2. Operation of the bench scale reactors

Both reactors were operated under 2 daily cycles, which was controlled by a computer-assisted program. The 12 h cycle comprised by the following steps:

1. 2 h feeding of the reactor with stirring
2. 6 interchanging aerobic and anoxic stages having 4 h of aeration and 2 h of anoxic stage
3. 2 h for settling of the biomass
4. 2 h decanting

2.3. Wastewater composition

Two types of wastewater influent were fed to reactors in two different periods each comprised of 90 days operation time. In the first period synthetic wastewater was prepared daily and supplied to the systems. The synthetic wastewater used in the study was composed

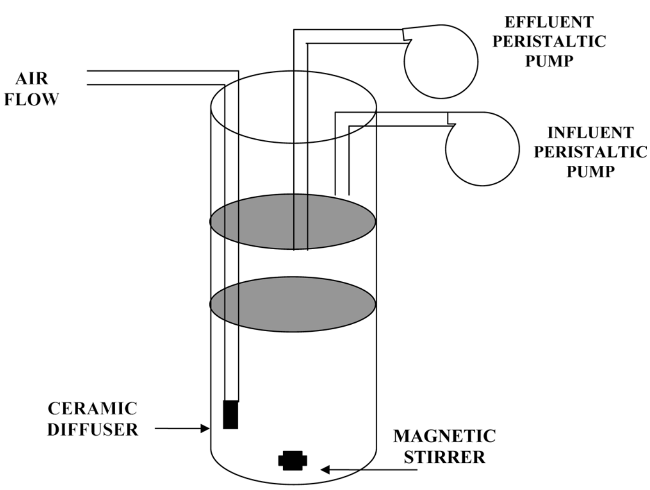

Fig. 1. Schematic diagram of lab-scale sequencing batch reactors (SBR).

of sodium acetate CH_3COONa 2.472 g/L (PANREAC) as carbon and energy source, while NH_4Cl 60 mg/L (PANREAC), Na_2HPO_4 18.7 mg/L (PANREAC), $MgSO_4{\cdot}7H_2O$ 100 mg/L (MERCK) and $NaHCO_3$ 1.3393 g/L (PANREAC) were used as the required nutrients. In order to obtain a nutritionally balanced wastewater, the composition of the synthetic wastewater was adjusted to yield a COD/N/P ratio of 100/5/1.5 with initial content of COD of 1200 ± 50 mg/L, TN = 60 ± 3 mg/L and P = 18 ± 2 mg/L [23].

In the second period wastewater was obtained 3 times a week from the primary sedimentation tank of a municipal activated sludge plant in the area of Thessaloniki. The composition was variable, COD values ranged from 156 to 800 mg/L, BOD_5 values ranged from 64 to 230 mg/L, nitrogen ammonia and total phosphorus ranged from 32 to 58 mg/L and from 15 to 27mg/L respectively.

2.4. Physicochemical analysis

Samples were collected from the influent, effluent and the mixed liquor of the aeration tank and were analysed for the measurement of the following parameters according to standard methods of analysis [24] COD, BOD_5, mixed liquor suspended solids (MLSS), effluent suspended solids (SS), nitrogen nitrate, nitrogen ammonia and phosphorus.

2.5. Microfauna identification, enumeration and biovolume calculation

For the analysis of protozoan community, aliquots of 200 μL were collected from the activated sludge of both bench scale reactors, during the aeration phase. Analysis was conducted for the identification of species in vivo using an optical microscope (OPTIKA). Direct counting procedures were carried out at magnifications of 10×, 40× and 100× magnifications, according to the sizes of species, and were expressed in number of individuals per ml of mixed liquor. Identification of protozoa was mainly based on morphology and movement based on identification keys [25,26]. Protistans were counted by placing the sample on a Fuchs–Rosenthal 3.2 μL chamber. The results for each sample were determined by averaging the counts of three replicates.

In the case of SBBR the protistan community on the plastic biofilm carriers was also examined. Calculation of the biovolume was conducted by the measurement of the dimensions of each species under the microscope. The shape of each organism was simulated to the nearest 3-D geometrical shape and the mean genus biovolume was estimated in μm^3. Conversion of the cells abundance (cells/mL) to biovolume (μm^3/mL) was necessary because of the differences in cell size of eukaryotic microorganisms [27].

2.6. Statistical analysis

Two statistical techniques were selected for data analysis and evaluation: Pearson's correlation coefficients and factor analysis (factor extraction method: principal components with varimax rotation), enabled by STATISTICA 7.0®. The abiotic variables selected for the correlation analysis were effluent organic loading (BOD_5, COD); effluent nitrogen content (NH_3-N, NO_3-N, TP); effluent phosphorus; mixed liquor suspended solids (MLSS). Factor analysis enables the correlations between variables of interest as reflecting putative underlining of causes or factors [28]. Because variables (species abundance) are assumed to respond linearly to the underlying factors effective results are expected for data with a small range of community variation such as that of the activated sludge. The biotic factors were selected according to protistan species frequency and abundance of each taxon [28].

3. Results and discussion

The operation time of the bench scale reactors was divided into two periods depending upon the influent wastewater composition:

1. Introduction of a synthetic wastewater
2. Introduction of municipal wastewater

3.1. Introduction of synthetic wastewater

In the SBR removal of COD ranged from 80 to 90% and BOD_5 ranged from 80 to 100%, while nitrogen ammonia ranged from 45 to 85% and phosphorus removal ranged from 60 to 80%. It should be underlined that in both systems the treatment efficiency increased with the operation time, suggesting the significance of acclimatization of the activated sludge organisms both to intermittent feeding conditions and to the synthetic wastewater as a carbon source. Protistan community of the activated sludge was co dominated by sessile and carnivorous species (Fig. 2). Free swimming ciliates and amoebae were present in 13 and 7% of the total protistan community.

In the SBBR COD, BOD_5 and nitrogen ammonia showed comparable removal efficiencies to SBR, reaching up to complete removal however, phosphorus removal

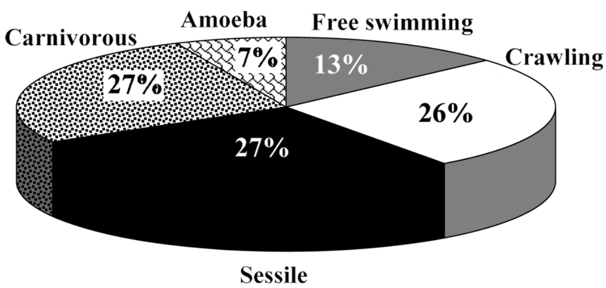

Fig. 2. Mean abundance of protistan community in SBR.

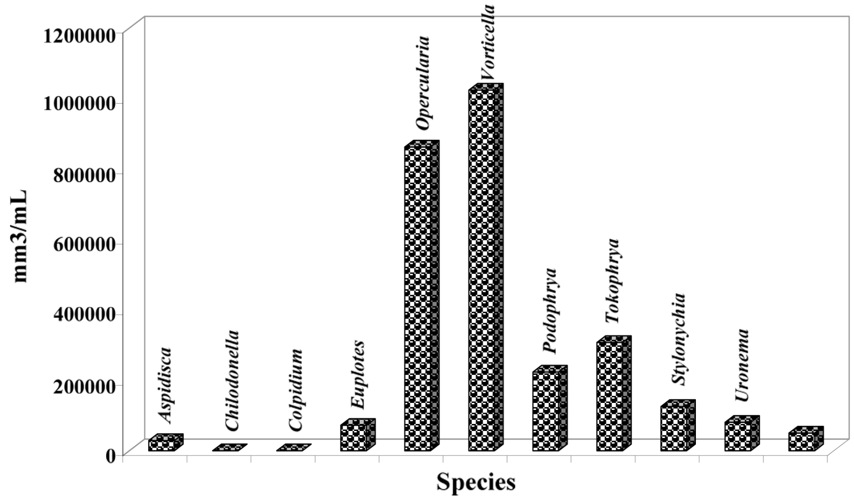

Fig. 3. Biovolume of each species found in SBR.

was achieved up to 89%. Protistan community comprised mainly of sessile species which occupied 46% (Fig. 4). Carnivorous species were present at 24% of the total protistan community, while free swimming and crawling ciliates were present at 6 and 12% respectively, however, increased presence of amoebae was observed in the SBBR reactor compared to the SBR.

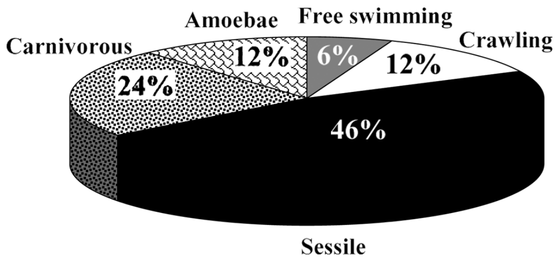

Fig. 4. Mean abundance of protistan community in SBBR.

The biovolume of each of the species found in the reactors are shown in Figs. 3 and 5 for the SBR and SBBR respectively. Eleven and nine ciliate species were observed in SBR and SBBR respectively. *Opercularia* and *Vorticella* sp. were the most dominant species in both reactors and their biovolume was significantly higher comparing to the rest of the species, however, in the case of SBBR both species occupied percentage of the total ciliated community and thus their presence is of increased importance in the treatment process. *Podophrya* and *Tokophrya* sp. were also found to occupy increased volume of the activated sludge protistan microfauna. It must also be noted that biovolumes of all species were higher in SBBR rather than in SBR, suggesting that the presence of the biofilm carriers enhance the increase of sessile colony forming species abundance.

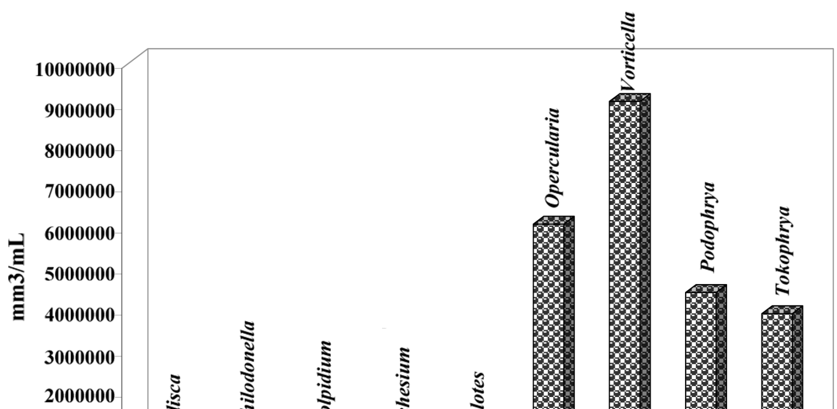

Fig. 5. Biovolume of each species found in SBBR.

Factor analysis for the SBBR and SBR is presented in Figs. 6 and 7 respectively. In the case of SBR, the two factors explained 66.96% of the total variance. *Aspidisca*, *Euplotes* and *Colpidium* sp. showed the strongest negative correlation with the effluent BOD_5 values, suggesting that these species mostly contribute to the organic content removal efficiency of the SBR system. Stylonychia and *Chilodonella* sp. exhibited inversely proportional relationship

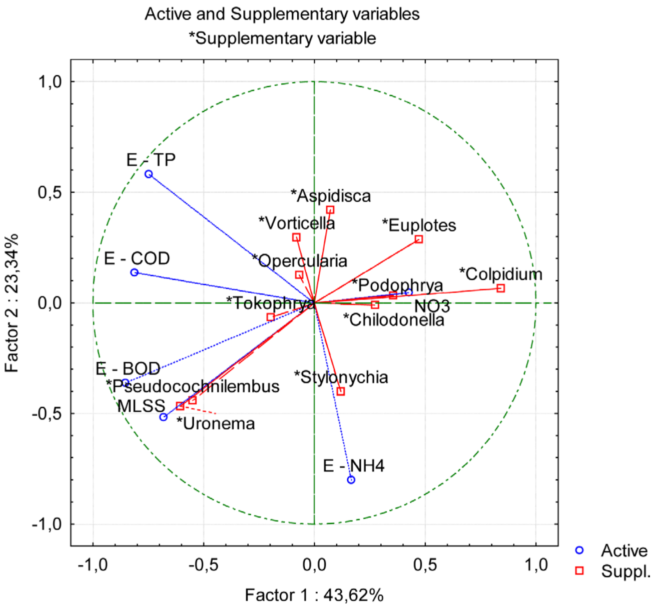

Fig. 6. Factor analysis of SBR operating under synthetic wastewater influent, explaining 66.96% of the total variance.

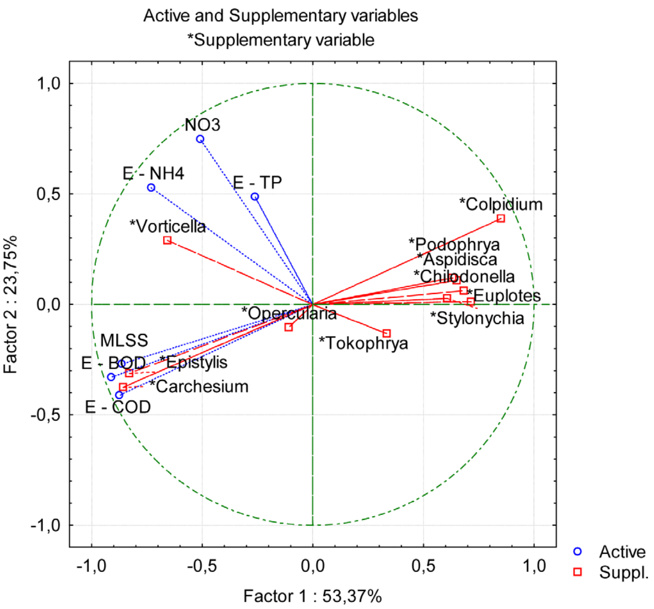

Fig. 7. Factor analysis of SBBR operating under synthetic wastewater influent, explaining 77.1% of the total variance.

to effluent COD, while *Stylonichia* sp. was also positively correlated to the efficiency of nitrogen ammonia removal.

In the SBBR factor analysis explained 77.1% of the total variance. *Podophrya*, *Colpidium*, *Chilodonella*, *Aspidisca*, *Euplotes* and *Stylonychia* sp. showed negative correlation to effluent organic load suggesting that these species are mostly responsible for the organic load removal. *Epistylis* and *Carchesium* sp. showed strongly positive relationship to MLSS content suggesting that the increase of these species attribute to the increase of the MLSS. Nutrient removal in the case of SBBR operating under synthetic influent showed no strong relationships with any of the species found.

3.2. Introduction of municipal wastewater

Variations of the treatment efficiencies were observed as a result of the variable influent quality. Although the organic load removal efficiency was comparable to that of the period in which it operated under synthetic wastewater, the nitrogen and phosphorus removal efficiency was decreased and variable throughout the operation time. In general variability of the removal efficiencies were in accordance to influent composition variations. Increased nutrient removal was observed in the SBBR comparing to the SBR, where TN and $N\text{-}NH_3$ reached 85 and 90% removal respectively.

The composition of the protistan communities in SBR and SBBR operating with raw sewage are shown in Figs. 8 and 10 respectively. Higher abundance and biodiversity of species was observed in SBR during this period. Families with the higher abundances were represented by more species than those present in the period of operation with the synthetic substrate. Sessile species were more abundant in the SBBR, while species such as *Vorticella* and *Opercularia* were present in comparable abundances both occupied 30% and 42% of the total sessile species abundance in SBR and SBBR respectively. Carnivorous species and free swimming ciliates were more abundant in the case of SBR than in the SBBR. Aspidisca sp showed increased abundance in the SBR system. *Podophrya* and *Tokophrya* sp during this period exhibited an inversely proportional relationship. In the SBR *Podophrya* occupied a significant part of the protistan microfauna (Fig. 9), while in SBBR *Tokophrya* co dominated in comparable

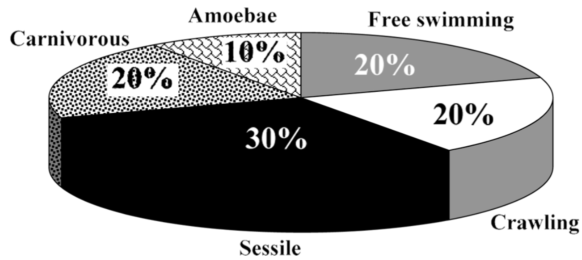

Fig. 8. Mean abundance of protistan community in SBR.

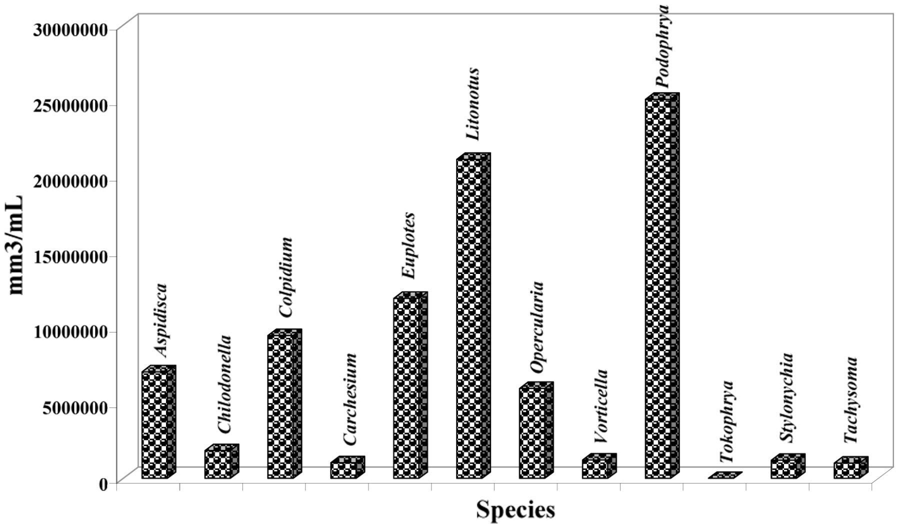

Fig. 9. Biovolume of each species found in SBR.

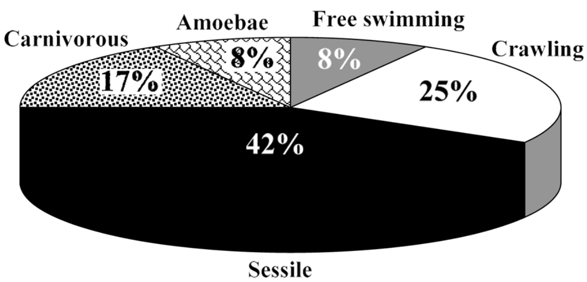

Fig. 10. Mean abundance of protistan community in SBBR.

biovolumes with *Opercularia* sp., with *Litonotus* sp., while Metopus sp to occupied greater part of the activated sludge protistan community (Fig. 11).

Factor analysis revealed two factors explaining 50.47% and 65.06% of the total variance for SBR and SBBR respectively (Figs. 12 and 13). The most significant negative relationships for the SBR were between *Euplotes* sp. and nitrate removal as well as between *Podophrya* sp. and COD and MLSS removal. In SBBR Pokophrya and *Tokophrya* sp. showed significant correlations between COD and MLSS removal, however, the strongest correlation was observed for the latter. *Metopus* sp. showed the strongest relationship to the nitrification process. However, it should be noted that the strongest relationships between the protistan species were found in the first period of operation rather than the second one.

Microfauna community structure of activated sludge in both reactors under different influent conditions pre-

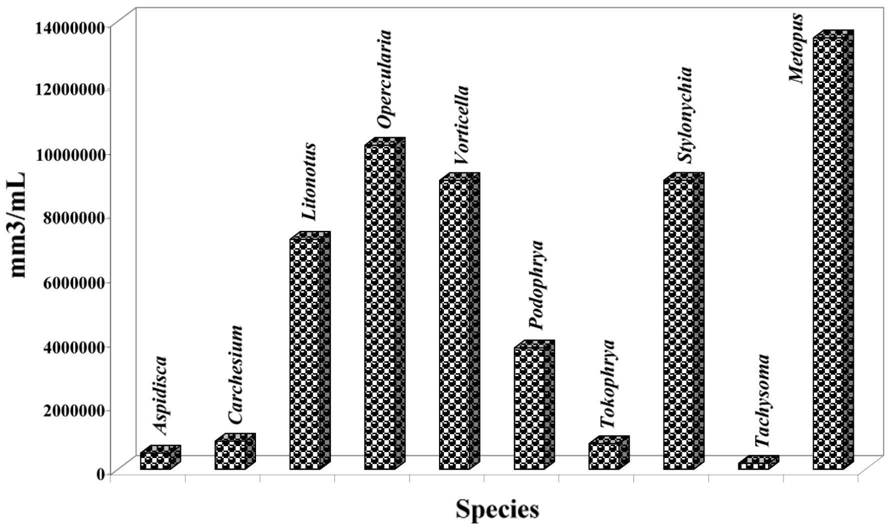

Fig. 11. Biovolume of each species found in SBBR.

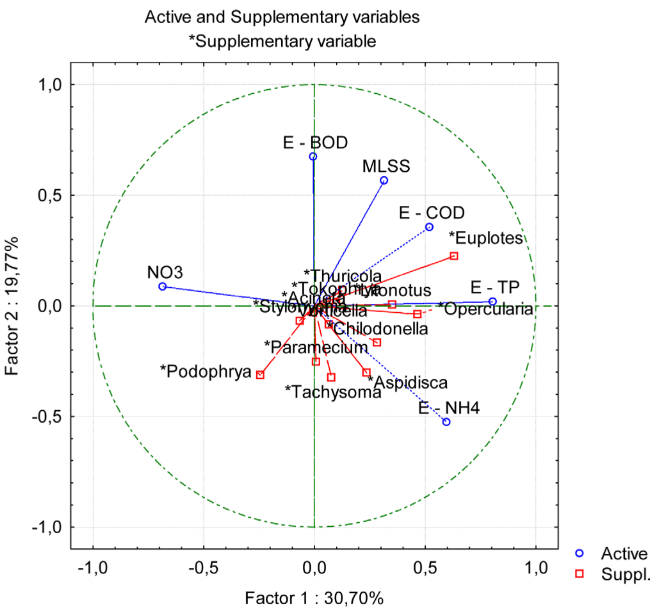

Fig. 12. Factor analysis of SBR operating under municipal wastewater influent, explaining 50.5% of the total variance.

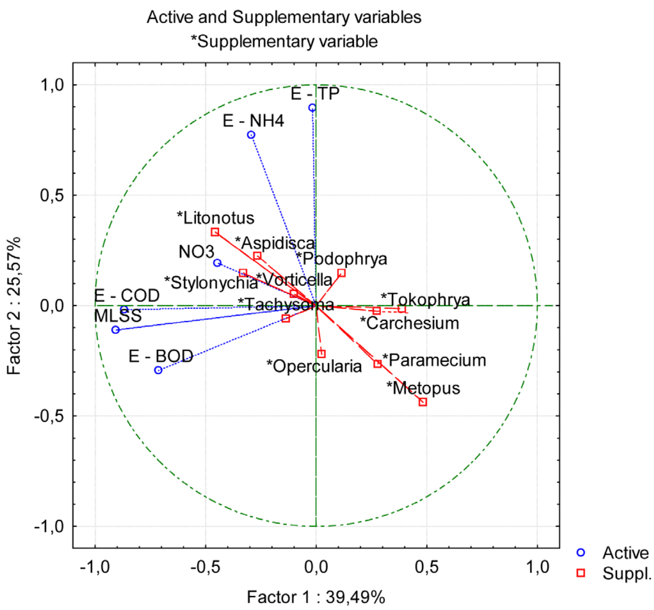

Fig. 13. Factor analysis of SBBR operating under municipal wastewater influent, explaining 65.1% of the total variance.

sented a general common characteristic, the dominance of sessile species. However, significant differences were observed in the diversity and abundance of the species found not only between the different periods of operation but also in the same period at the different reactors. The differences in protistan community and species abundance between various operational parameters and/or influent quality have been also observed in other studies [29]. The raw wastewater influent was able to support greater biocenosis than the synthetic influent, this may be attributed to the greater biodiversity of bacteria, which constitute the basic prey for most of the protistan species [1,2]. Moreover the increased biovolume of the species found in the reactors operating under raw wastewater increased sedimentation ability of the sludge [27], which under great fluctuations of F/M ratio may be deteriorated [28]. In the case of the reactors operating under synthetic wastewater the good sedimentation ability can be attributed to the increased abundance of the carnivorous protistan species that are usually favoured under synthetic wastewater influent [30]. The parameters assessed in this work are mainly related to the removal of organic matter and nutrients. The statistical analysis showed very good explanation percentages 66.96 and 77.1% of the total variance for the SBR and SBBR respectively in the cases of synthetic wastewater influent, although that low species diversity was observed. However, in the second period of operation under the municipal wastewater the greater biodiversity didn't enhance the relationship between the treatment efficiency and the protistan community, since low correlations were revealed by the statistical analysis. It should be underlined that changes in the C:N:P ratio, which are frequent in the case of raw wastewater entering a wastewater treatment plant may shift the feeding preferences of protozoan species stimulating on the one hand C-mineralization and on the other hand nutrient-mineralization, depending on the prevailing limitations. Enhanced C-mineralization only is possible when carbon is not limited and a nutrient limitation (N or P) is neutralized [9], thus the "stability" of C:N:P ratio of the influent in the case of synthetic wastewater, result in a more acclimated and stable biocenosis that will be acclimated towards either C or nutrient mineralization and thus correlate better to the treatment efficiency. In the case of competition between protozoa and bacteria for carbon mineralization, which may occur in the cases of high organic load, fast carbon-mineralization is inhibited by the uptake of substrate by protozoa because of shortening of the food chain. In every link of a food chain, part of the food is converted into biomass. The remaining carbon-compounds are used as energy source, inter alia for maintenance. When the chain becomes longer, less energy will remain locked into biomass. This means more carbon-mineralization and less biomass production [9].

Crawling and sessile species were increased in the operation under raw sewage, the increase of *Podophrya* sp. in conjunction to the reduction of *Tokophrya* sp. may suggest that these species compete for the same food resources consuming the same protozoan species [16]. It should be underlined that both species are carnivorous, while the percentage of carnivorous species in the total protistan community is relatively low in the case of raw sewage influent. However, the biovolume of these species, which was high, may give an indication about the importance of their ecological niche in the activated sludge. The distinctive dominance of sessile species in the SBBR can be attributed to the greater surface area offered by the plastic biofilm material as well as to the succession of the species in the activated sludge aggregates on the biofilm. The observations of all SBR approaches documented that peritrichous ciliates were crucially implemented in the granule structure-forming process, since they served as the basis for bacterial biofilm growth. Thus, the question of which specific interactions occur between ciliates and bacteria in granular biofilms arises. It is known that protozoa can excrete growth-stimulating compounds which enhance bacterial activity [9].

4. Conclusions

From the present study it was evident that the biofilm reactors were able to support increased protozoan biodiversity and abundance of sessile species due to the supply of greater surface area, and to the development of integrated food webs on the surface of the biofilm material. Thus the reactor configuration can be one of the most important factors for the determination of the activated sludge microfauna. Another factor that played a significant role in the configuration of activated sludge microfauna characteristics was the influent composition. The fluctuations of organic load may initiate competition between bacteria and protozoa for carbon resources and that fact may affect the removal efficiency of the wastewater treatment unit. Finally statistical analysis revealed that protozoan species observations may serve as indicators for the determination of trophic relations in the activated sludge and thus comprise precursors for the prediction of effluent quality.

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