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# Limitation of Sludge Biotic Index application for control of a wastewater treatment plant working with shock organic and ammonium loadings

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## Abstract

This study aimed to determine the relationship between activated sludge microfauna, the sludge biotic index (SBI) and the effluent quality of a full-scale municipal wastewater treatment plant (WWTP) working with shock organic and ammonium loadings caused by periodic wastewater delivery from septic tanks. Irrespective of high/low effluent quality in terms of COD, $BOD_5$, ammonium and suspended solids, high SBI values (8–10), which correspond to the first quality class of sludge, were observed. High SBI values were connected with abundant taxonomic composition and the domination of crawling ciliates with shelled amoebae and attached ciliates. High SBI values, even at a low effluent quality, limit the usefulness of the index for monitoring the status of an activated sludge system and the effluent quality in municipal WWTP-treated wastewater from septic tanks. It was shown that a more sensitive indicator of effluent quality was a change in the abundance of attached ciliates with a narrow peristome (*Vorticella infusionum* and *Opercularia coarctata*), small flagellates and crawling ciliates (*Acineria uncinata*) feeding on flagellates.


***Keywords:*** Protozoa; Metazoa; Activated sludge microfauna; Sludge Biotic Index; WWTP; Septic tanks

## Introduction

The main factors influencing biocenosis of activated sludge are organic sludge loading and sludge retention time (SRT). These parameters determine both the time required for the growth of organisms and the amount of food available to them. Low organic loading rate (OLR) is associated with long sludge retention time, stable aerobic conditions and poor feeding substrate. These factors result in a smaller number of dispersed bacteria, a high abundance of species with a small diversity, predominance in the taxonomic structure of a group of protozoa consisting of shelled amoebae and crawling and attached ciliates and the presence of small metazoa (Salvadó and Gracia 1993; Jenkins et al. 1993; Salvadó 1994). Because of the lower abundance of dispersed bacteria, these groups are able to obtain enough food to thrive through high efficiency wastewater clarification (attached ciliates with a wide peristome and rotifers), feeding within sludge flocs (rotifers, nematodes, and amoebae) and feeding on the lightly adherent bacteria of the floc surface (crawling ciliates). Long sludge retention time provides adequate time for growth of the organisms. The increase of organic load improves feeding conditions but is associated with the reduction of sludge retention time and often with obstacles related to aerobic conditions, such as faster growth of dispersed bacteria, increases in the overall size of the microfauna, the decline of species diversity and the domination of taxa, characterised by low feeding efficiency: small flagellates, free swimming ciliates

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and/or attached ciliates with a narrow peristome (Klimowicz 1970; Poole 1984; Madoni et al. 1993). These organisms require a high concentration of dispersed bacteria and are able to tolerate oxygen deficiency. A high rate of proliferation (flagellate and swimming ciliates) and a sedentary way of living (attached ciliates) protect them against leaching from the system due to reduced sludge retention time. The individual taxa are characterised by different levels of feeding efficiency, and to some extent by their ability to clarify wastewater. Thus, they are associated with differences in effluent quality. Also, observations of the microfauna community composition in activated sludge may be used to predict the effect of wastewater treatment.

The relationships described above have been used by many researchers to develop methods for biological monitoring of the progress and effects of the wastewater treatment process by activated sludge. In 1994, Madoni summarised the knowledge on the ecology of activated sludge (Curds and Cockburn 1970; Sladka and Sládeček, 1985; Al-Shahwani and Horan 1991), and his own previous research (Madoni 1991) and developed the sludge biotic index (SBI) to display the results of microscopic analysis of activated sludge and the numerical values for the quality of sludge.

The proposed method is based on the assumption that the dominance of key groups and the abundance and number of indicator taxa of microfauna in activated sludge vary depending on the physicochemical and technological parameters and the effects of the treatment process. A specially constructed table allows for defining the biological quality of the sludge using numerical values (biotic index) of 0–10, and to group SBI values into four quality classes. Class I (very well colonized and stable sludge, excellent biological activity, very good performance) includes SBI values of 10, 9 and 8, class II (well colonized and stable sludge, biological activity on decrease, good performance) includes 7 and 6, class III (insufficient biological depuration in the aeration tank, mediocre performance) includes 5 and 4. Class IV (poor biological depuration in the aeration tank, low performance) includes the remaining values.

In the last decade, several studies have reported the applicability of the SBI to be a useful monitoring tool that can assess the health of the activated sludge successfully. Samaras et al. (2009) investigated the response of the activated sludge protozoan community to chromium (VI) in the influent (synthetic wastewater). Increased chromium concentrations (up to 50 mg/l) stimulated a sharp decrease of the SBI values. A significant increase of SBI occurred after 60 days of operation. However, addition of chromium did not affect the organic removal and nitrification efficiency, resulting in high quality effluents. Similarly, Papadimitriou et al. (2007) investigated the effects of toxic substances (phenol, thiocyanides, cyanide) on the activated sludge microfauna. During the treatment of municipal wastewaters, the SBI corresponded to quality classes I and II. However, the addition of toxic compounds resulted in the deterioration of the SBI to quality classes III or IV, although high treatment efficiencies were observed. According to Papadimitriou et al. (2007), application of the SBI may be limited upon the addition of toxic influent, especially in cases of co-treatment of municipal and industrial wastewaters.

Nicolau et al. (2005) studied the response of the activated sludge community to the introduction of copper in synthetic and real wastewater. They showed that in synthetic wastewater, the microfauna was negatively affected by 50, 20 and 8 mg Cu/l. 4 mg Cu/l did not significantly decrease the value of the SBI. However, after seven days, only the community exposed to 50 mg Cu/l did not recover to the value of SBI referring to the quality class I. Passed into real wastewater, the same concentrations of copper caused a decrease in the SBI value, and, by the end of the assay, only the microfauna exposed to 50 mg Cu/l had not recovered. However, 4 mg Cu/l affected the SBI value more drastically than in the experiment with synthetic wastewater, lowering the SBI values to quality class II. Moreover, *Acineria uncinata* and *Aspidisca cicada* showed a remarkable tolerance of low concentrations of copper, in synthetic and real wastewater, respectively. On the other hand, *Opercularia* sp. was exceptionally tolerant of much higher concentrations of copper. According to the authors, the obtained results emphasise the resistance of these species to stressed environmental conditions. Other authors (Salvadó et al. 1995, 2001; Papadimitriou et al. 2007) pointed out that some species of ciliates can be useful indicators of toxic substances in treatment systems and/or effluent quality. Papadimitriou et al. (2004) studied the microfauna compositions in two bench-scale activated sludge units (continuous and SBR) treating high-strength wastewater ($BOD_5$ up to 2000 mg/l) and examined their correlation with operation modes and process efficiency. These findings were then compared to the respective findings from municipal activated sludge plants. Sessile and swimming ciliates correlated well to effluent $BOD_5$ values, and, according to the authors, can be used as indicators of process efficiency of the treatment systems. Among the protozoa species, *Vorticella* was the most abundant genus, especially in the SBR system. However, certain species were observed in the bench-scale systems, such as *Chilodonella uncinata* and *Opercularia coarctata*, but were not identified in the samples from the full-scale plants. Therefore, these species could be correlated to the addition of highly concentrated wastewaters in the bench-scale systems.

Although there are some experimental studies concerning the effect of toxic compounds on activated sludge microfauna (including some that describe the acclimatisation capacity of the microfauna to toxic substances, including heavy metals), limited information is available regarding the harmful effect of highly concentrated wastewater from septic tanks on microfauna communities from activated sludge processes. Therefore, the extent to which these wastewaters affect the wastewater treatment process by changing the activated sludge microfauna community needs further investigation. Sudden shock loadings could cause the destabilisation of the microfauna community of activated sludge and adversely affect the quality of treated wastewater. Therefore, the aim of this study was to evaluate the usability of the SBI to control treatment in the WWTP working with shock loadings of organics and nitrogen caused by high shares of wastewater delivered from septic tanks in the overall quantity of influent.

## Material and Methods

### Characteristics of the monitored WWTP

The monitored WWTP was designed for an average daily flow rate of wastewater of 2000 $m^3$/d and employs a mechanical–biological system. The mechanical stage contains coarse screen, grit chamber and primary settling tank, whereas the biological stage comprises one chamber with aerobic and anoxic zones and a secondary settling tank. The WWTP is operated at the following technological parameters: MLSS 4360 ± 412 mg/l; MLVSS 3440 ± 280 mg/l; DO (aerobic zone) 2.5–3.0 mg/l; DO (anoxic zone) <0.5 mg/l; HRT 16 h; and SRT 18 ± 3 d.

Wastewater is supplied to the monitored WWTP from a tourism and recreation community. Basic parameters of raw wastewater showed high daily, weekly and seasonal variability that was caused by periodical increases of pollutants from septic tanks. The amount of wastewater from septic tanks constituted 15 ± 8% of the influent. It resulted in a significant increase of organics and nitrogen concentration in wastewater and multiple increases of organic sludge loading.

Organic and ammonium sludge loadings without septic tank wastewater were on average 0.14 kg COD/kg MLVSS d, 0.086 kg $BOD_5$/kg MLVSS d and 0.009 kg $N_{NH_4}$/kg MLVSS d, respectively. However, within periods of wastewater delivery from septic tanks, the parameters were a few times higher (0.42 kg COD/kg MLVSS d, 0.29 kg $BOD_5$/kg MLVSS d and 0.026 kg $N_{NH_4}$/kg MLVSS d, respectively). During the experiments, no disturbance in the final sedimentation tank (i.e., bulking, rising) was observed.

Within the analysed period (6 months), 24 samples were collected; of these, 17 were collected at a time when only domestic wastewater was supplied to the WWTP (samples No. 1; 2; 3; 4; 5; 6; 7; 8; 9; 12; 13; 14; 16; 17; 18; 20; 23), and 7 were collected during a delivery of wastewater from septic tanks (samples No. 10; 11; 15; 19; 20; 21; 24). The frequency of sample collection was once a week.

### Analytical methods

Both raw and treated wastewater were analysed for pH (pH-meter HI 8818), chemical oxygen demand (COD; following APHA (1992) standard methods), biochemical oxygen demand (OxiTop, made by the WTW company according to DIN EN 1899-1/EN 1899-2 official EPA methods), and lastly, Kjeldahl nitrogen, ammonia-N, total suspended solids and volatile suspended solids (APHA 1992).

Both influent and effluent samples were taken every two hours for 12 h, following shock loading, and 12, 24 and 48 h afterwards.

### Biological analyses

Samples of mixed-liquor were collected for microscopic examinations (grab sampling) from the biological chamber; wastewater was delivered from septic tanks at least every 3 h. Collected samples were transported to the laboratory, where they were kept continuously aerated. All biological sample identifications were completed within 6 h after collection.

The composition of the microfauna in activated sludge was determined ‘in vivo’ according to Kahl (1930–1935), Kutikova (1984), Foissner et al. (1991, 1992, 1994, 1995), Foissner and Berger (1996) using phase contrast microscopy (Zeiss) at 100–400× magnification, depending on the size of each taxon. The abundance of flagellates was estimated along the diagonal of a Fuchs-Rosenthal chamber (Madoni 1994). The abundance of other protozoa and metazoans was determined as an arithmetic average obtained from the analysis of four subsamples with 0.05 $cm^3$ volume each of mixed liquor taken using a gravimetrically calibrated automatic micropipette. Finally, the abundance of individual taxa was counted in 1 $cm^3$ of activated sludge. Calculations of the SBI were undertaken in accordance with Madoni’s (1994) guidelines.

### Statistical analyses

Assuming the null hypothesis ($H_0$) that the average abundance of individuals in each taxon with high- and poor-quality effluent is the same, the Kruskal–Wallis test was used for statistical verification. Statistical significance of the relationship between the abundance of selected taxa of protozoa and wastewater quality was assessed using correlation coefficients (Pearson). Statistical data analysis was performed using the PC program STATISTICA 9 PL package.

## Results

Analysis of the basic parameters in raw wastewater showed relatively high contents of organic compounds (expressed as $BOD_5$ and COD) and ammonia, amounting to 546 mg/l, 856 mg/l and 67.4 mg/l, respectively. However, organics and nitrogen concentrations in the effluent did not exceed permissible values. $BOD_5$, COD and suspended solids concentrations equalled 12.0 mg/l, 78 mg/l and 22.3 mg/l on average (“high effluent quality”) (Table 1). Moreover, complete nitrification was obtained, and ammonia concentration in the effluent was below 1 mg/l.

**Table 1.** Composition of raw and treated wastewater.

| Wastewater constituent | Unit | Value | | | |
|---|---|---|---|---|---|
| | | Domestic wastewater; $n = 17$ | | Domestic wastewater with wastewater from septic tank; $n = 7$ | |
| | | Influent | Effluent | Influent | Effluent |
| pH | – | 7.56 ± 0.28 | 7.48 ± 0.14 | 7.33 ± 0.49 | 7.42 ± 0.18 |
| COD | $g/m^3$ | 856 ± 343 | 78 ± 35 | 2342 ± 647 | 274 ± 86 |
| $BOD_5$ | $g/m^3$ | 546 ± 89 | 12 ± 5.8 | 1582 ± 452 | 48.2 ± 13.8 |
| Total Kjeldahl nitrogen | $g/m^3$ | 119.2 ± 16.1 | 17.6 ± 4.8 | 208.9 ± 68.2 | 56.3 ± 26.1 |
| Ammonium nitrogen | $g/m^3$ | 67.4 ± 28.3 | 0.7 ± 0.26 | 162.4 ± 48.1 | 34.7 ± 17.2 |
| Total suspended solids | $g/m^3$ | 368 ± 197 | 22.3 ± 12 | 852 ± 326 | 156 ± 62 |

*Abbreviation*: *n*, number of samples.

A different situation was observed when the wastewater treatment plant was supplied with wastewater from septic tanks. Wastewater comprised, on average, 15% of the total quantity of influent and occasionally exceeded 20%. This caused multiple increases of organics and nitrogen concentrations in the influent (Table 1), and in consequence, a rise in organic sludge loading from 0.086 kg $BOD_5$/kg MLVSS d (0.14 kg COD/kg MLVSS d) to 0.29 kg $BOD_5$/kg MLVSS d (0.42 kg COD/kg MLVSS d). Therefore, despite the relatively long hydraulic retention time (HRT 16 h), the concentration of $BOD_5$ and COD in the effluent was as high as 43.6 mg/l and 215.5 mg/l, respectively. These concentrations exceeded permissible values ($BOD_5$ 25 mg/l, COD 125 mg/l) for discharge in surface water (according to the regulations of the Minister of Environment (on 24 July 2006) regarding conditions to be met for the introduction of sewage into the water or soil and on substances particularly harmful to the aquatic environment (Statute No. 137, item 984)). High concentrations of organics and ammonium were noted 24 h after the wastewater supply from septic tanks, followed by a decrease in their values observed prior to shock loading.

Within the 24 samples analysed over six months, 19 microfauna taxa were identified, including 11 ciliates, 4 shelled amoebae, 1 naked amoebae, rotifers and nematodes. The number of taxa varied from 9 to 15. Small flagellates and ciliates, such as *O. coarctata*, *Vorticella infusionum*, *Vorticella convallaria*, and *A. cicada*, occurred most frequently. The average density of microfauna without flagellate species was 17,709 ± 3927 ind./ml. The most prevalent species was *A. cicada*. The population of small flagellates ranged from one to several dozen individuals (Table 2).

Attached ciliates with a narrow peristome (*V. infusionum*, *O. coarctata*) and small flagellates and crawling ciliates (*A. uncinata*) feeding on flagellates indicate the deterioration of the effluent quality. Given poor wastewater quality, the protozoa were two to three orders more abundant in activated sludge than with high wastewater quality (Table 2; Fig. 1). All of them showed highly significant positive correlations with effluent BOD5, COD, ammonium nitrogen and total suspended solids (Table 3).

Regardless of the effluent quality, the SBI calculated on the basis of the microfauna composition was in the range of 8–10, which represents the first class of sludge quality. The high value of the SBI correlates with rich taxonomic composition of the microfauna and domination of the Madoni key group scored highest: crawling and sessile ciliates and/or testate amoebae, abundance $\geq 10^6$ ind./l. When these criteria were met, an increased abundance of flagellates on the level of 10–100 ind., estimated along the diagonal of a Fuchs-Rosenthal chamber during the period of shock loading, was unable to reduce the value of the SBI below 8 (Fig. 2).

## Discussion

This study revealed that shock organic and ammonium loadings connected with periodic wastewater delivery from septic tanks caused an increase of organics ($BOD_5$ and COD), suspended solids and nitrogen in the effluent. High concentration of organic substances in the effluent, despite rather long hydraulic retention time, could have been a result of two factors. First, a portion of the organic compounds from septic tanks was characterised by low biodegradation susceptibility and was not oxidised at the assumed retention time. Secondly, there was a high concentration of suspended solids in the treated wastewater (156 ± 62 mg/l), which would cause high COD in the final effluent (1 mg VSS = 1.42 mg COD; Dionisi et al. 2005). Moreover, although measured over a relatively long (18 d) sludge retention time, complete nitrification was not obtained, and ammonia concentration in the effluent attained the average value of 33.4 mg/l. This could be caused by the fact that activated sludge nitrification activities decrease with increasing organic concentration, as the presence of a high concentration of that type of substrate often prevents oxygen from being used for nitrification. Moreover, a high concentration of ammonia can limit the metabolism of nitrifiers, which have a relatively slow growth rate. Similarly, Hamoda and Al-Sharekh (1999) studied the four-compartment aerated submerged fixed-film (ASFF) process under both normal operation with domestic wastewater

**Table 2.** Frequency of occurrence and abundance of the protozoa and small flagellates at high and low effluent quality.

| Functional groups | Taxons | High effluent quality; $n = 17$ | | Low effluent quality; $n = 7$ | | $P$-value |
|---|---|---|---|---|---|---|
| | | Frequency (%) | Abundance (ind./ml) | Frequency (%) | Abundance (ind./ml) | |
| Smalle flagellates | Flagellates <20 μm | 100 | 6 ± 4[a] | 100 | 43 ± 24[a] | **0.0004** |
| | *Aspidisca cicada* | 100 | 12,000 ± 3251 | 100 | 9165 ± 1627 | **0.0454** |
| Crawling ciliates | *Chilodonella uncinata* | 50 | 29 ± 37 | 43 | 65 ± 105 | 0.8119 |
| | *Acineria uncinata* | 92 | 53 ± 45 | 100 | 2079 ± 715 | **0.0001** |
| | *Vorticella convallaria* | 100 | 870 ± 286 | 100 | 651 ± 292 | 0.0922 |
| | *Epistylis coronata* | 75 | 786 ± 813 | 86 | 205 ± 228 | 0.1790 |
| Attached ciliates | *Carchesium polypinum* | 83 | 1655 ± 1249 | 86 | 526 ± 903 | 0.0979 |
| | *Vorticella infusionum* | 100 | 302 ± 237 | 100 | 2042 ± 786 | **0.0002** |
| | *Opercularia coarctata* | 100 | 632 ± 369 | 100 | 5126 ± 1831 | **0.0002** |
| Carnivorous ciliates | *Holophrya discolor* | 13 | 8 ± 21 | | | |
| | *Tokophrya quadripartita* | 50 | 14 ± 17 | 43 | 9 ± 11 | 0.5238 |
| Shelled amoebae | *Euglypha* sp. | 58 | 26 ± 26 | 57 | 20 ± 23 | 0.6639 |
| | *Arcella* sp. | 67 | 49 ± 61 | 57 | 20 ± 23 | 0.2404 |
| | *Pyxidicula* sp. | 38 | 7 ± 10 | 43 | 9 ± 11 | 0.7335 |
| | *Cochliopodium* sp. | 33 | 273 ± 463 | 43 | 114 ± 213 | 0.9698 |
| Naked amoebae | *Acanthamoeba* sp. | 29 | 5 ± 9 | 43 | 14 ± 22 | 0.2800 |
| Rotifers | Philodinidae | 54 | 13 ± 16 | 71 | 20 ± 16 | 0.3040 |
| Nematodes | Nematoda | 42 | 12 ± 20 | 43 | 9 ± 11 | 0.9707 |

Bold font indicates significantly different values.

[a]Number of flagellates counted along the Fuchs-Rosenthal chamber diagonal; $n$, number of samples; ±, standard deviation; $P$, probability of $H_0$.

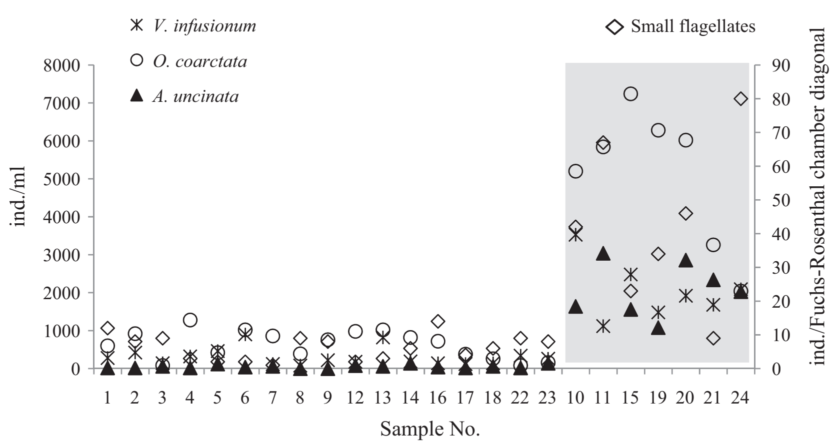
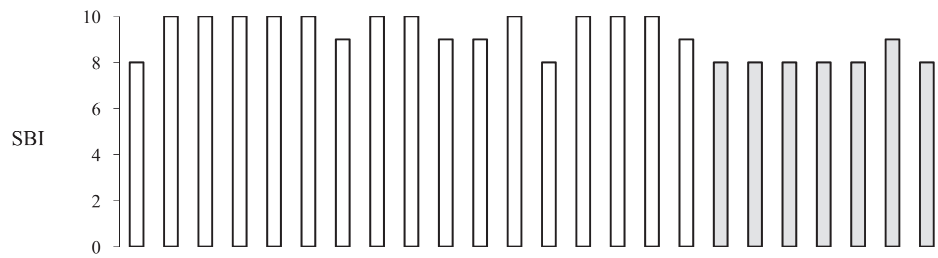



**Fig. 1.** Population changes of *Vorticella infusionum*, *Opercularia coarctatata*, *Acineria uncinata* [ind./ml] and small flagellates [ind./Fuchs-Rosenthal chamber diagonal] throughout the study (shaded in grey – samples with low effluent quality). Small flagellates were counted along the Fuchs-Rosenthal chamber diagonal <10 = <50,000 ind./ml; 10–100 = 50,000–500,000 ind./ml.

**Table 3.** Correlation analyses between the average population density of selected species of microfauna and some physico-chemical parameters.

| Taxons | Effluent $BOD_5$ | COD | Ammonium nitrogen | Total suspended solids |
|---|---|---|---|---|
| *Flagellates < 20 μm* | 0.670*** | 0.635** | 0.753*** | 0.620** |
| *Vorticella infusionum* | 0.867*** | 0.883*** | 0.712*** | 0.887*** |
| *Opercularia coarctata* | 0.842*** | 0.837*** | 0.821*** | 0.874*** |
| *Acineria uncinata* | 0.892*** | 0.852*** | 0.883*** | 0.665*** |
| *Aspidisca cicada* | −0.340*** | −0.467* | −0.373 | −0.462* |

$n = 24$;* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.




| Sample No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 13 | 14 | 16 | 17 | 18 | 22 | 23 | 10 | 11 | 15 | 19 | 20 | 21 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of taxonomic units that constitute microfauna | 13 | 11 | 11 | 15 | 12 | 11 | 10 | 11 | 11 | 9 | 10 | 13 | 12 | 12 | 13 | 11 | 10 | 12 | 12 | 12 | 12 | 12 | 9 | 13 |
| Number of small flagellates | 12 | 8 | 9 | 3 | 2 | 2 | 1 | 9 | 8 | 2 | 3 | 6 | 14 | 4 | 6 | 9 | 8 | 42 | 67 | 23 | 34 | 46 | 9 | 80 |
| Dominant keygroup, abundance | Crawling + sessile ciliates and testate | | | | | | | | | | | | | | | | | amoebae, > $10^6$ ind./ml | | | | | | |

**Fig. 2.** SBI analysis (shaded in grey – samples with low effluent quality).

and under organic shock loads with sugar wastewater. They showed that the ASFF process was able to cope with organic loads increasing from 5 to 120 g BOD/m$^2$ d; however, organic removal efficiency decreased from 97.9% to 88.5% for BOD and from 73.6 to 67.8% for COD. Nitrification similarly decreased, but at higher rates. Recovery to normal operation was attained in 10–24 h following shock loading.

Hu et al. (2011) compared the influence of organic and hydraulic shock loadings on the performance of submerged aerated filters (SAFs) packed with wool and Kaldness ring media during synthetic wastewater treatment. They showed that after the short-term shock load, ammonia effluent concentrations from the two bioreactors packed with wool and Kaldness rapidly increased from 0 mg/l to 17 mg/l and 25 mg/l, respectively. The ammonia concentration from the SAF with wool rapidly decreased on the first day and was restored to pre-shock operation performance by the third day. The recovery period of the SAF with Kaldness was apparently one day longer than that of the SAF with wool.

It is known that heterotrophic bacteria have a maximum growth rate of five times and yield two to three times higher than autotrophic nitrifying bacteria (Grady and Lim, 1980; after Ling and Chen 2005). Nitrification inhibition at high organic loading is commonly known and was observed by many authors. Ling and Chen (2005) determined the nitrification rate with and without the interaction of organic matter in three types of biofilters of laboratory scale (floating bead filter, fluidised sand filter, and submerged bio-cube filter). They showed that with the addition of organic carbon, the nitrification rate of all three types of biofilters decreased exponentially. The reduction of nitrification rates of the biofilters was about 60–70% when the COD/N ratio increased from 0 to 3. In our study, after supplying wastewater from septic tanks, the ammonium concentration increased from 0.7 to 34.7 mg/l, which corresponded to a decline of the nitrification efficiency from 98.9% to 78%. By applying fluorescent *in situ* hybridisation (FISH) technique and microelectrodes in the nitrification biofilm analysis, Ohashi et al. (1995) and Satoh et al. (2000) found that the proportion of nitrifiers decreased with an increasing C/N ratio (after Ling and Chen 2005).

The observed microfauna consisted of taxa belonging to two opposed bioindicator groups. The first group, composed of shelled amoebae, crawling ciliates and attached ciliates with wide peristomes, nematodes and rotifers, indicates a healthy, low-loaded, sufficiently aerated and well-flocculated activated sludge that results in a high-quality effluent (Madoni et al. 1993; Martin-Cereceda et al. 1996; Chen et al. 2004; Lee et al. 2004; Zhou et al. 2006; Papadimitriou et al. 2007; Tyagi et al. 2008). On the contrary, the second group were defined in all samples by attached ciliates with narrow peristomes (*V. infusionum*, *O. coarctata*) and small flagellates, indicating a high-loaded activated sludge with a low oxygen concentration, high content of dispersed bacteria, and poor effluent quality as a consequence (Poole, 1984; Madoni, 1991; Salvadó et al. 1995; Ginoris et al. 2007). The composition of the microfauna of activated sludge is primarily a result of the feeding conditions, depending on organic sludge loading. *Opercularia* spp., *V. infusionum* and flagellates are characterised by large feeding requirements and therefore can be abundant only in an environment rich in dispersed bacteria for consumption. Such conditions can occur on conditions of high sludge loading, favouring the rapid growth of bacteria. The abundance of dispersed bacteria can also increase in the presence of toxic compounds in the influent, interfering with the flocculation of activated sludge flocs and causing their decay. The regular observation of the simultaneous presence of indicators from the opposed bioindicator groups is probably the result of the low-loaded sludge working, periodically shocked with an increased load of wastewater from septic tanks.

Our results showed that increases in abundance of attached ciliates with a narrow peristome (*V. infusionum* and *O. coarctata*), small flagellates and crawling ciliates (*A. uncinata*) feeding on flagellates indicate the deterioration of the effluent quality. Therefore, these four taxa are likely to be useful as bioindicators for treatment effectiveness in WWTP with shock organics and ammonia loadings. Our results corroborate the observations of previous authors. Puigagut et al. (2005) studied the harmful effect of ammonia nitrogen concentration (9, 20, 30 and 50 mg $N–NH_4$/l) on activated sludge microfauna in terms both of abundance and diversity. They showed that ammonia nitrogen caused a clear but reversible toxic effect on microfauna abundance when its concentrations are about three times higher than that to which the microfauna is accustomed. *C. uncinata* and *A. uncinata* were the dominant species and the ciliates least affected by the ammonia nitrogen toxicity. Moreover, they showed that activated sludge microfauna abundance recovered after 48 h. According to these authors, this shows that the toxic effect of ammonia nitrogen in most conventional urban WWTP could remain undetected. Moreover, Salvadó et al. (1995) found an association between *Opercularia* species and poor effluent quality from a municipal WWTP, whereas Madoni (1994) observed a negative correlation between the abundance of small flagellates, nitrifying ability and BOD removed. Hul and Drzewicki (2001) examined the relationship between the intensity of aeration and the microfauna composition of activated sludge in a municipal WWTP and noticed an increase in the number of *V. infusionum* with the deterioration of the organic efficiency of and ammonia removal.

The high values of the SBI (8–10), obtained on the basis of microfauna composition, referred to the first quality class of the activated sludge and indicated a stable, very well-inhabited microfauna. This quality level is associated with high biological activity and a high treatment effectiveness, independent from effluent quality. This discrepancy limits the use of the SBI in the case of the wastewater treatment process using low-loaded activated sludge under shocked loadings. Similarly, Papadimitriou et al. (2007) showed that applicability of the SBI may be limited upon the addition of toxic influents. The authors observed that the addition of toxic compounds resulted in deterioration of the SBI to quality classes III or IV, despite high treatment efficiency. Arévalo et al. (2009) showed that for MBR systems, the quality of effluent obtained at SRT values over 25 days is not associated with the SBI. In spite of the change in the SBI, the quality of the treated wastewater did not vary, particularly with regard to the levels of COD and $BOD_5$. Shock sludge loads caused an increase in dispersed bacteria density and, consequently, an increase in the abundance of flagellates and attached ciliates with a narrow peristome. A low efficiency of these protozoa feeding produces a poor-quality effluent. Changes in taxa abundance and crawling ciliates (*A. uncinata*) feeding on them are believed to be more sensitive indicators of process destabilisation than the SBI value.

## Conclusions

During wastewater treatment in the WWTP working with shock loadings of organic substances and nitrogen (attributed to the high share of wastewater from septic tanks in the total quantity of influent), the presence of microfauna belonging to two opposite groups of indicators of activated sludge performance were observed. The SBI calculated on the basis of microfauna composition obtained high values of 8–10, irrespective of high/low effluent quality. The high value of the SBI was caused by abundant taxonomic composition of the microfauna and domination of the Madoni key group scored highest: crawling and sessile ciliates and/or testate amoebae, with abundances $\geq 10^6$ ind./l. After assuming these components of the SBI, observed on the condition of shock loading, an increased abundance of flagellates (10–100 ind./Fuchs-Rosenthal chamber diagonal) during the period of shock loading, was unable to reduce the value of the SBI below 8 (<class I of activated sludge).

Changes in the abundance of attached ciliates with a narrow peristome (*V. infusionum* and *O. coarctata*) and small flagellates can be considered more sensitive indicators of effluent quality and process destabilisation. These taxa increased their abundance during the period of high concentration of dispersed bacteria connected with the influent of delivered wastewater. Crawling ciliates (*A. uncinata*) feeding on flagellates were also sensitive indicators of process changes.

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