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A SLUDGE BIOTIC INDEX (SBI) FOR THE EVALUATION OF THE BIOLOGICAL PERFORMANCE OF ACTIVATED SLUDGE PLANTS BASED ON THE MICROFAUNA ANALYSIS

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Abstract—An objective index, the Sludge Biotic Index (SBI), based on the microfauna associated with activated-sludge mixed liquor, has been devised to monitor activated-sludge plant performance. The proposed method makes it possible to define the biological quality of the sludge using numerical values (biotic index) of 0–10, and to group SBI values into four quality classes. The method is based on two principles. First, the dominance of the microfauna keygroups changes in relation to the environmental and operational conditions of the plant. In particular, some protozoa such as small flagellates and the ciliates *Vorticella microstoma* and *Opercularia* spp have a negative influence on the index value. Secondly, the number of morphological species is reduced as the performance of the plant gets worse. The advantage of this method over other schemes is that it provides numerical values which enable the operator to monitor the prevalent plant operating conditions on a daily basis.

Key words—protozoa, activated sludge, Sludge Biotic Index, indicator species, plant performance

INTRODUCTION

Numerous biotic indices have been developed to assess the degree of pollution in freshwater ecosystems. For rivers, the Extended Biotic Index (EBI) by Woodiwiss (1980), based on macroinvertebrates, is one of the most widely used in Europe. This method is based on two phenomena: the change in the composition of the taxocenosis as the pollution level rises, and the reduction in number of taxonomic groups as pollution increases. Objective indices such as the Extended Biotic Index and the Saprobic Index (Sladecek, 1973), present methods for calculating fixed index values for any given community, independent of the analyst's personal evaluation. Both the random fluctuations found in samples taken under the same conditions and the personal subjectivity in sampling and counting techniques, represent two factors possibly introducing a certain degree of uncontrolled variability.

The routine analysis of the microfauna as an indicator of activated-sludge plant performance is becoming more and more common. This analysis quickly gives useful information on the biological activity of the sludge based on the community structure of the microorganisms present. However, the microfauna is also used to indicate changes in the performance of specific activated-sludge plants (Al-Shahwani and Horan, 1991; Esteban *et al.*, 1991) but these methods cannot necessarily be applied directly to other similar plants. On the contrary, other methods, based on microscopic analysis can be applied to any plant to estimate both the quality of the effluent (Curds and Cockburn, 1970b) and the performance of the plant (Drakides, 1978; Madoni, 1981, 1988). Some of these methods, however, are subjective indices not being based on rigid calculations, but on the analyst's personal interpretation of the microfauna that colonize the activated sludge under investigation. Objective indices have a great advantage over subjective ones in that index values assessed by different operators are comparable.

In this paper an objective index is proposed to estimate the biological quality of the sludge in an aeration tank of activated-sludge plants. The method, that is inspired by the Extended Biotic Index (Woodiwiss, 1980) is applicable to all types of activated-sludge plants.

MICROFAUNA IN ACTIVATED SLUDGE

Biological sewage-treatment plants may be regarded as man-made ecosystems subjected to extreme conditions. As in every other biological system, the community living in the aeration basin of an activated-sludge plant has a precise structure (components and factors) and follows exact dynamics (in time and space). In activated sludges, biotic components are represented by decomposers (bacteria, fungi) which utilize the dissolved organic matter in the wastewater, and by consumers (heterotrophic flagellates, ciliates, rhizopods, and small metazoans) which feed on dispersed bacteria and other organisms. It is well-known that activated sludges develop

Table 1. List of ciliated protozoa commonly found in activated sludges and their food habit

	Bacterivorous		
Carnivorous	Free-swimming	Crawling	Attached
Holotrichs	*Colpoda* sp.	*Aspidisca cicada*	*Carchesium* spp
Acineria incurvata	*Colpidium colpoda*	*Aspidisca lynceus*	*Epistylis* spp
Amphileptus sp.	*Colpidium campylum*	*Chilodonella uncinata*	*Opercularia coarctata*
*Coleps hirtus**	*Cinetochilum margaritaceum*	*Euplotes affinis*	*Opercularia microdiscus*
Litonotus spp	*Cyclidium glaucoma*	*Euplotes moebiusi*	*Opercularia minima*
Spathidium spp	*Dexiotricha* sp.	*Euplotes patella*	*Stentor* spp
Suctorians	*Glaucoma scintillans*	*Stylonychia* spp	*Vaginicola crystallina*
Acineta spp	*Loxocephalus* sp.	*Trithigmostoma cucullulus*	*Vorticella convallaria*
Metacineta sp.	*Paramecium* spp	*Trochilia minuta*	*Vorticella microstoma*
Podophrya spp	*Pseudocohnilembus pusillus*		*Vorticella octava*
Tokophrya spp	*Sathrophilus* sp.		*Zoothamnium* spp
	Spirostomum teres		
	Tetrahymena pyriformis complex		
	Uronema nigricans		
	Acineria uncinata†		
	Drepanomonas revoluta†		
	Trachelophyllum pusillum†		

*Omnivorous; †*incertae saedis*: these species are free-swimming forms but their grazing activity seems to be linked to the floc.

specific communities of protozoa which are sustained by large populations of bacteria. Ciliated protozoa are very numerous in all types of aerobic biological-treatment systems; they are commonly found in densities of about 10,000 cells/ml of activated sludge mixed liquor, and constitute approx. 5% of the dry weight of suspended solids in mixed liquor. Although about 230 species of protozoa have been observed in the various types of aerobic treatment systems, only few have been observed frequently (Curds and Cockburn, 1970a; Madoni and Ghetti, 1981). Table 1 shows the list of the most important ciliates found in activated sludge. The majority of ciliates present in biological water treatment plants feed upon dispersed populations of bacteria. The bacterivorous ciliates in activated sludge can be subdivided into three groups on the basis of behaviour: free-swimmers, crawlers, and attached.

All bacterivorous ciliates rely upon ciliary currents to force suspended bacteria into the oral region. So, while free-swimming and attached ciliates are in competition for bacteria dispersed in the liquid phase, crawling forms, that are in close proximity to surface growths, feed upon particles that only lightly adhere to the sludge and that are dislodged by the feeding currents very easily.

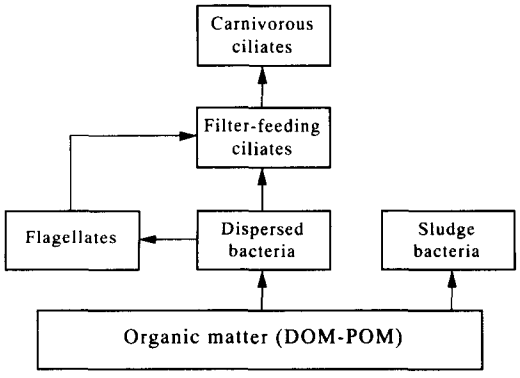

Fig. 1. Trophic web in the activated-sludge process.

Ciliated protozoa improve the quality of the effluent. In their absence the effluents flowing from the system, have an elevated BOD and are highly turbid due to the presence of many dispersed bacteria (Curds *et al.*, 1968). In the aeration tank of biological processes a true trophic web is established. A simplified diagram of this is illustrated in Fig. 1. The biological systems of these plants consist of populations in continuous competition with each other for food. The relationships of competition and predation create oscillations and succession of populations until dynamic stability is reached (Fig. 2). This is strictly dependent on plant management choices based on design characteristics aimed at guaranteeing optimum efficiency (Madoni, 1986).

Results from research on the modalities of colonization and of population succession in activated sludge have been of great importance in understanding the role of protozoa as plant performance indicators and demonstrated the determining effect of environmental conditions in the aeration tank on the ciliate community established (Curds, 1966, 1971; Madoni and Poli, 1981; Madoni, 1982; Madoni and Antonietti, 1984).

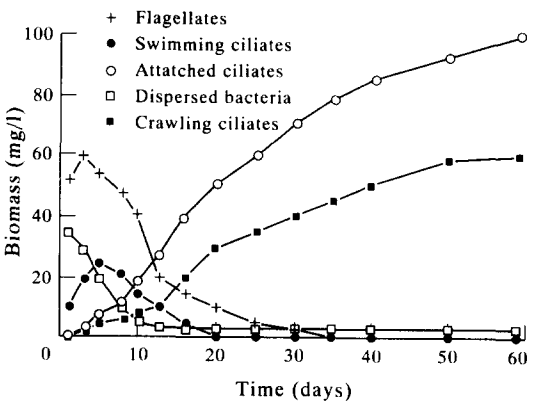

Fig. 2. Successions of microorganisms during the establishment of an activated sludge.

Table 2. Some situations regarding the plant performance indicated by the dominant group of the microfauna (from Madoni, 1986, 1988 modified)

Dominant group	Performance	Possible causes
Small flagellates	Low	Poorly aerated sludge; overloading; fermenting substances involved
Small swimming ciliates	Mediocre	Too short sewage retention time; poorly aerated sludge
Large swimming ciliates	Mediocre	Overloading; poorly aerated sludge
Crawling ciliates	Good	
Sessile and crawling ciliates	Good	
Sessile ciliates	Decreasing	Transient phenomena (discontinuous load, recent sludge extraction)
Small naked amoebae and flagellates	Poor	Very high load, not easily degradable
Testate amoebae	Good	

The structure of the microfauna is indeed a valid indicator of purification plant performance. In synthesis, an efficient activated sludge plant presents the following characteristics:

(1) High numbers of microfauna cells ($\geqslant 10^6$ organisms/l);
(2) Microfauna composed chiefly by crawling and attached ciliates, with almost no flagellates;
(3) The species and ciliate groups are highly diversified and none dominates numerically over the others by a factor greater than 10.

When this is not the case, the identification of the dominant group (keygroup) of the microfauna allows diagnosis of the particular state of functionality of the plant (Madoni, 1986). Some examples of these situations are reported in Table 2.

THE KEYGROUPS

Quantifying the indicator value of the microfauna is a difficult task because there are groups which are more or less tolerant to a wide range of environmental factors, whilst the plant performance results from the simultaneous action of many of these external and operational conditions. In this investigation, the relationship between the various groups of the microfauna and the main operational conditions of the plant were studied by a wide survey. Forty-four activated sludge plants, including oxidation ditches, conventional aeration plants and extended aeration units, were sampled and their choice was made in order to explore a diverse spectrum of different operational conditions. Table 3 shows the correlation coefficients between nine groups of the microfauna and six physico-chemical and operational parameters. These results corroborate the observations of previous authors, and enable one to select and group microfauna organisms into positive and negative keygroups. The positive keygroups are crawling and attached ciliates, and testate amoebae; negative keygroups are small flagellates, swimming bacterivorous ciliates, and the peritrich ciliates *Vorticella microstoma* and *Opercularia* spp. Density and diversity of the microfauna, moreover, results to be highly correlated to the plant performance.

Density and diversity

Many recent studies have demonstrated that the number of ciliated protozoa living in a normally functioning plant is about 10^6 individuals/l. When the number falls below 10^4/l, it indicates insufficient purification (Curds, 1975; Drakides, 1980; Madoni, 1981). In this case, there is a proliferation of dispersed bacteria which make the effluent turbid and consequently cause a greatly increased BOD in the output water. A high number of ciliates ($>10^7$/l) on the contrary, almost always indicates good purification and optimum plant performance. The microfauna of a normally functioning system is almost always highly diversified, namely composed by different groups of organisms, each group with several species. No group or species is ever numerically dominant over the other components, even if the ratios between various groups or species can differ. On the contrary, a microfauna which is dominated by one species or group is almost always an index of trophic imbalances due to the existence of limiting factors impeding the development of most of the other species and favouring the growth of forms more tolerant to these factors. The most common limiting conditions are generally the presence of a shock load of toxic

Table 3. Correlation coefficients between protozoans and plant operational conditions, obtained from 44 activated sludge plants ($^*P < 0.01$, $^{**}P < 0.001$)

	DO	Nitrifying ability	BOD removed	Effluent colour	Sludge age	MLSS
Small flagellates	−0.652**	−0.596**	−0.798**	0.836**	−0.583**	−0.736**
Swimming ciliates	−0.651**	−0.549**	−0.829**	0.725**	−0.668**	−0.753**
Crawling ciliates	0.616**	0.620**	0.784**	−0.611**	0.534**	0.534**
Sessile ciliates	0.340	−0.029	0.432*	−0.516**	0.171	0.266
V. microstoma	−0.676**	−0.504**	−0.679**	0.648**	−0.638**	−0.722**
Opercularia spp	−0.745**	−0.597**	−0.763**	0.676**	−0.008	−0.181
Testate amoebae	0.727**	0.912**	0.760**	−0.583**	0.464*	0.464*
Microfauna density	0.626**	0.429*	0.762**	−0.768**	0.335	0.495**
No. of species	0.778**	0.645**	0.923**	−0.841**	0.487**	0.591**

discharge, the under- or the overloading, the strong sludge extraction, the lack of aeration.

Recent data obtained from a survey of protozoa in several activated-sludge plants (De Marco *et al.*, 1991), corroborate these results obtained in previous works. These authors classified the abundance of the microfauna into three classes ($<10^4$, 10^4–10^6, $>10^6$) according to the efficiency of the plant (not efficient, poorly efficient, well efficient respectively) and, by means of the Kruskal–Wallis test of variance, were able to point out that both organic load removed and metal concentration in the mixed liquor were significantly different in the treatment plants, according to the three classes of protozoa abundance. Statistical analysis applied to the study of ciliates and of physico-chemical variables in activated sludge sewage treatment plants showed that the number and diversity of ciliate communities change according to the quality of entering settled sewage and operating conditions of the plant (Esteban *et al.*, 1991; Esteban and Tellez, 1992).

Crawling and attached ciliates

These two ciliate groups normally co-dominate the microfauna in activated sludge plants. This is due to their different food habits preventing their competition. Nevertheless, the ratio between the two groups tends to change with sludge loading. Crawling ciliates diminish their presence as sludge loading increases so that above 0.6 kg BOD/kg MLSS · d, most species of this group disappear (Curds and Cockburn, 1970b; Klimowicz, 1970). Crawling ciliates are also inversely related to SVI. High densities of crawling ciliates ($>2000\ ml^{-1}$) are always associated to SVI values smaller than 200; at SVI values greater than 400 crawling ciliates reduce drastically in number (Pagnotta and Tommasi, 1979).

Testate amoebae

Testate amoebae in activated sludge are represented by the three genera: *Arcella*, *Difflugia* and *Euglypha*. These protozoa colonize the sludge of plants at very low sludge load, in particular they are found normally in the aeration basin of N removal plants (Poole, 1984). Testate amoebae are more abundant or dominant in sludges characterized by low loading, long retention time, and high DO in aeration tank that enable complete nitrification (Drakides, 1978; Chierici and Madoni, 1991). Under these conditions, the quality of the effluent is excellent and high performance of the plant is reached. Testate amoebae are more abundant in plants with a large sludge age since testates have low growth rates. They often are seasonal being more common in summer when temperature and growth rates increase. Sasahara and Ogawa (1983) found that *Euglypha* and *Difflugia* were always abundant in activated-sludge plants for brewery effluent with low sludge load and good quality of the effluent. When the sludge load was high (> 1 kg BOD/kg MLSS · d) and the COD of the effluent was elevated, these species were replaced by the peritrich ciliate *Opercularia* and by free-swimming ciliates. Madoni *et al.* (1993) were able to find high correlation coefficients between the presence and abundance of testate amoebae and some prevailing environmental factors and nitrifying conditions of the plant. These species, in fact, were associated with low ammoniacal-N concentrations, high DO values and low sludge load and SVI values.

Attached ciliates (> 80%)

Peritrich ciliates are normally co-dominant in the activated sludge. Nevertheless, a massive increase in their number (>80% of the whole microfauna) occurs in occasion of transient situations that reduce the plant performance (Drakides, 1978; Madoni, 1981). Such transient conditions are (1) a quick increase of the sludge load due to loss of sludge and (2) a discontinuous input of organic load from the influent. Curds and Cockburn (1970b) found that sessile ciliates were able to grow throughout a large range of sludge loadings; nevertheless, at values ranging from 0.3 to 0.6 kg BOD/kg MLSS · d, these ciliates dominated, and for sludge loadings of 0.6–0.9 sessile ciliates and flagellates co-dominated. Evidence was found by Bedogni *et al.* (1991) which suggested that the abundance ratio between crawling and attached ciliates was associated with the performance of the plant. When ratio values were high (>0.5) a better quality of the final effluent was achieved.

Among attached ciliates some forms such as *Vorticella microstoma* and *Opercularia* spp can survive and grow in activated sludge subjected to severe conditions (lack of oxygen, toxicants, etc.). When these species are present in high numbers, they must be considered as separate keygroups (see below).

Opercularia spp

Three species are commonly found in activated sludge: *O. coarctata*, *O. microdiscus*, and *O. minima*. Low numbers of these ciliates are often observed in activated sludge. Nevertheless, *Opercularia* shows a close association with the variables concerned with the quality of the activated sludge and statistical analysis confirms that these ciliates are quite useful as bioindicators, because their number increases when the activated sludge is of bad quality (Esteban *et al.*, 1991). Curds and Cockburn (1970b) found that *Opercularia* spp were associated with high final effluent BOD concentrations, and Klimowicz (1970) found that these species were among the most abundant forms at high loadings. *Opercularia* spp moreover can survive in stressed environments better than other protozoans. Some authors (Antonietti *et al.*, 1982; Becares, 1991; Cardinaletti and Zitelli, 1991), in fact, found that large numbers of *Opercularia* occur in sludge of plants receiving industrial waste containing toxic substances. *Opercularia coarctata* may be the only component of the microfauna, in sludges of plants treating industrial waste containing metal

salts. *O. microdiscus* is able to survive to severe lack of oxygen found in sedimentation tanks with low return-sludge ratio. Moreover, these species were associated with high final effluent BOD and ammoniacal-N concentrations (Poole, 1984; Madoni *et al.*, 1993). *Opercularia* spp often are associated with *V. microstoma*.

Vorticella microstoma

The ciliate *V. microstoma* is quoted in the saprobic system as a polysaprobic species (Sladecek, 1973; Foissner, 1988). This ciliate is frequently present in the plant during the first phase of colonization but is substituted by other species (*V. convallaria*) which become dominant during stable conditions. When there is a drastic reduction in the dissolved oxygen concentration in the mixed liquor, an alternation of the two species is observed, due to their different degree of tolerance to the lack of oxygen (Madoni and Antonietti, 1984). *V. microstoma* thus, indicates a lack of dissolved oxygen in the aeration tank; the greatest resistance to the influence of anoxic environment was noticed for this species also by other authors (Bick, 1972; Toman and Rejic, 1988). Massive growth of this sessile ciliate was observed in the aeration tanks in occasions of high wastewater flow arrival to the sewage plant and low values of massic loading and water colour of the effluent (Esteban *et al.*, 1990). Large numbers of *V. microstoma* in occasions of low values of DO, MLSS, and high values of sludge load and SVI, were found also by Poole (1984) and Esteban *et al.* (1991).

Free-swimming ciliates

Swimming bactivorous ciliates are more abundant in the early phases of a developing plant, when sludge flocs are still scarce and, consequently, sessile ciliates are absent. Nevertheless, they are soon substituted by attached ciliate population owing to competition for dispersed bacteria. Attached ciliates, in fact, are filter-feeders more efficient than free-swimming forms to enforcing suspended bacteria into the oral region by means of ciliary currents. Curds (1971) was able to show, by means of computer simulation of microbial population dynamics, that high-quality effluent was produced when attached and crawling ciliates were dominant, a slightly worse effluent quality when free-swimming ciliates were dominant, and a low-quality effluent when no ciliates were present at all. Small free-swimming ciliates (such as *Colpidium*, *Cyclidium*, *Tetrahymena*, and *Uronema*) sometimes dominate the microfauna of plants operating at sludge too short aged or at both high sludge loading and lack of oxygen. These bacterivorous protozoans require high concentrations of dispersed bacteria but survive better than other components of the microfauna to the toxicity of the influent and to lack of oxygen. Small free-swimming ciliates ever couple with heterotrophic flagellates and sometimes these two keygroups co-dominate the microfauna. Curds and Cockburn (1970b) found that swimming forms were dominant at high sludge loadings (0.6–0.9 kg BOD/kg MLSS · d).

It should be emphasized that to this keygroup belong only bacterivorous ciliates; free-swimming carnivorous forms such as *Amphileptus*, *Litonotus*, *Spathidium*, etc. (see Table 1) must not be inserted into this keygroup.

Flagellates

Small heterotrophic flagellates such as *Bodo*, *Polytoma*, and *Tetramitus*, normally dominate the microfauna during the starting phase of the plant when floc-forming bacteria are still scarcely present. They feed on dispersed bacteria and, in time, are substituted by bacterivorous ciliates. By contrast, the massive presence of these protozoans in a mature activated sludge, is associated to bad performance of the biological depuration, due to the following causes: (a) poorly aerated sludge, (b) over loading, (c) fermenting substances involved (Drakides, 1978; Madoni, 1986). Flagellates become the only protozoan form present in sludges strongly loaded (>0.9 kg BOD/kg MLSS · d) (Curds and Cockburn, 1970b). Flagellates continuously enter the plant by the sewage influent where they are very numerous. In a normally-functioning activated sludge these protozoa outcompete the bacterivorous ciliates and, in addition, they are strongly subjected to predaceous activity of other protozoa; so, their presence in the activated sludge is limited to few individuals (<10 ind. counted along the diagonal in the Fuchs–Rosenthal chamber). In the case of disfunctions of the plant, their number can increase (>10 ind. counted) and, consequently, this indicates a decrease in the plant performance. The dominance of flagellates become apparent when they reach the density of more than 100 individuals along the diagonal in the Fuchs–Rosenthal chamber. In this last case they should be considered as the dominant keygroup.

Large flagellates such as *Euglena* and *Peranema*, are infrequently observed in the activated sludge where they grow hardly in large numbers. Their presence is related to influents characterized by very diluted organic matter.

THE SLUDGE BIOTIC INDEX

The sludge biotic index (SBI) here proposed in Tables 4 and 5, has been set up on the basis of the results obtained both by the numerous research conducted on the activated-sludge microfauna during the last 20 years and in the present research as well (Table 3), as summarized above. The sensitivity of the proposed method was assessed on the basis of physical, chemical, and operating conditions of the plants. This method is based both on the different sensitivity showed by some keygroups to principal physico-chemical and operating parameters, and on

Table 4. Two-way table to determine the Sludge Biotic Index (SBI) on the basis of keygroups, density and number of taxonomic units of the microfauna

Dominant group and density of the microfauna that define the horizontal row of entrance in the table		Total number of taxonomic units that constitute the microfauna of the activated sludge and number of small flagellates (F) counted along the Fuchs–Rosenthal chamber diagonal							
		>10		8–10		5–7		<5	
Dominant keygroup	Density (ind./l)	$F<10$	$10<F<100$	$F<10$	$10<F<100$	$F<10$	$10<F<100$	$F<10$	$10<F<100$
Crawling + sessile* ciliates and/or testate amoebae	$\geq 10^6$	10	8	9	7	8	6	7	5
	$<10^6$	9	7	8	6	7	5	6	4
Sessile ciliates* >80%	$\geq 10^6$	9	7	8	6	7	5	6	4
	$<10^6$	8	6	7	5	6	4	5	3
Opercularia spp	$\geq 10^6$	7	5	6	4	5	3	4	2
	$<10^6$	6	4	5	3	4	2	3	1
Vorticella microstoma	$\geq 10^6$	6	4	5	3	4	2	3	1
	$<10^6$	5	3	4	2	3	1	2	0
Swimming bacterivorous ciliates	$\geq 10^6$	5	3	4	2	3	1	2	0
	$<10^6$	4	2	3	1	2	0	1	0
Small-swimming flagellates (>100)†	$\geq 10^6$	4		3		2		1	
	$<10^6$	3		2		1		0	

*_Opercularia_ spp and _Vorticella microstoma_ not abundant; †along the Fuchs–Rosenthal chamber diagonal.

the abundance and the diversity of the microfauna: it enables us to define the biological quality of the sludge by means of conventional numerical values (biotic index). The SBI considers also the following points:

—the species richness tends to change with the sludge load. The higher number of species has been observed when loadings are ranging from 0.2 to 0.3 kg BOD/kg MLSS · d (Curds and Cockburn, 1970b);

—the density of the microfauna drops down as the sludge load lowers. In the aeration basin of N removal plants, a microfauna less abundant than in conventional plants is expected.

The index to be ascribed to the activated sludge under test is obtained by means of a two-way table (Table 4). The keygroups following one another from the upper part toward the lower part of the table, indicate a worse and worse biological quality of the sludge. In the column headings four ranges in which the total number of taxonomic units of the microfauna falls are reported. The two-way table moreover considers the abundance of both microfauna (excluding flagellates) and flagellates. To determine the SBI it is necessary to select the horizontal way-in on the row that corresponds to the dominant keygroup occupying the lower position, taking into account its density (more or less than 10^6 ind./l). The vertical way-in is determined by the total number of taxonomic units and density of flagellates as well.

Once both horizontal and vertical ways are identified, the SBI value can be determined and this is situated in correspondence of their cross. The two-way table allows us to put in a number from 0 to 10 for the biological quality of the activated sludge on the basis of two indicators: the different sensitivity showed by some microfaunal groups to environmental conditions of the sludge, and the effect that these conditions produce on both abundance and richness of the protozoan community.

Finally, the values of SBI have been grouped into four quality classes marked by a Roman number (Table 5). These classes allow to represent the biological quality of the activated sludge through four ranges of judgements rather wide, and thus of reliable diagnostic value.

Table 5. Conversion of SBI values into four quality classes and respective judgements

SBI value	Class	Judgement
8–10	I	Very well colonized and stable sludge, excellent biological activity; very good performance
6–7	II	Well colonized and stable sludge, biological activity on decrease; good performance
4–5	III	Insufficient biological depuration in the aeration tank; mediocre performance
0–3	IV	Poor biological depuration in the aeration tank; low performance

VERIFICATION

The strict applicability of the proposed method was tested by investigating 45 activated sludges. In each activated sludge the microfauna was both identified and enumerated, and the principal physical, chemical, and operational parameters such as BOD and ammonium removal, DO, sludge and sewage retention time, MLSS, nitrifying ability, etc. were registered. This test showed that the method gives

correct analysis of the plant performance, in all the cases investigated. The two-way table displays 88 different situations that can be observed, and these events fall into the four quality classes in the proportion of 9, 18, 24, and 37, respectively. The test, obviously, was unable to verify all situations; nevertheless, since it was possible to verify the reliability of the method at the extreme SBI values for each quality class, it seems well-founded to expect a full reliability of the method to the intermediate SBI values too.

PROCEDURE PROTOCOL

Since the method here proposed is based on diversity and density of the microfauna, particular attention should be paid to both identification and enumeration of these organisms.

Identification of the microfauna

Although a large number of organisms can be observed in the activated sludge process, some forms such as both naked amoebae and drifting organisms (algae, crustaceans, insects), are not considered in this method. Organisms to include in the microfauna are: small and large flagellates, ciliates, testate amoebae, rotifers, and nematodes. All the species of ciliated protozoa and testate amoebae contribute to the determination of the microfauna diversity (vertical way in the SBI table). Since flagellates, rotifers and nematodes are very difficult to identify at species level, these groups contribute each with 1 systematic unit only.

Concerning keygroups (horizontal in the SBI table), it should be remembered that only bacterivorous ciliates belong in the three functional groups (free-swimming, crawling, attached). Carnivorous ciliates contribute only to the total density and diversity of the microfauna. Swimming predaceous ciliates such as *Litonotus*, *Amphileptus*, *Spathidium*, must be excluded by the "free-swimming ciliates" keygroup; in the same way attached predaceous ciliates such as the suctorians *Podophrya*, *Tokophrya*, and *Acineta*, must be excluded by the "sessile ciliates" keygroup. The identification of the various species of protozoa is important, in order to obtain an accurate SBI value; however, some keys written specifically about the protozoa found in activated sludge treatment process and polluted waters are available (Curds, 1969; Madoni, 1981, 1988; Streble and Krauter, 1981; Foissner *et al.*, 1991, 1992).

In order to list the species present in the sludge, the following procedure is suggested:

—to complete analyses within 5 h from collection. Samples of mixed liquor for microscopical observations must be kept alive during both carriage and analysis period, by aerating them sufficiently to keep all solids in suspension;

—to take a little drop (50–100 μl) of mixed-liquor for examination under microscope. Cover glasses 24 × 24 mm or 24 × 32 mm are recommended.

Enumeration of the microfauna

Two different counts are required: estimate of the microfauna abundance (excluding small flagellates), and estimate of the abundance of small flagellates. The first count is necessary to establish both the class of abundance into which the microfauna falls ($<$ or $\geqslant 10^6$/l), and the abundance ratio (%) among groups and species. Estimate of population density should be based on enumeration from sub-samples extracted with an automatic micropipette. For the most appropriate drop size and number of replicate counts, to refer to sub-sampling techniques described by Madoni (1984) and Augustin *et al.* (1989) specifically for the sludge microfauna. Mostly, 25 μl sub-samples of activated sludge and 1 or 2 replicates of this volume are sufficient. In this case, coverglasses of 18 × 18 mm are recommended in order to prevent a quick drying of the sub-samples. Counts should be made under microscope at low magnification (× 100). When colonial species are observed (*Carchesium*, *Epistylis*, *Opercularia*, *Zoothamnium*), all individuals of the colony must be counted. In the case of species found in the screening but not observed during counts, these should be considered as present in the proportion of 1 individual/ml.

Estimate of small flagellate population requires an appropriate count technique, due to their very reduced size and high density, often reaching 10^7–10^8 ind./l. For this purpose, a 3.2 μl Fuchs–Rosenthal chamber can be usefully employed. The chamber is 4 × 4 × 0.2 (depth) mm sized and is sub-divided into 256 squares, 250 μm sized: flagellates inside the 16 squares along the diagonal of the chamber should be counted. When flagellates counted along the diagonal are less than 10, it means that their density in the activated sludge mixed liquor is less than 50,000 ml; over 100 flagellates along the diagonal correspond to the density of $5 \cdot 10^8$ ind./l. Flagellate counts should be made under microscope at × 200 magnification.

An example of enumeration of the microfauna and utilization of the SBI is reported in Table 6.

FINAL REMARKS

It was possible to construct a Sludge Biotic Index based on both structure and abundance of the microfauna inhabiting the activated sludge mixed liquor. As methods, the SBI was also set up on the basis of the relationship occurring between plant performance and operating conditions on one hand, and structure of the microfauna within the activated sludge reactor on the other. The method was formulated with the needs of a treatment plant operator in mind, so both identification and enumeration of the microfauna were suitable. A further advantage of this method over other schemes is that it provides numerical values which enable the operator to compare day by day the prevalent plant operating conditions.

Table 6. Example of the estimation of the microfauna density and use of the sludge biotic index SBI to predict the biological quality of the sludge

Taxa	No. 25 µl	No./ml	%
Swimming ciliates			
Paramecium caudatum	1	40	<1
Crawling ciliates			
Aspidisca cicada	312	12,480	45
Aspidisca lynceus	23	920	3
Chilodonella uncinata	16	640	2
Euplotes affinis	—	1	—
Trochilia minuta	24	960	3
Attached ciliates			
Vorticella convallaria	203	8120	29
Vorticella microstoma	3	120	<1
Vorticella octava	71	2840	10
Epistylis plicatilis	9	360	1
Opercularia coarctata	18	720	3
Carnivorous ciliates			
Litonotus fasciola	8	320	1
Podophrya sp.	2	80	<1
Testate amoebae			
Arcella	3	120	<1
Euglypha	—	1	—
Large flagellates	1	40	<1
Rotifers	3	120	<1
Total microfauna	697	27,882	100
Small flagellates*	<10		
Free-swimming ciliates	1	40	<1
Crawling ciliates	375	15,001	54
Attached ciliates	304	12,160	44
Testate amoebae	3	121	<1

Dominant keygroup: crawling + attached ciliates; microfauna density: $>10^6$/l; total number of taxa = 17; small flagellates: <10; SBI = 10; quality class = 1.
*Along the Fuchs-Rosenthal chamber diagonal.

It should be emphasized however that the SBI was set up specifically for the evaluation of the biological reactor performance. This index thus, is unable to reveal any disfunction in the final sedimentation tank (i.e. bulking, rising), even if some of these problems, such as losses of sludge, can cause, in time, changes in the community structure.

The index was designed to be used in all activated sludge plants including oxidation ditch, conventional aeration plants and extended aeration units. The microfauna used in the index generally have a cosmopolitan distribution; we therefore assume that the index is applicable to the activated sludge plants in all the continents.

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