ORIGINAL ARTICLE

Study of the correlation between microfauna and the macrostructure of activated sludge and the efficiency of biological wastewater treatment plants

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Key words

Wastewaters •Activated sludge • Microfauna

Summary

Assessment of protozoan populations is an important tool in evaluating the efficiency of activated sludge in the treatment of wastewater. In this process, protozoa play a significant role by grazing on dispersed bacteria, supporting a healthy food web in activated sludge artificial ecosystems. The objective of this study was to verify how the success of the purification process in activated sludge plants, mainly in terms of TSS, BOD_5 and COD, is related to ciliate protozoa communities and the presence of filamentous bacteria.

Samples were collected from five water treatment plants in the Puglia region, in the period May 2007 - April 2008. Microfauna and filamentous bacteria were identified and quantified, and the sludge biotic index calculated.

The data show a correlation between the biological components of activated sludge and traditional chemical parameters. Our results indicate that biological analyses represent a valid alternative to traditional chemical testing in assessing the performance of activated sludge systems.

Introduction

Biological wastewater treatment plants may be considered artificial ecosystems subject to extreme conditions. As in any other biological system, the biocoenosis that develops in the aeration basin of a plant based on the "activated sludge" principle has a specific structure and follows precise space-time dynamics. Recently activated sludge microfauna is widely used to assess the operational conditions of biological wastewater treatment plants [1-4]. Furthermore, microfauna has recently been reported to reduce sludge production, affect floc distribution size via predatory activity and decrease the sludge volume index (SVI), without compromising effluent quality [5-8].

Activated sludge consists of a complex biological community – including viruses, bacteria, protozoa, fungi and metazoan – which breaks down organic compounds in waste water [9]. Quantitatively, bacteria represent around 95% of the total microbial population [10] and are essential for biological removal of organic carbon, ammonium and phosphate in the aeration tank [11].

The aeration basins of these plants develop their own trophic network, which consists of various populations competing with each other for food. The relations of competition and predation create oscillations and successions of populations until a dynamic equilibrium is reached. This is closely dependent on the management and design of the plant, the objective of which is obviously to achieve the greatest efficiency in terms of purification.

Ciliate protozoa are highly numerous in all types of process used in aerobic wastewater treatment: they typically reach densities of 10,000 cells per ml of aerated activated sludge, meaning that they account for about 9% of suspended solids in the mixed liquor.

Most of the ciliates present in biological wastewater treatment plants feed on free-floating bacteria, but there are also ciliates with ventral mouths that are able to scrape bacteria off the surface of flocculated sludge [12].

Ciliate protozoa have been observed to improve the quality of the final effluent from treatment plants by preying on most of the bacteria present in the aerated mixture that continuously enter the system with the liquid, leading to a more clarified effluent [13, 14]. In addition, ciliate protozoa also assist in the process of sludge flocculation by secreting mucilaginous substances with flocculating properties [15, 16].

By combining chemical-physical testing with biological analysis techniques, information can be obtained on the biological history of the plant and its efficiency, because the structure of the biological community in terms of functional groups or in species is representative of the way the plant functions [12]. Thus the species structure of the microfauna is a valid diagnostic tool, enriching the parameters that are typically used to evaluate the biological efficiency of treatment plants [14]. The pres-

ence or disappearance of particular species, as well as the structure of the microfauna as a whole, may be considered a good indicator of the biological effectiveness of activated sludge in terms of water purification [17].
Briefly, efficient activated sludge should have the following characteristics:high density of microfauna ($\geq 10^6$ organisms per litre); microfauna composed of both mobile and sessile forms (organisms that are able to attach themselves or remain fixed to flakes of sludge), with practically no small free-swimming flagellates and ciliates (organisms that swim in the liquid fraction and are subject to being flushed out of the system in the final effluent); a highly diversified community, where no group or species numerically dominates the others by a factor of more than 10.
When these criteria are not met, the identification of the dominant group makes it possible to diagnose the functional state of the plant and any dysfunctions that may be affecting it.
As well as analysing the microfauna colonising the flocs, it is important to check the filamentous bacteria present, as these may be responsible for problems of bulking and foaming [18].
Filamentous bulking, caused by excessive proliferation of filamentous bacteria, can produce interfloc-bridging – where the filaments extend from the floc surface and physically hold the floc particles apart – or open-floc structures – where the filaments grow mostly within the floc and the floc grows around them and attached to them [19].
Filamentous foaming is seen when there is persistent, brown and viscous foam on the surface of the aeration basin, which is subsequently transferred to the secondary clarifier. Persistence of this foam results in decreased transfer of oxygen through the surface in mechanically aerated basins, odour problems and a reduction in the quality of the effluent due to increased suspended solids and BOD_5 [20].
The aim of this study was thus to verify how the success of the purification process – in terms of total suspended solids (TSS), BOD_5 and COD – in treatment plants based on the activated sludge principle is related to communities of ciliate protozoa and the presence of filamentous bacteria.
This represent a new approach of analysis, alternative to the chemical parameters usually used as indicators of the purification process efficiency.

Materials and methods

This study was carried out in the period May 2007-April 2008 in five water treatment plants in the Puglia region characterised by a range of process dysfunctions linked to both the quality of the microfauna and the presence of filamentous bacteria. Specifically the following cases were analysed:

1. a well-functioning treatment plant;
2. a treatment plant with problems of filamentous bulking;
3. a treatment plant with high chloride concentrations (750 mg/l Cl^-) due to infiltrations of seawater in the influent;
4. a treatment plant with a double oxidation basin with poor treatment efficiency due to problems with the sludge line;
5. a treatment plant with problems of filamentous foaming.

The receiving environment of the treated waters is different for each plant monitored: in the first case it is field drainage ditches, in the second case the subsoil, in the third and fifth cases the sea and in the fourth case a fresh water channel – a non-significant water body, as per Annex 1 of Italian law D. Lgs. 152/06 governing superficial water courses [21] and the WFD, Water Framework European Directive [22].
Sampling was carried out by automatic samplers, programmed to gather material at fixed times during the day, installed at both the entrance to the wastewater treatment line and the exit of the oxidation basin.
Concerning biological testing, an optical microscope was used to characterise and quantify the microfauna, and the sludge's biotic index (SBI) was calculated in accordance with Madoni's method [1]. This kind of microscopy analysis allow us to get important indication about the biological activity of the sludge, considering its own microorganism community structure.
The activated sludge biotic index can be obtained using the table indicated by Madoni [1], where SBI values are assembled in 4 classes of quality (I, II, III and IV); through them we can represent, widely and completely, the biological quality of activated sludge.
In order to estimate the microfauna, 25 μl of sample were observed at the optical microscope at 100 X.
We carried out the analysis on 4 replicates.
Filamentous bacteria were identified and their abundance estimated with reference to the manual by Jenkins et al. [10].
The average number of organisms was standardised per litre of sample.
The density of small flagellates was determined putting 32 μl of sample in a Fuchs-Rosenthal counting chamber and observed at the optical microscope at 200 X.
Protozoa were identified with reference to the manual "Microbiologia e Depurazione" by Fantei et al. [23] and the manual by Madoni [24].
As well as protozoa, filamentous bacteria populations present in the activated sludge were identified with reference to the atlas published by the AGAC (Azienda Gas Acqua Consorziale) company of Reggio Emilia [25].
As well the study of the biological facies, the waste water was also tested for certain chemical parameters – TSS, BOD_5, and COD – in accordance with analytical methods described by APAT and IRSA-CNR [26].
The data obtained from these analytical studies were used to measure the pollutant load of waste waters entering the plants and the performance of the purification process.

Tab. I. Average density of protozoa found in oxidation basins of five water treatment plants and relative quality class of activated sludge.

Microfauna	Treatment plant				
	1	2	3	4	5
Taxa	n/ml	n/ml	n/ml	n/ml	n/ml
Acineria uncinata		40	40		
Testate amoebae	2600	3600		80	220
Aspidisca sp.		20			2360
Chilodonella uncinata		60			
Epistylis spp	5600			160	
Euglypha sp.	360	160			
Euplotes sp.		20			
Flagellates			200		
Gastrotricha		140			
Rotifers	240	380			
Vorticella convallaria	920		50	452	140
Total	9720	4420	290	692	2720
Functional groups					
Free-swimming ciliates	0	40	0	0	0
Sessile ciliates	6520	0	50	612	140
Crawling ciliates	0	140	40	0	2360
Dominant group	**Sessile ciliates**	**Testate amoebae**	**Flagellates**	**Sessile ciliates**	**Crawling ciliates**
Density of microfauna (n/l)	> 10^6	> 10^6	< 10^6	< 10^6	> 10^6
Total number of taxa	5	8	3	3	3
Small flagellates	< 10	< 10	> 10	> 10	< 10
SBI	8	9	0	3	7
Quality class	I	I	IV	IV	II

CLASS I Well colonised and stable sludge, optimal biological activity, high treatment efficiency

CLASS II Well colonised and stable sludge, sub-optimal biological activity, fair treatment efficiency

CLASS III Insufficient biological activity, mediocre treatment efficiency

CLASS IV Poor biological activity, poor treatment efficiency

Results and discussion

Tables I-V show the results of the study of the biological facies of the activated sludge and the chemical tests carried out on the influent and effluent of the five treatment plants under study.
Analysis of the data obtained shows the following:

a) Case n. 1
- optimal microfauna (SBI = 8, class I);
- macrostructure with filamentous bacteria dominated by no single species;
- excellent reduction of TSS, COD and BOD_5 with respect to the limits laid down in table IV of Italian law D.L. 152/06 [19].

b) Case n. 2
- optimal microfauna (SBI = 8, class I);
- macrostructure characterised by excessive presence of filamentous bacteria of *type 021N* and *Nostocoida limicola*;
- excellent reduction of TSS, COD and BOD_5 with respect to the limits laid down in Table IV of Italian law D.L. 152/06 [19], despite the presence of a high concentration of filamentous bacteria with the potential to destabilise the general functioning of the plant. A lack of adequate controls may have led to poor sedimentation of the mud and leakage of suspended solids with the final effluent.

Tab. II. Filamentous bacteria found in oxidation basins of five activated sludge water treatment plants.

Treatment plant	Filamentous bacteria present	Importance	Abundance
1	021N	S	F
	Nostocoida limicola	D	A
2	021N	D	A
	Thiothrix	S	M
3	021N	S	Rare, almost absent
4	H. Hydrossis	D	A
	0041/0675	S	M
5	Microthrix parvicella	D	A

Importance: D: Dominant; S: secondary
Abundance: F: few; M: Moderate; A: abundant.

c) Case n. 3
- poor microfauna composed almost exclusively of flagellates, typical of plants which are still in the start-up phase (SBI = 0, class IV);
- macrostructure compromised by toxic effects of chlorides (present in high concentrations), which

Tab. III. Concentration of total suspended solids (ppm) in influent and effluent of five activated sludge water treatment plants.

Treatment plant	TSS in influent	TSS in effluent	% reduction
1	269	6	98
2	261	6	97.70
3	76	32	57.89
4	292	72	75.34
5	297	98	67

Tab. IV. BOD_5 values (ppm) in influent and effluent of five activated sludge water treatment plants.

Treatment plant	BOD_5 in influent	BOD_5 in effluent	% reduction
1	290	11	97
2	410	13	96.83
3	85	85	0
4	360	84	76.67
5	350	52	85.14

Tab. V. COD values (ppm) in influent and effluent of five activated sludge water treatment plants.

Treatment plant	COD in influent	COD in effluent	% reduction
1	703	35	95
2	810	35	95.68
3	200	180	10
4	723	183	74.69
5	715	142	80.14

prevented the development of both filamentous bacteria and microfauna;

- extremely limited reduction of pollutant load, especially in terms of COD and BOD_5, both of which had values above the legal limits laid down in table 4 of Italian law D.L. 152/06 [19] (Tab. III), as a consequence of the poor quality of the microfauna present in the system.

d) Case n. 4

- microfauna with low species diversity dominated by sessile ciliates, whose growth is generally favoured by plants with rapid increases in sludge load in the secondary clarifier and subsequent extraction of biomass (SBI = 3, class IV);
- non-optimal macrostructure with large, irregularly-shaped sludge flocs due to the presence of *Spirilla* and *H. hydrossis*, typical of sludge with low concentrations of dissolved oxygen;
- insufficient reduction of TSS, COD and BOD_5, in excess of the limits laid down in Table IV of Italian law D.L. 152/06 [19]. This was mostly due to problems in the management of the sludge line and to the inactivity during the monitoring of one of the two oxidation basins, causing negative effects on the development of the microfauna and compromising the entire treatment process.

e) Case n. 5

- microfauna dominated by crawling ciliates, such as *Aspidisca cicada* and testate amoebae, typical of high-age sludge (SBI = 7, class II);
- macrostructure characterised by excessive presence of *M. parvicella*, responsible for the foaming observed on the surface of the final sedimenter and the aeration basin;
- insufficient reduction of TSS and BOD_5, in excess of the legal limits laid down in Table III of Italian law D.L. 152/06 [19], and turbid effluent due to the presence of organic substances and sludge-containing foam.

Discussions and conclusions

Despite the study was limited at a single experience on 5 waste water treatment plants, our results show a clear correlation between the biological facies data of the activated mud and the chemical parameters traditionally used for evaluating the purification efficiency of treatment plants. Indeed, the treatment plants with optimal microfauna and a sludge floc macrostructure without excessive filamentous bacteria were characterised by an equally optimal reduction of TSS, COD and BOD_5, which were all within legal limits. Conversely, in treatment plants characterised by poor biological constituents or the dominance of filamentous bacteria, an inadequate reduction of the chemical parameters was observed.

These considerations indicate that biological analyses may constitute a valid alternative to traditional chemical testing in assessing the efficiency of activated sludge treatment plants.

In addition, these analyses may be a crucial tool for the correct management of such plants, as has been argued by some authors [4, 27, 28]: the immediacy with which this biological information can be acquired enables a plant's technicians and operators to carry out corrective measures in real time, averting prolonged or unsustainable malfunctions.

In conclusion, it may be asserted that the study of the biological constituents of activated sludge provides information regarding the history of a treatment plant and the mutations occurring in it over time.

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