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Water Practice & Technology Vol 12 No 4
doi: 10.2166/wpt.2017.092

Discharge quality from municipal wastewater treatment plants and the *Sludge Biotic Index* for activated sludge: integrative assessment

M. Ostoich[a,*], F. Serena[b], C. Zacchello[c], L. Falletti[d], M. Zambon[a] and L. Tomiato[a,b]

[a] Venice Provincial Department, ARPAV, Veneto Regional Prevention and Protection Agency, via Lissa 6, 30171 Mestre-Venice, Italy

[b] Treviso Provincial Department, ARPAV, via Santa Barbara, Treviso, Italy

[c] Regional Laboratory Department, ARPAV, via Lissa 6, 30171 Mestre-Venice, Italy

[d] Industrial Engineering Department (DII), University of Padova, Via Marzolo, Padova, Italy

*Corresponding author. E-mail: marco.ostoich@arpa.veneto.it

Abstract

Wastewater treatment plants (WWTPs) are scrutinized by Environmental Authorities particularly regarding the compliance to discharge limit values fixed by national and local regulations. An integrated approach is necessary to achieve the objectives established with Directive 2000/60/EC (WFD) considering the ecological status of the receiving water body and the quality of the discharge. Specifically, documentary, technical, management and analytical controls should be developed. Moreover, integrative information on the behaviour of the activated sludge in the aeration tank can be useful for plant managers as well as for the regulating Authorities. The study presents the experience concerning WWTP regulation considering the analytic assessment of the discharge as well the monitoring of the *Sludge Biotic Index* (SBI) for activated sludge. Data from monitoring during the period 2008–14 on SBI values and chemical and microbiological data on the discharges of a sample of 35 WWTPs in the province of Venice (north-east Italy, Veneto region) are presented and discussed. Together with chemical and microbiological analysis, the SBI appears to be a highly useful index for the integrative assessment of plant functionality, in particular when monitoring and identifying critical situations that can determine the exceedance of discharge limit values. The SBI method, in an integrated control approach, can be used for small and medium sized WWTPs that only treat domestic wastewaters. In a case by case assessment this may even substitute part of the analytical monitoring carried out in the WWTPs' control process.

Key words: activated sludge process (ASP), bacteria, discharge limit value, protozoa, Sludge Biotic Index (SBI), wastewater treatment plant (WWTP)

INTRODUCTION: INTEGRATED APPROACH FOR MONITORING WASTEWATER TREATMENT PLANTS

Humans and their activities produce wastewaters that are generally referred to as 'urban wastewaters', that is the mix of metabolic residues from humans and drainage waters. In an urban, industrialized context, together with domestic wastewaters (WW), industrial ones may also be present. Municipal wastewater is generally treated by means of activated sludge processes (ASPs), that facilitates the biological transformation of dissolved and colloidal organic compounds into biomass. The ASP aims to obtain maximum reduction of BOD_5, suspended solids (SS) and nutrient (N, P) levels in treated effluents and the lowest possible sludge production. The ASP consists of bacteria and other organisms naturally present in water bodies that can utilize dissolved and colloidal organic matter to grow and produce biomass; when bacteria and other organisms are grown the biologic biomass can be

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separated from the remaining water by allowing it to settle. Thus, organic pollution is separated from wastewater; residual WW should be discharged respecting the specific limit values defined, in Italy, in the *River Basins Management Plan* (RBMP) and in the *Water Protection Plan* (WPP) drawn up respectively by the district/basin Water Authority and by the Region in accordance with the Water Framework Directive 2000/60/EC (WFD; EC 2000).

Biological treatment processes are based on the natural self-purification process, typical of water bodies and performed by bacteria and micro-fauna already present in the water body. In the micro-fauna, protists are crucial for the effective treatment of the organic load. The importance of protists in ASPs has been described by many authors; Foissner (2016) observes that protists have been identified as bioindicators in activated sludge. Moreover, this author highlights that for the use of protists as indicators in wastewater treatment three different periods can be identified: 1) age of discovery and exploitation (discovery and use of activated sludge for wastewater purification); 2) age of bloom (development of saprobic index and practical tools for performance of sewage plants); 3) age of decline. Foissner claims we are currently in phase three; however, his conclusions reveal that the study of protists in ASP systems can also give a valuable contribution to the performance and management of wastewater treatment plants (WWTPs). To monitor the performance of ASP plants, Madoni (1994a) proposed the *Sludge Biotic Index* (SBI), defined as an 'objective index' similar to the *Extended Biotic Index* (EBI) (Woodiwiss 1980) developed for rivers. SBI is based on the consideration that the analysis of micro-fauna can give quantitative, standardized information regarding ASP plant performance. It is founded on both the structure and the abundance of the micro-fauna inhabiting the activated sludge in mixed liquor.

For WWTP monitoring Ostoich *et al.* (2010) proposed an integrated approach using *documentary*, *technical*, *management* and *analytical* controls and functionality assessment of the plant to assess its reliability in ensuring compliance with discharge limit values. In the official regulation of WWTPs, analytical discharge monitoring is not always sufficient to obtain a satisfactory overview of the real situation and of the reliability of the WWTPs monitored. This study presents experimental data from institutional monitoring activity in which analytical controls on the effluent, as well as the application of the SBI on activated sludge, have been performed. In particular, case studies and the usefulness of the index are discussed for plants along coastal areas in the Veneto Region (province of Venice, north-east Italy). This study consists of the preliminary investigation of a sample of 35 small (<2,000 PE), medium (<10,000 PE) and large WWTPs in the province of Venice in the period 2008–14 performed by the regulatory Authority (Veneto Regional Environmental Prevention and Protection Agency-ARPAV). The reliability of WWTPs in avoiding excessive discharge limit values is very important, in particular along the coast where they can directly impact bathing water quality which is strategic for the tourist industry. Integrative SBI analyses can be useful in supporting functionality analysis in large plants where analytical discharge is delegated to the plant manager as it is allowed by the Italian Regulation on urban wastewater discharges (Italian Decree n. 152/2006 Part III Annex V) with integrated requirements. Moreover, in small and medium sized ASP treatment plants, SBI monitoring can be integrative to standard, planned monitoring activities or in substitution of some analytical controls, with the aim to gain more information on the general functionality of the plant and its reliability in guaranteeing effective treatment and satisfactory performance.

ACTIVATED SLUDGE PROCESSES

Protozoa play an important role in the trophic web of wastewater treatment processes (Curds 1973, 1982). Decomposers account for nearly 91% of the microbial population in activated sludge, while consumers represent the remaining 9%. Biotic indices have been developed for the evaluation of the quality of water bodies according to the state of health of their ecosystems (Madoni 1994a).

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The biomass in the oxidation tank is really a complex ecosystem and its efficiency in organic load removal depends on its structure. Ciliates can be used as indicators of effluent quality (Curds & Cockburn 1970a) The presence of ciliate protozoa reflects an increase in effluent quality (Curds 1982) and microscopic analysis can be applied to activated sludge to estimate effluent quality (Curds & Cockburn 1970b). Changes in the structure of the community of activated sludge reflect changes in wastewater quality and the relationship between protozoan species in the aeration tank was quantified to predict plant performance (Al-Shahwani & Horan 1991). The majority of ciliates present in biological water treatment plants feed on dispersed populations of bacteria (Madoni 1994a). Protozoa analysis is used to determine the organic load (Salvadó & Gracia 1993).

A low organic loading rate is associated with long sludge retention time, stable aerobic conditions and poor feeding substrate. These conditions are characterized by fewer dispersed bacteria, an abundance of species, high diversity with predominance of crawling and attached ciliates. Increased organic loading modifies the micro-fauna structure causing the decline of species diversity and the predominance of small taxa-like flagellates, free swimming ciliates and attached ciliates (Madoni *et al.* 1993). Madoni (1994a) observed that the performance of activated sludge WWTPs depends on the diversity and abundance of micro-fauna. The capability of ciliated protozoa as indicators of effluent quality in ASP plants has also been highlighted by other authors: ciliated protozoa are effective in wastewater purification because they feed off dispersed bacteria (Salvadó *et al.* 1995). Plant behaviour and performance can be predicted by analysing activated sludge and protozoa (Al-Shahwani & Horan 1991; Madoni *et al.* 1993; Salvadó *et al.* 1995; Drzewicki & Kulikowska 2011). Salvadó *et al.* (1995) presented the relationship between the ciliate population density and effluent quality in activated sludge plants and the analysis of ciliate protozoa and small metazoa. Effluent quality was monitored with BOD_5 and SS. The higher the ciliate species population density, the better the capability of the species as an indicator. The correlation coefficients between ciliates and effluent quality depend on the range of the studied physico-chemical values (Salvadó *et al.* 1995).

THE SLUDGE BIOTIC INDEX

SBI is based on the structure and abundance of the micro-fauna present in the activated sludge in the mixed liquor. SBI is based on protistan assemblage and is founded on two factors: 1) changes in protistan key-groups triggered by environmental and operational conditions; and 2) plant efficiency correlated to cell density and number of taxa present in the activated sludge. Protistan key-groups in activated sludge plants, which feed on dispersed bacteria, are bacterivorous ciliates and are classified into three functional groups: free-swimmers; crawling forms; attached forms (Madoni 2000).

The microbial community can be used as an indicator of various operational conditions present at the plants; the performance of the activated sludge sewage treatment process depends on the density and structure of the protistan community on the sludge flocs in the aeration tank (Madoni 2000). No information is given on dysfunction in the final settlement tank (phenomena such as bulking, rising sludge, pin-point floc, etc.). The micro-fauna considered in the SBI have a cosmopolitan distribution; it is assumed therefore that the SBI is applicable to activated sludge plants all over the world. The SBI presents as a standardized method but, as stated by Drzewicki & Kulikowska (2011), the response is not always simple and straightforward. In fact, structural, design and operational factors must be considered. They can influence plant performance and the classification of some ciliates may also be difficult (Madoni 1996, 2000).

Basically, the microbiological analysis of the activated sludge diagnoses the functionality state of an ASP plant. The SBI was proposed as a useful tool for plant managers as well for conducting research on ASPs. The SBI method is based on the taxonomic identification of Protozoa in activated sludge with microscopic analysis, which has already been used to estimate the quality of the effluents and the performance of the plant (Madoni *et al.* 1993). This method assumed that the dominance of

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key-groups, the abundance and quantity of indicator taxa micro-fauna present in the activated sludge varied depending on the physico-chemical and technological parameters and the effects of the treatment process. Madoni (1994a) presented the correlation between protozoan and plant operational conditions; he proposed and used a double-entry table where horizontal data represented diversity and vertical data population density. The SBI method was set up and based on the relationship between plant performance and operating conditions on the one hand, and the structure of the micro-fauna within the activated sludge reactor on the other. Density and biomass can be measured with an optic microscope. Key-groups of bacterivorous ciliates are recorded horizontally on the SBI table; while the enumeration of taxa and biomass density are recorded vertically (Madoni 1994a). The SBI is an 'objective index' as the quantitative values determined by different operators are wholly comparable. This method, is based on the different sensitivity shown by some groups of protists in the micro-fauna towards main chemical, physical and operational parameters as well as on the abundance and diversity of species in activated sludge micro-fauna. The number of ciliated protozoans in a normally-functioning activated-sludge plant is about 10^6 individuals/l; when ciliated protozoans are fewer, there is insufficient purification (Madoni 1994b). For an efficient ASP the structure of the micro-fauna should comprise (Madoni 1994a) a high concentration of micro-fauna cells $\geq 10^6$ cells/L, the presence of crawling and attached species, and a highly diversified micro-fauna structure.

Toxicity levels for activated sludge micro-fauna of different substances has been observed by various authors, for example Papadimitriou *et al.* (2007), Madoni *et al.* (1996), Madoni (2000) and Nicolau *et al.* (2005). The activity of aerobic bacteria and micro-fauna in an ASP can be inhibited by the presence of certain organic and inorganic substances introduced in industrial discharges. Heavy metals are particularly toxic for activated sludge (Madoni *et al.* 1996); in fact, Cd, Cu, Pb, Ni, Cr(VI) can negatively affect the aerobic processes at ppm level or even at the ppb level (Madoni & Romeo 2006). Other inorganic substances that can be found in industrial discharges and that inhibit the ASP also include chlorine and cyanides while, among the toxic organic substances, there are phenols, formaldehyde, herbicides and phyto-pharmaceuticals. In some cases, microorganisms can be forced to cohabitate with increasing doses of toxic substances, after a sufficiently long period of adaptation.

According to available literature and to the experimental activity performed, the SBI can be used to monitor and assess plant performance, reduce the load of organic and SS, monitor operative conditions and plant reliability, in particular for small and medium sized plants treating domestic wastewater (for industrial discharges the situation appears different). The SBI is negatively influenced by the following factors: toxic discharges of heavy metals (Madoni *et al.* 1996; Nicolau *et al.* 2005) and organic compounds (Papadimitriou *et al.* 2007; Drzewicki & Kulikowska 2011); situations of under- or over-loading; excessive sludge extraction and lack of aeration (Madoni 1994a). Moreover, as a general consideration, Foissner (2016) observes that for both the *Saprobic Index* and the SBI, it does not appear possible to reduce the organism communities to a single numerical value; in fact, these indices should be interpreted cautiously. This author himself suggests that an effort should be made to integrate the following aspects: the biological analysis of activated sludge, operational conditions, physico-chemical data, the technology applied and design data. This aspect has been considered and discussed in the present study, although more plant functioning data are necessary to conduct a satisfactory statistical analysis.

INTEGRATED CONTROL APPROACH FOR WWTPS AND FUNCTIONALITY VERIFICATION

The approach to environmental monitoring of WWTPs developed and applied by the control Authority (ARPAV) since 2000 has been inspired by the hierarchical principle derived from Italian regulations and from the risk assessment of the different pressure sources. This is a preventive integrated approach, or more specifically, a control approach where the aim is not to verify just one

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environmental aspect (for example analytical monitoring), but to help gather all 'diagnostic' data and information required to assess the functioning levels of the plant (point pressure source). The developed approach is based on the following control typologies (Ostoich *et al.* 2010): *Documentary*; *Technical*; *Management*; *Analytical*.

Operative protocol for WWTP monitoring has been developed and applied based on the above criteria. Figure 1 presents the elements of the performance assessment: it refers to theoretical verification at mean loads and is carried out using a precompiled electronic spreadsheet. The functionality assessment, which uses data obtained during the inspection visit including self-certification data, analytical determinations, structural and management data, provides information regarding plant behaviour and information on each of the single sections in extreme conditions. Functionality verification integrates the above-mentioned operative protocol.

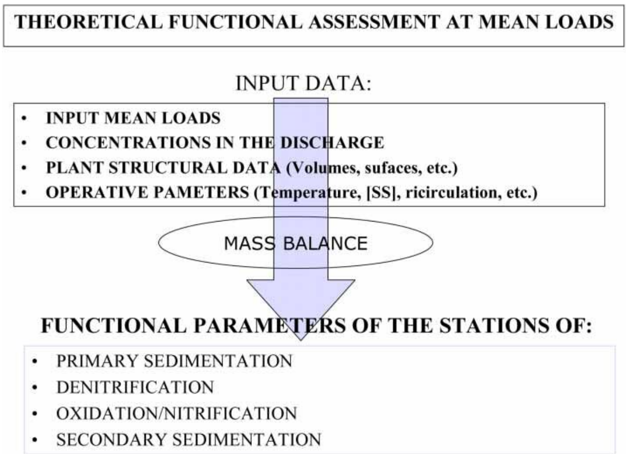

Figure 1 | WWTP theoretical functional verification at mean loads.

The use of the SBI could support the integrated monitoring of small and medium sized plants, as well as could reduce the quantity and frequency of analytical activities but it also improves knowledge of how effective and reliable plants are at respecting discharge limit values.

The functional parameters

Microorganisms in an ASP system follow a typical growth curve in which we can identify the following phases: acclimation, logarithmic growth, reduced growth and the endogenous phase. In the initial phases there is a reduced biomass with a high concentration of food, while in the last phase food is insufficient for the whole population. Micro-organisms present in the Activated Sludge differ depending on the growth phase and the specific plant and when the process actually occurs during the growth phase. In general, the higher the diversity, the better the process performs in removing the organic load. The growth curve is characteristic and each plant, depending on its design, functions within a specific range of this curve i.e. young, middle or old sludge. The parameter to be considered is the sludge load F_c (kg BOD_5/kg MLSS*d).

The choice of the organic sludge load (Vismara & Butelli 1999) determines which micro-organisms' growing phase occurs within the plant. Increased values show an abundance of organic substance as far as the biomass is concerned and therefore rapid growth of the active sludge. Reduced values however, show limited availability of organic substance which is highly stabilized. Efficient treatment is bound to the sludge load: greater efficiency is obtained when organic load values are lower. Based on the value of the adopted F_c there are different types of processes as indicated in Table 1 (Masotti 1999). If a plant is classed as an 'extended aeration' treatment plant retention times are extended and volumes high.

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Table 1 | Biological treatment processes at different values of the sludge organic load

F_c value (kg BOD_5/kg MLSS*d)	Process type	Process description
$0.02 < F_c < 0.15$	Extended aeration (sludge stabilization)	Non putrescible excess sludge Nitrification with very high performances
$0.2 < F_c < 0.3$	Low load	Nitrification with very high performances
$0.3 < F_c < 0.5$	Middle load	Oxidative processes
$F_c > 0.5$	High load	Enhanced oxidative processes

MATERIALS AND METHODS

The WWTPs selected for the study

The WWTPs considered in this study are localized in the province of Venice (north-east Italy). The Veneto Regional Environmental Prevention and Protection Agency-ARPAV carried out analytical monitoring on the final discharges (chemical and microbiological measures) and SBI determination in the period 2008–14. Information concerning the WWTPs in the province of Venice was supplied by the Veneto Region Environmental Informative System (SIRAV) – Cadastre, managed by ARPAV, and obtained during monitoring activity. Table 2 reports the WWTPs census for the province of Venice, which is the area under observation in this study (Figure 2(a) and 2(b)). In Table 3 the 35 WWTPs situated in the province of Venice, and for which the SBI was determined in the period 2008–13 (in one case 2014), are listed together with their population equivalents (PE). To better understand the operative and performance conditions, data from plant managers was requested and used for the following WWTPs: Venice-Fusina, Venice-Campalto, Venice-Lido, Cavallino-Treporti and Chioggia. As a result, a deeper and more satisfactory assessment was possible but only for these plants, regardless of the differences between them.

Table 2 | Number of WWTPs (census) in the province of Venice distributed by PE size (source: SIRAV Cadastre, 2016)

Province	<2,000 PE	2,000–9,999 PE	10,000–49,999 PE	≥50,000 PE	*Tot. Prov.*
Venice – Numb. of WWTPs	22	18	5	9	54

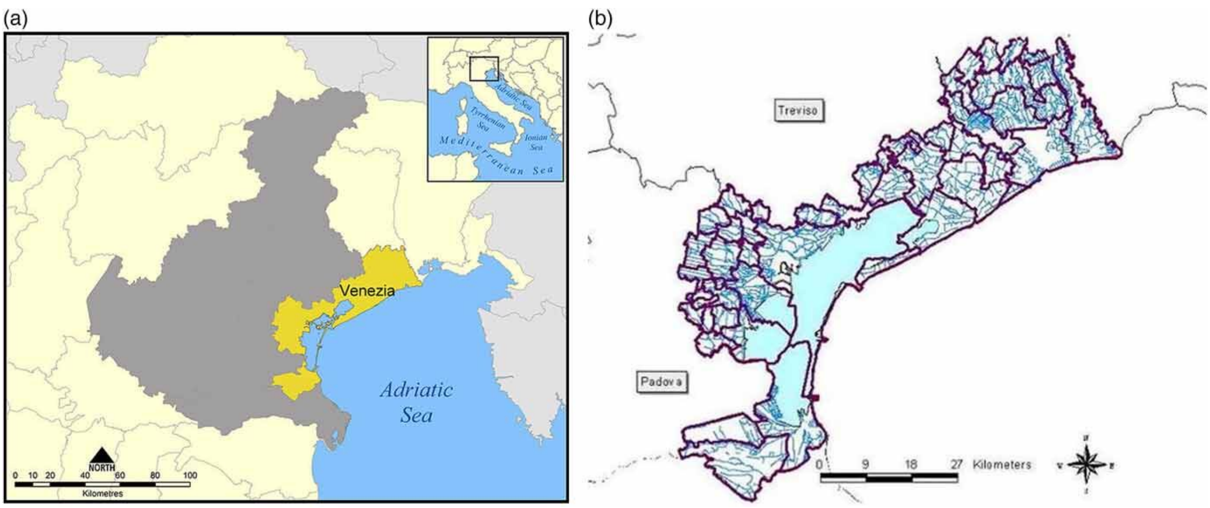

Figure 2 | (a and b) The area of study (province of Venice) in the Veneto Region, north-east Italy (source: ARPAV, 2016).

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Table 3 | WWTPs investigated for SBI determination in the period 2008–14 in the province of Venice (source: ARPAV, 2016)

WWTP	PE	WWTP	PE
ANNONE VENETO, Capoluogo	2,000	NOVENTA DI PIAVE, Capoluogo, Via Torino	4,500
CAORLE, Capoluogo, Via Traghete	120,000	SAN MICHELE AL TAGLIAMENTO, Capoluogo, via A. Moro	8,000
CAORLE, San Giorgio	3,000	SAN STINO DI LIVENZA – LA SALUTE, via L. da Vinci.	2,500
CAVARZERE, Via Piantazza	17,500	PORTOGRUARO, loc. Destra Reghena, Viale Venice	8,700
CAVARZERE, Rottanova	1,000	PRAMAGGIORE, Blessaglia	2,500
CAVARZERE, S. Pietro	1,000	GRUARO, La Sega via Giai	2,800
CEGGIA, Via I Maggio	5,000	MUSILE DI PIAVE, Capoluogo via Rovigo	10,000
CINTO CAOMAGGIORE, Via dei Prati	2,000	SAN STINO DI LIVENZA, Capoluogo, via Canaletta	10,000
CHIOGGIA, Val da Rio	160,000	SAN DONÁ DI PIAVE, Capoluogo via Tronco	45,000
CONA, Pegolotte	6,000	TORRE DI MOSTO, via Xola	3,000
CONCORDIA SAGITTARIA, Capoluogo, Via Basse	3,300	VENICE, Fusina, via dei Cantieri	400,000
CONCORDIA SAGITTARIA, Capoluogo via Gabriela	3,000	VENICE, Campalto, Via Scantamburlo	130,000
ERACLEA, Ponte Crepaldo	4,700	VENICE, Lido di Venice, via Galba	60,000
ERACLEA, loc. Eraclea mare, via Pioppi	32,000	JESOLO, via Aleardi	185,000
FOSSALTA DI PIAVE, Capoluogo via Cadorna	3,600	BIBIONE, via Parenzo	150,000
FOSSALTA DI PORTOGRUARO, Villanova, Via Zecchina	1,800	QUARTO D´ALTINO, Capoluogo via Marconi	50,000
FOSSALTA DI PORTOGRUARO, Capoluogo, Via Europa	3,000	CAVALLINO – TREPORTI, via Fausta	105,000
MEOLO, Capoluogo, via Marteggia	9,000		

Sampling and analytical methods for chemical parameters

Official sampling and analytical methods adopted in Italy were applied during this study: *Analytical methods* (APAT 2003) used since 2004. Where analytical methods were lacking in the Italian national legal framework international official methods were also used (i.e. APHA *et al.* 1998). The sampling techniques for WWTP effluents were the following: instantaneous sampling for microbiological parameters and mean-composite sampling on a 24 hour basis for all chemical parameters. When data are reported as lower than the limit of quantification (<LOQ) the value of the parameter has been substituted with half of its value, as suggested in literature, to allow for elaboration (Spaggiari & Franceschini 2000).

SBI assessment

The SBI method is based on the relationship between plant performance and operating conditions on the one hand and on the structure of the micro-fauna within the activated sludge reactor on the other; the index is calculated using a two-way table (Madoni 1994a). Along the horizontal lines the dominant/prevalent groups are considered (diversity) and are associated with decreasing sludge quality from top to bottom; in the upper part of the vertical columns the number of systematic units (micro-fauna density) for vertical entry are reported. The total density of micro-fauna for horizontal entry is also considered (< or $>10^6$ organisms/l) along with the abundance of flagellates for vertical entry. The SBI value, from 0 (worst quality) to 10 (best quality), can therefore be determined in the

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intersection of the selected line and the selected column; the index values are grouped into four extensive quality classifications from I (the best) to IV (the worst) as reported in Table 4 (Madoni 1994a; Drzewicki & Kulikowska 2011).

Table 4 | WWTPs SBI method – quality classes for activated sludge

SBI scores	Quality class	Judgement
8–10	I	Very well colonized and stable sludge; optimal biological activity; very high abatement efficiency
6–7	II	Well colonized and stable sludge; good biological activity; moderate abatement efficiency
4–5	III	Insufficient biological treatment and poor performance of the plant; poor abatement efficiency
0–3	IV	Poor biological treatment and low performance, low abatement efficiency

In aerated ASP, the mixed liquor contained in the aeration tank should be sufficiently homogeneous to allow for the sampling of sludge at any point in the tank. However, the real situation is not so as particular conditions such as building conditions, eddy diffusion, etc. still influence the findings. Therefore, sampling was carried out at points that were not too close to the tank walls nor to the aeration systems. Sampling was performed by using 1 liter plastic bottles. If the bottle is not completely filled, the remaining air is enough to guarantee aerobic conditions at a temperature of 4 °C and to avoid modifications of the microbiological communities during transport. Sludge observations were performed within five hours from sampling. An optic 100X microscope was used. Firstly, screening identification was performed for the forms present in the sludge sample and followed by the estimation of the relative abundance of each species or group, with a complete count of the forms present in a known volume of sludge.

RESULTS AND DISCUSSION

SBI data for the WWTPs' sample

In the period 2008–2013 (2014 for the Venice-Fusina plant) the SBI was determined for 35 WWTPs in the province of Venice as listed in Table 3. The frequency distribution of SBI scores is reported in Figure 3(a), while the frequency distribution of the corresponding quality classes are reported in Figure 3(b). SBI scores are reported in Figures 4 and 5 (presented in two groups each of 16 WWTPs). The majority of the SBI scores are in the range 8–10 and therefore, according to Madoni (1994a) they are in quality class I. Despite the good result with reference to activated sludge quality, some scores were below 8, in some cases as low as 3, for 15 WWTPs out of the 35 plants assessed, as reported in Table 5. From this table it is possible to see that SBI scores below 8 were reached in both

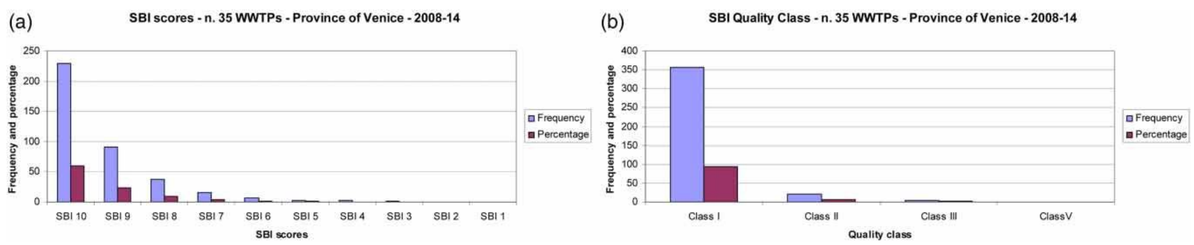

Figure 3 | (a) SBI scores for 35 WWTPs in the province of Venice (source: ARPAV). (b) SBI Quality classes for 35 WWTPs in the province of Venice (source: ARPAV).

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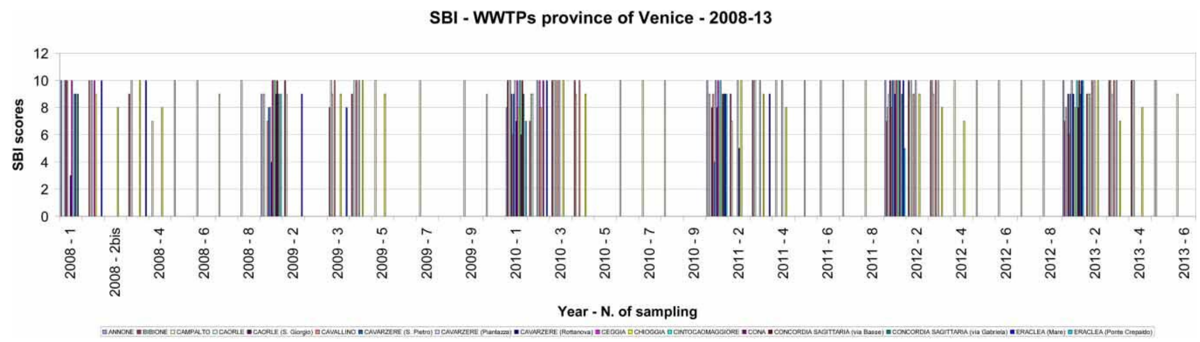

Figure 4 | First group of 16 WWTPs in the province of Venice – SBI scores – 2008–13.

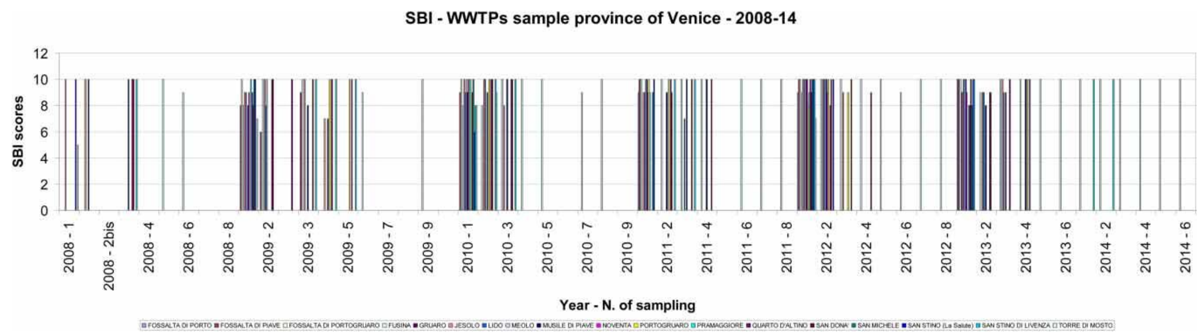

Figure 5 | Second group of 16 WWTPs in the province of Venice – SBI scores – 2008–14 (2014 only for Venice-Fusina plant).

Table 5 | WWTPs with SBI values below 8 in the province of Venice – Period 2008–14

WWTP	WWTP capacity in PE	Number of cases SBI <8	SBI Scores
Bibione SM Tagliamento	150,000	3 cases	7
VeniceVenice – Campalto	130,000	2 cases	7
Caorle – Via Traghete	120,000	1 case	6
Cavallino-Treporti	105,000	1 case	6
		2 cases	7
Cavarzere – San Pietro	1,000	1 case	4
Cavarzere – Rottanova	1,000	1 case	7
		1 case	5
		1 case	4
		1 case	3
Chioggia Val da Rio	160,000	2 cases	7
Cona	6,000	1 case	6
Eraclea Ponte Crepaldo	4,700	1 case	7
Fossalta di Piave	3,600	1 case	6
Jesolo	185,000	1 case	7
Venice-Lido	60,000	1 case	7
Musile di Piave	10,000	1 case	7
S. Stino di L. la Salute	2,500	1 case	6
Torre di Mosto	3,000	1 case	7
		2 cases	5

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large and small plants. It appears that very small plants are subject to phases of lower efficiency (there can be fluctuations of the entering flow and/or fluctuations of the organic load).

Quality of the final discharge

Analytical data on the final discharges were assessed to give a complete assessment of the causes of the behaviour registered with SBI investigation on the sample of 35 WWTPs in the period 2008–14. Data were performed by the control Authority (ARPAV). Due to limited space, only two of the WWTPs whose SBI scores were below 8 have been here considered for chemical parameters: Venice-Campalto and Caorle-Palagon plants. The trends of BOD_5, COD and TSS parameters are presented in Figure 6(a) and 6(b). Limit values for discharges have been introduced only for indicative purpose, given that each discharge must be assessed according to the vulnerability of the specific area where discharge occurs and as established by the regional *WPP* (BOD_5, = 25 mg/l; COD = 125 mg/l; TSS = 35 mg/l).

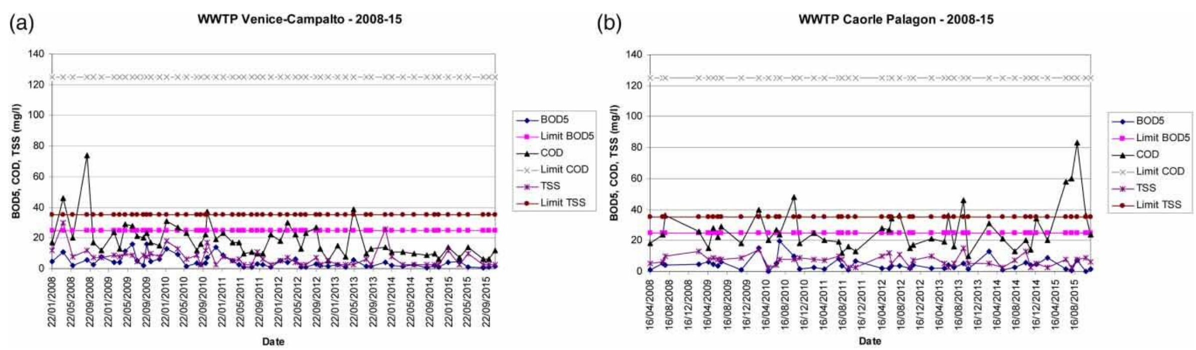

Figure 6 | (a) Trend of WWTP discharge 2008–15 (source: ARPAV, 2016). (b) Trend of WWTP discharge 2008–15.

Analysis of the activated sludge micro-fauna

Before extending the assessment to a larger number of WWTPs, a preliminary study was performed on a sample of WWTPs in the province of Venice (35 plants in the period 2008–14; analytical data on the final discharge were available according to institutional regulating activities performed by ARPAV at only 32 of these plants). In general, the higher the sludge age the more numerous are the protozoan species present in the activated sludge, conversely, the lower the sludge age, the fewer the species present characterized by a very high growth rate. Out of the 35 plants on which SBI investigation was carried out by ARPAV in the period 2008–14, 5 were selected for a deeper analysis using plant manager data (self-monitoring). The selected plants are (see Table 3): Venice-Fusina (400,000 PE), Venice-Campalto (130,000 PE), Venice-Lido (60,000 PE), Chioggia (160,000 PE) and Cavallino-Treporti (105,000 PE). The wastewaters treated at these plants are not homogeneous as they are domestic, mixed domestic and industrial. Moreover the plants at the Lido and Chioggia are subject to sizeable fluctuations (hydraulic and organic load variations) due to increased loads during the summer tourist season.

The parameters supplied by plant managers comprise the following: the concentration of influent and effluent wastewaters and, in particular, the mean concentration in the period 2008–14; the values measured in the sampling date for SBI determination concern the following parameters: BOD_5, COD, TSS, N_{tot}, P_{tot}, N-NH_4, N-NO_2, N-NO_3 and TKN entering the plant and treated effluent, VSS (volatile suspended solids) in the aeration tank, sludge load, sludge age and the concentration of SS in the aeration tank (MLSS). In many cases it was not possible to obtain the required functional information/data from the plant managers. Missing data includes influent flow, effluent flow, VSS in the aeration tank, TSS, sludge load and age, etc.

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Regarding the assessments performed on the activated sludge, in addition to the SBI values, the following parameters were also considered: micro-fauna density, with reference to the protists present in the activated sludge; the total number of systematic units present and the relative dominant groups; the flocs' diameter (considering the larger diameter of the flocs in the bulk of activated sludge where dissimilarities were present). All these values have been recorded for each sampling date of the discharge and of the activated sludge. It must be noted that this is just a preliminary study that aims to investigate the possibility of identifying a relationship between sludge status and the classic functional and management parameters used in the plant's operational activities. To this purpose plant manager data together with discharge data from the regulating Authority have been assessed. In a subsequent phase a statistical study will be performed to verify the relationship, should it exist, between the SBI and the main functional parameters. The present study has been carried out using data supplied by plant managers derived from self-monitoring activity as well as from management data and plant design data. Analytical data supplied by plant managers were produced by the same laboratory for all four of the selected WWTPs. The parameters BOD_5, COD, P_{tot}, N_{tot} or TKN were sampled on the same date in which activated sludge sampling was carried out to determine the SBI. The SBI values were produced by the monitoring authority (ARPAV). Comparison of SBI values that were determined by analysing activated sludge micro-fauna in a WWTP using the main functional parameters, can frequently present situations that are very difficult to explain. For example, in situations where there are high SBI values and where activated sludge is characterized by a large number of systematic units and with a moderate presence of filamentous bacteria, these are characteristics of a well-colonized, stable sludge that has very good biological activity but which can contemporarily present poorly efficient removal of pollutants, bulking phenomena, and a high presence of TSS in the final discharge. On the other hand, situations of sludge with non-optimal conditions can be found, where SBI scores are low, flocs are small, but where pollutants removal is good or moderately efficient and treatment efficiency is, on the whole, good.

Analysis of data supplied by plant manager

In order to analyse data supplied by plant managers, certain parameters, such as the efficiency of organic load removal (in terms of COD removed) have been determined by comparing data regarding influent and effluent, the efficiency of N removal (as N_{tot}), the efficiency of P removal (as P_{tot}), the ratios COD/BOD_5, C/N, N/P, and when possible, the SBI value. All these parameters' values were compared and trend analysis revealed a relationship. However, based on the analysed data derived from the selected 4 WWTPs, some anomalies can be observed and should be investigated more thoroughly using new physical and process data.

As an example, we refer to the parameters measured in the Venice-Fusina WWTP on 07/06/2010: in this case the SBI reached a score of 10 and removal efficiency reached values that were lower than the mean values registered in other periods of the year (N removal efficiency had been registered $\eta_{Ntot} = 0.73$, organic load removal efficiency $\eta_{COD} = 0.87$, phosphorous removal efficiency $0\eta_{Ptot} = 0.68$). Moreover, the situation registered on 3/12/2013 also gave a SBI value of 10, 16 systematic units and values of removal efficiencies of $\eta_{Ntot} = 0.63$, $\eta_{COD} = 0.87$ and $\eta_{Ptot} = 0.74$ respectively. At the same time as heavily influencing the WWTP, it is evident that there are situations that require further investigation. Furthermore, data recorded at the Lido's WWTP on 12/12/2012 gave an SBI score of 10 and 11 systematic units with the following removal efficiencies: $\eta_{Ntot} = 0.53$, $\eta_{COD} = 0.73$ and $\eta_{Ptot} = 0.74$. Figure 7(a–d) present the characteristics of the micro-fauna and the functionality parameters of the WWTPs selected for specific assessment where data was supplied by plant managers. Graphs of micro-fauna as well functional parameters were possible only for Venice-Fusina and Venice-Lido, while for the Cavallino, Chioggia and Campalto plants information was incomplete and therefore not reported.

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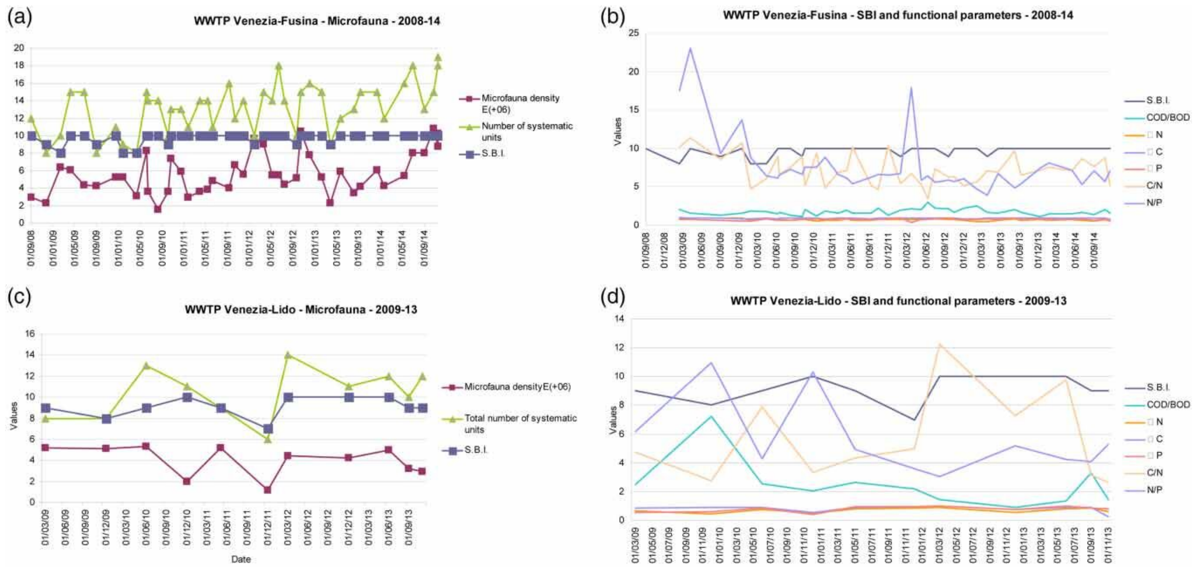

Figure 7 | (a) Venice-Fusina plant – Micro-fauna characteristics. (b) Venice-Fusina plant – Functional parameters. (c) Venice-Lido plant – Micro-fauna characteristics. (d) Venice-Lido plant – Functional parameters. Source: (Source: plant managers).

Considerations on the WWTPs analysed

The objectives of this study were to understand if it was possible to establish relationships between the observed phenomena and the environmental conditions in which the sludge is developed and whether or not they are the result of management or structural aspects. It was also motivated by the need to understand if the micro-fauna or other bacteria present in the activated sludge could modify and if so, what the causes might be (changes in environmental conditions, pH, T, changes in nutrient substances, presence of toxic compounds, etc.), and moreover if the response of the micro-fauna to external changes might make it difficult or impossible to determine direct correlation. Despite some limitations, SBI appears to be a useful integrative monitoring tool. Particularly where small and medium sized WWTPs are concerned, it can be used to reduce analytical control frequency once a specific correlation parameter has been defined for each plant and after a significant historical series of data has been made available for the characterization of the discharge. Plant functional data must be gathered and elaborated. However, more information is needed regarding the functionality of the plants. Specific comments on the plants investigated with functional data from the plants' managers can be pointed out as follows:

- Venice-Lido WWTP: wastewater appears diluted, often with influent concentrations already below discharge limit values; efficiency removal is high; the high dilution appears responsible for the abundance of filamentous organisms and SBI scores always tend to be around 9 or 10 with only one score of 7; sludge age of 80 days is too long.
- Venice-Fusina WWTP: wastewater appears concentrated and removal efficiencies are high; filamentous organisms are few or plentiful and SBI values always tend to be around 9 or 10 with a minimum of 8.
- Chioggia WWTP: influent wastewater appears concentrated, removal efficiencies are high, filamentous organisms are plentiful; SBI scores are 8–10 with a minimum value of 7.
- Venice-Campalto WWTP: influent wastewater is diluted, removal efficiencies are high, filamentous organisms are few or plentiful but SBI always tend to be around 9–10 with a minimum value of 7.

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CONCLUSIONS

The SBI has been applied as a useful monitoring and management tool that can assess the health of activated sludge in aerobic wastewater treatment processes. According to Papadimitriou *et al.* (2007), SBI calculations can be effectively applied in the treatment of municipal wastewaters, while this is not possible when toxic substances (inorganic or organic) are present. The SBI can be used to monitor and assess plant performance, the load reduction of organic and SS, to monitor operative conditions and plant reliability with regard to compliance of discharge limit values. This applies particularly to small and medium sized plants that only treat domestic wastewaters. Often anomalous situations can occur where SBI values are particularly high, where ciliates protozoa present are highly diverse or where the activated sludge is good but removal efficiencies are unsatisfactory. These situations could be linked to specific environmental factors such as variations of T, pH, presence of toxic substances or growth inhibitory substances, variations in nutrient compositions, etc., or structural and/or management elements. The capability of ciliates as indicators of effluent quality is limited by a number of factors, like over or underloading (Salvadó *et al.* 1995) or shock organic and ammonium loadings (Drzewicki & Kulikowska 2011).

The general situation regarding the SBI for 35 WWTPs in the Province of Venice (north-east Italy) has been presented as a preliminary investigation conducted in the period 2008–14. Functionality data were supplied by plant managers but only for four WWTPs in this group; the main findings of the assessment are as follows: 1) the SBI confirmed the satisfactory performance of the four WWTPs considered and evaluated by means of physical, chemical and microbiological analysis in the same period; 2) the activated sludge analysed for SBI assessment was determined within the quality class I, which corresponds to a very well colonized, stable sludge with excellent biological activity and, consequently, very good abatement efficiency (SBI scores $\geq$8); 3) for each plant considered, the variations of the SBI and of the micro-fauna composition were minimal during the year and apparently insensitive to variations in operative parameters (temperature, sludge age, sludge load, etc.); 4) for the type of WWTPs considered (frequently monitored by regulatory Authorities but continuously monitored by plant managers) the SBI method confirms the general good functioning of the plants. The current study needs for more information that requires greater collaboration from plant managers in gaining and assessing data on plant abatement efficiency. In particular, a statistical analysis concerning the identified parameters is called for but was not possible in this study, due to the lack of data from plant managers.

Traditional analytical controls should be integrated and in certain cases partially substituted with other controls including the determination of the SBI. This index could be used to directly monitor the activated sludge present in aeration tanks and gather information on the functionality and performance of the whole biological process. SBI can be a useful indicator of plant performance but cannot be used alone. It appears useful as an integrative monitoring and management tool. For small and medium sized WWTPs in particular, it can be used to reduce the frequency of analytical monitoring once a specific correlation parameter has been determined for each plant and after a significant quantity of historical data has been made available for the characterization of the discharge. With available data regarding contradictory situations highlighted in the discussion (for example where SBI values are high but functional performance parameters are unsatisfactory) SBI monitoring appears to be a useful complementary tool, particularly for small and medium sized plants. More data are required to define a statistical analysis between SBI data and functional plant parameters in order to produce a mathematical relationship. At this level SBI appears complementary to the other monitoring tools but cannot substitute them.

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ACKNOWLEDGEMENTS

The authors wish to thank the personnel of the ARPAV Venice laboratory service for analysis performed and plants' managers for supplied data.

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