

**IMPLEMENTATION OF THE SLUDGE BIOTIC INDEX FOR CONTROL
AND OPTIMIZATION OF THE BIOLOGICAL TREATMENT PROCESS**

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Abstract. The article examines the methodology for determining the Sludge Biotic Index (SBI) to assess the quality of activated sludge at treatment plants. The Sludge Biotic Index is a tool for quantitatively evaluating the functionality of sludge, allowing for monitoring and detection of critical conditions that may affect the quality of wastewater treatment. The determination of SBI is based on the analysis of the microfauna of activated sludge, where organisms are grouped into positive and negative key groups depending on their impact on the treatment process. The methodology allows for comparisons between different treatment facilities and identifying exceedances of discharge limits. Experimental studies were conducted at wastewater treatment facilities in Kharkiv. Samples of sludge were collected over several months, allowing for the investigation of changes in sludge quality over time. It was established that using the SBI allows for determining the degree of stability of activated sludge, as well as identifying adverse phenomena such as sludge bulking, which can lead to a decrease in treatment efficiency. The results of the studies confirm that the application of the SBI contributes to improving control and optimizing the biological water treatment process, which is especially important for the preservation of natural water resources. The obtained data indicate the high effectiveness of using the biotic index for monitoring the condition of activated sludge, allowing timely measures to be taken to improve wastewater treatment quality. This confirms the feasibility of implementing European methodologies in the management practices of treatment facilities in Ukraine.

Keywords: activated sludge, biological treatment, treatment efficiency, biotic index, microfauna, European experience.

1. Introduction

Biological wastewater treatment is a process involving the decomposition, oxidation, and mineralization of colloidal and dissolved organic and inorganic substances that pollute wastewater by an active microbiocenosis (biofilm, activated or granular sludge). Activated sludge is a biomass capable of autoflocculation, consisting of bacteria, actinomycetes, fungi, algae, protozoa (flagellates, sarcodines, ciliates, and suctorians), and multicellular organisms. This artificially cultivated biocenosis is dominated by capsulated, Gram-negative, rod-shaped bacteria. The activity ensures the sorption and oxidative destruction of pollutants and the effective separation of the cleaned liquid from the biomass (Madoni et al., 1993).

Technological control of biological treatment facilities, including the quality control of activated sludge, ensures the reliability and efficiency of this technology for protecting natural water bodies. Testing the state of the sludge, i.e., quantitatively assessing its quality and potential activity in oxidizing pollutants, is a crucial tool for both research and the practical operation of treatment facilities. This involves various biological and technological disciplines, developments from fundamental sciences, and wastewater treatment practices. However, to date, the tasks of methodological development and practical application of activated sludge state tests

have not been fully resolved (Madoni et al., 1993; Cupak et al., 2019).

Several convenient and reliable biological tests currently used at industrial treatment plants have been developed for analyzing the state of sludge that oxidizes organic compounds (Madoni, 1994). The most well-known and widespread is the hydrobiological analysis, which uses indicator microscopic organisms to assess the state of the sludge. Unfortunately, this analysis only qualitatively characterizes the overall state of the biocenosis.

Currently, scientific and technical publications by European experts in the field of biological wastewater treatment often refer to the determination of the Sludge Biotic Index (SBI) (Surerus et al., 2014; Gulshin, 2017). The SBI is a very useful index for quantitative integrative assessment of sludge functionality, particularly for monitoring and detection of critical conditions that may lead to exceeding discharge limits. It allows for comparing SBI indicators determined at biological treatment plants in different cities and technical facilities. SBI is established based on the results obtained from studies of the microfauna of activated sludge. According to this method, the organisms of the sludge microfauna are grouped into

positive and negative key groups (Ostoich et al., 2017; Eikelboom, 2000; Pedrazzani et al., 2016).

The SBI methodology asserts that the dominance and abundance of key groups, as well as the presence of indicator taxa, are shaped by the physical, chemical, and technological parameters, and the processes involved in purification. The term “key” groups refers to fundamental groups distinguished by their movement and ecological roles in the activated sludge environment. These groups encompass free-swimming, crawling, and attached infusoria, shell amoebae, small heterotrophic flagellates, and infusoria such as *Vorticella microstoma* and *Opercularia* spp. When applying SBI, individual species of infusoria are treated as separate entities, whereas flagellates, ciliates, nematodes, rotifers, gastropod mollusks, and oligochaetes are evaluated without specific species differentiation (Mesquita et al., 2013; Mikkelsen, 2002; Van Dierdonck et al., 2013). The evaluation of flocs focuses on their morphology, including form, strength, structure and size (Ntougias et al., 2011; Drzewicki, Kulikowska, 2011; Winkler et al., 2012).

The index determined for the activated sludge is obtained using a two-sided table (Table 1).

Table 1

A two-way table for calculating the SBI using key groups, their densities, and the total number of taxonomic units comprising the microfauna present in activated sludge (Madoni, 1994)

Dominant keygroup	Density (ind./l)	Aggregate count of taxonomic units comprising the microfauna found in activated sludge			
		> 10	8–10	5–7	< 5
Crawling + sessile ciliates and/or testate amoebae	≥ 10 ⁶	10	9	8	7
	< 10 ⁶	9	8	7	6
Sessile ciliates > 80 %	≥ 10 ⁶	9	8	7	6
	< 10 ⁶	8	7	6	5
Opercularia spp	≥ 10 ⁶	7	6	5	4
	< 10 ⁶	6	5	4	3
<i>Vorticella microstoma</i>	≥ 10 ⁶	6	5	4	3
	< 10 ⁶	5	4	3	2
Swimming bacterivorous ciliates	≥ 10 ⁶	5	4	3	2
	< 10 ⁶	4	3	2	1
Small-swimming flagellates (>100)	≥ 10 ⁶	4	3	2	1
	< 10 ⁶	3	2	1	0

The table ranks key groups in descending order, indicating the biological quality of the sludge. Column headers categorize the total number of taxonomic units of microfauna into four ranges. The table also differentiates between the abundance of microfauna (excluding

flagellates) and flagellates. To calculate the SBI, select a horizontal path corresponding to the lowest-positioned dominant key group and its density (greater or less than 10⁶ individuals/l). The vertical path considers both the total number of taxonomic units and flagellate density.

Once both paths are identified, determine the SBI value at their intersection. This two-way table assigns a score from 0 to 10 to assess the biological quality of activated sludge based on two criteria: the

sensitivity of specific microfauna groups to environmental conditions and their impact on the abundance and diversity of the protozoan community. SBI values are categorized into four quality classes (Table 2).

Table 2

Conversion of SBI values into four quality categories (classes) and corresponding evaluations (Madoni, 1994)

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

These classes enable the assessment of activated sludge's biological quality through four broadly defined evaluative ranges, providing dependable diagnostic insights.

The goal of the work is to validate the SBI methodology for assessing activated sludge in operational biological treatment plants in Ukraine.

2. Experimental Part

The object of the research was the activated sludge obtained from the aeration tank of the municipal treatment facilities in Kharkiv. The collected sludge samples were kept under aeration conditions and analyzed within 4 hours after sampling. To assess the biotic index and the morphology of the flocs, a microscopic analysis was conducted using a Lomo Mikmed-1 biological microscope. The counting and identification of protozoa were carried out in 25 ml mixed samples of sludge liquid (each in two replicates) from two aeration tanks using light microscopy at magnifications of 100x or 400x, depending on the size of the species. Identification was performed using identification keys from specialized scientific literature (Ostoich et al., 2017). For counting small flagellates, a Goryaev chamber was used. The calculation of the SBI was conducted according to the recommendations of Madoni (Madoni, 1994). Sludge index values, dry residue, and nitrate concentration were determined according to the methodologies recommended by the normative documents of Ukraine. Statistical analysis was performed using Microsoft Excel software.

To assess the quality of wastewater treatment, integral coefficients recommended by foreign scienti-

fic and technical literature (Karczmarczyk, Kowalik, 2022; Madoni, 2011) were used: the index of technological purity (TPI) and the reliability coefficient (RF).

The index of technological purity and the reliability coefficient were calculated using formulas 1 and 2 (Karczmarczyk, Kowalik, 2022).

$$TPI = \frac{n_z}{(N+1)}, \quad (1)$$

where TPI is the index of technological purity; n_z is the number of test outcomes meeting the limit values;

N is the total number of test outcomes pertaining to the specified indicator.

$$RF = \frac{m_x}{X_{acc}}, \quad (2)$$

where RF is the reliability coefficient; m_x – average value of the given indicator in wastewater, mg/l;

X_{acc} is the permissible value of the indicator in wastewater, mg/l.

An RF value below 1.0 indicates proper operation of the treatment facilities. A lower RF value indicates a more effective treatment outcome. A TPI index of 1.0 indicates that all test results meet the standards for treated wastewater quality. A lower TPI value indicates a higher number of samples exceeding the specified values.

3. Results and Discussion

3.1. Characteristics of Wastewater

The values of the reliability coefficient and the index of technological purity calculated for COD, NO_3^- , and NH_4 with respect to GDS values are presented in Table 3.

Table 3

Characteristics of wastewater quality, reliability coefficient, and index of technological purity calculated for the studied treatment facility

Parameter	Characteristics of wastewater quality (mg/l)			Reliability detectors	
	N	Mean	$\frac{MIN \div MAX}{SD}$	RF	TPI
COD	20	88.80	$\frac{35 \div 154}{30.71}$	1.11	0.43
NO ₃ ⁻	28	47.35	$\frac{7.77 \div 59.3}{14.99}$	1.05	0.24
N-NH ₄	26	1.02	$\frac{0 \div 3.9}{0.94}$	0.45	0.89

As can be seen, the RF value for N-NH₄ is significantly below 1, reflecting a high effect of wastewater treatment for this pollutant during the study period. The TPI value indicates that, for N-NH₄, exceedances of the limit values occurred infrequently (less than 8 % of samples).

The RF values for COD and NO₃⁻ are somewhat above 1 and significantly higher than the RF for NH₄, indicating a relatively lower treatment effect. The low TPI values for COD and NO₃⁻ reflect frequent exceedances of the limit values for COD and NO₃⁻ in the wastewater.

3.2. Physical Parameters of the Sludge

The specifications of the activated sludge are detailed in Table 4. As shown in the data, the concentration of activated sludge in the facility was below the optimal level, which, according to (Karczmarczyk, Kowalik, 2022), is 3–7 g/L. However, operational experience with biological treatment systems in Ukraine and domestic regulatory documents recommend other optimal values: 2–3 g/L.

Table 4

Characteristics of the studied activated sludge

Parameter	N	Mean	$\frac{MIN \div MAX}{SD}$	Optimal values
Dry weight (sludge dose) (DM), g/L	28	1.20	$\frac{0,6 \div 2,6}{0,46}$	3–7
Sludge index by volume (Vo), mL/L	8	561.25	$\frac{130 \div 910}{267,49}$	300–700
Sludge index by weight (SI), mL/g	28	442.75	$\frac{43 \div 1187}{354,23}$	50–150

The sludge index (SI) for standard activated sludge typically ranges from 50 to 150 ml/g. The values of this indicator established in the studied aeration tanks were significantly higher, typical for bulking sludge, often due to a high concentration of filamentous bacteria. The bulking of the sludge likely also explains the low concentration of activated sludge in the facility, due to its partial washout. Exceedances of the optimal sludge index values were found in 17 out of 28 analyzed samples.

The oxygen consumption rate of the studied activated sludge was 11–15 mg O₂/(g·d·h), indicating its normal oxidative capacity (Karczmarczyk, Kowalik, 2022).

3.3. Floc Morphology

The characteristics of activated sludge flocs encompass their shape, structure, strength, and size (Eikelboom, 2000). Floc shapes range from round to irregular, with round shapes being most common. Irregularly shaped flocs have a reduced settling rate. Irregular shapes, such as star-shaped, feathered, or net-like structures, often indicate the presence of filamentous microorganisms (primarily bacteria and fungi), which can cause sludge bulking.

The structure of the flocs can be open (with water flowing through the floc particles) or compact, with compact flocs settling more rapidly. In conditions of hypoxia or nutrient deficiency, flocs

tend to become compact and develop grayish formations, which diminish their settling efficiency. Factors such as the presence of filamentous organisms, oxygen excess or deficiency, hydraulic overload, or nutrient starvation can all contribute to deteriorating floc structure (Tocchi et al., 2012).

Flocs can be classified as strong or weak based on their strength.

The size of the flocs varies significantly. There are three size classes for flocs: small (diameter < 25 µm), medium (25–250 µm), and large (> 250 µm) (Eikelboom, 2000). Small flocs occur under high aeration, intense mixing, and high loading. A large proportion of small flocs may be the reason for their presence in the treated wastewater.

The size of flocs influences two critical processes: biosorption (the absorption of pollutants) and

sedimentation. Biosorption, a precursor to biodegradation, depends on the surface area of the floc. The larger the surface area, the smaller the floc. However, excessively small flocs, despite having a large sorptive surface, settle poorly in secondary clarifiers, which can lead to contamination of the treated wastewater. Conversely, while large flocs settle effectively, the bacteria within the core of a large floc may have limited access to oxygen and pollutants necessary for effective biodegradation. This limitation reduces their efficiency in wastewater treatment processes.

The results of the determination of the morphological indicators of the flocs, conducted according to the recommendations of D. Eikelboom (Eikelboom, 2000), are presented in Table 5.

Table 5

Morphological Characteristics of the Flocs

Date	Floc morphology			
	Shape	Structure	Strength	Size, µm
08.07	Irregular	open	weak	30–50
09.10	Irregular	open	medium	30–50
24.10	Rounded	compact	medium	50–80
27.11	Rounded	compact	firm	50–80
08.12	Rounded	compact	firm	> 50

As seen from the data in Table 5, in the initial samples, the activated sludge exhibited an irregular floc shape due to bulking. Subsequently, as bulking decreased, the floc shape improved to a more rounded form. The structure varied from open to compact, with the latter dominating in the later samples. Open flocs settle at a slower rate compared to compact ones. The structure of the flocs can be affected by the existence of filamentous bacteria. When these bacteria predominate and are present both inside and outside the floc, sludge flotation may arise. Such sludge typically exhibits a high sludge index (SI). In our studies, the density of the flocs ranged from weak to strong. In the initial samples (08.07 and 09.10), floc sizes tended towards the lower values (30–50 µm). With the appearance of the “round” shape and “compact” structure, floc sizes increased (50–80 µm and >50 µm). Thus, the data presented show a clear trend towards improved morphological characteristics of activated sludge flocs over the course of the study.

3.4. Determination of SBI

The microscopic analysis concentrated on identifying microfauna to determine the sludge biotic

index (SBI) (Madoni, 1994). The examination of samples revealed a variety of organisms, including naked amoebae, worms, algae, crustaceans, and insects, which are not included in the SBI method (Madoni, 1994). The findings from the microscopic analysis of the studied activated sludge and the computed SBI values are presented in Table 6.

Analysis of sludge quality is an essential tool for optimizing wastewater treatment plant operations, helping to identify factors contributing to operational inefficiencies. For example, there is an inverse relationship between the abundance of flagellates and ciliates in activated sludge. Elevated flagellate counts often indicate sludge overload, whereas the presence of ciliates suggests effective sludge performance.

Following the identification of dominant key groups, density, and taxonomic units of microfauna, the Sludge Biotic Index (SBI) and corresponding sludge class were determined using Tables 1 and 2. Among the 22 analyzed activated sludge samples, 4 were classified under Class IV – III SBI (indicating poor or inadequate biological treatment in the aeration tank, with low to moderate performance), 12 under Class II (indicating well-established and stable sludge, with reduced biological activity but high

performance), and 6 under Class I–II (indicating very well-established and stable sludge, excellent biological activity, and very high performance). Based

on the trends observed in sludge classification, it can be concluded that the efficiency of wastewater treatment in the studied facilities varied from poor to high.

Table 6

Assessment of activated sludge quality and SBI determination

Date	Dominant keygroup	SBI
		Value/Class
08.07	Swimming bacterivorous ciliates	3,5/IV – III
09.10	Testate amoebae	6/II
24.10	Sessile ciliates or testate amoebae / Swimming bacterivorous ciliates	6,5/II
27.11	Crawling + sessile ciliates and/or testate amoebae / Swimming bacterivorous ciliates	7/II
08.12	Swimming bacterivorous ciliates / Sessile ciliates or testate amoebae / Sessile ciliates > 80 %	7,7/II – I

3.5. Change in SBI over the Study Period

Fig. 1 graphically presents the results from the assessment of activated sludge quality (sludge index and SBI) in the aeration tanks throughout the study period. The data shows that during the study period, the SBI tended to increase, which may indicate enhanced oxidative and destructive activity

of microorganisms in the sludge and improved sludge condition. The sludge index demonstrated positive changes over time, although the index values fluctuated. A sharp decrease in the sludge index in November may be related to the temperature factor, which affects the development of filamentous bacteria and the overall sedimentation properties of the sludge.

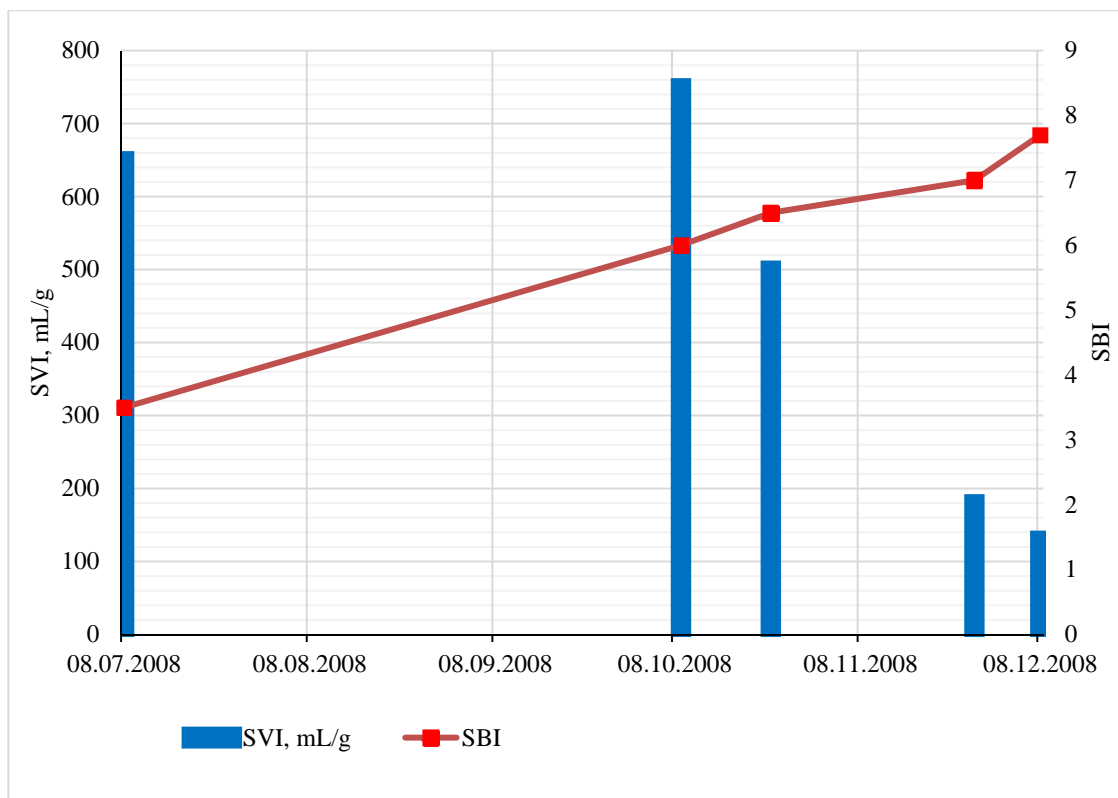


Fig. 1. Trends in controlling activated sludge bulking at municipal wastewater treatment facilities No. 2 in Kharkiv

4. Conclusions

1. Objective indices have a significant advantage over subjective ones as they allow for comparisons of index values determined by different operators. Madoni introduced a measurable criterion for evaluating the biological quality of sludge in the aeration tank, based on an extended biotic index, which can be applied to all types of activated sludge facilities. Method Basis: The Madoni method is based on relationship between wastewater treatment efficiency and various microfauna groups in activated sludge—particularly the ciliate community. The Sludge Biotic Index is a very convenient tool for integral evaluation of sludge functionality, particularly for overseeing and detecting critical conditions in biological treatment facilities.

2. During experimental research, integral coefficients recommended by foreign scientific and technical literature were used to assess wastewater treatment quality: index of technological purity (TPI) and the reliability coefficient (RF). RF and TPI Values: The RF and TPI values for N-NH₄ indicated a high effect of wastewater treatment from this pollutant during the research period and very rare exceedance of limit values. The RF and TPI values for COD and NO₃⁻ indicated a relatively lower effect of treatment and a relatively frequent exceedance of limit values.

3. Exceedance of optimal sludge index values was found in 17 out of 28 analyzed samples. The established values in the studied aeration tanks were significantly higher than the 50–150 ml/g range, typical for bulking sludge, resulting from a high content of filamentous bacteria. The bulking of sludge likely explains the low concentration of activated sludge in the facility, due to partial carryover.

4. Activated sludge flocs were described based on their size, shape, strength and structure. In initial samples, disordered floc shapes were observed, which improved to round shapes as bulking decreased. The structure of flocs ranged from open to compact throughout the study, with floc density varying from weak to strong, and floc sizes increasing from 30–50 μm to 50–80 μm and larger.

5. Over the 5-month study period, SBI values in the aeration tanks were determined. At the beginning of the study, the SBI value/class was 3.5/IV – III (insufficient biological purification), which changed to 6/II (good biological treatment), 6.5/II (good biological treatment), and 7/II (Well colonized and stable sludge, decreasing biological activity; good

performance). By the end of the study, it was 7.7/II – I (Very well colonized and stable sludge, excellent biological activity; very good performance).

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