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An overview of methods for evaluating the properties of activated sludge

Aleksandra Piasek[1], Grzegorz Łagód[1*]

[1] Faculty of Environmental Engineering and Energy, Lublin University of Technology, ul. Nadbystrzycka 40B, 20-618 Lublin, Poland
* Corresponding author's e-mail: g.lagod@pollub.pl

ABSTRACT

Stable and efficient wastewater treatment is increasingly important amid growing water scarcity and complex pollutant loads. Physicochemical parameters (BOD_5, COD, nutrient concentrations) remain the primary monitoring tool at treatment plants but provide limited insight into the biological condition of activated sludge, the core component of secondary treatment. This article reviews contemporary biological methods for evaluating activated sludge properties, focusing on the diagnostic value of bioindication. It first outlines the activated sludge process, floc structure, key operational parameters (sludge age, organic loading, sludge volume index, pH, temperature), and the ecological roles of indicator organisms such as ciliates, rotifers, nematodes, and filamentous bacteria. It then examines quantitative tools for assessing biocenosis structure, including classical biodiversity and dominance indices (Shannon, Simpson, Margalef, Pielou, McIntosh, Berger–Parker) and activated-sludge-specific indices, notably Madoni's Sludge Biotic Index, alongside methods for assessing filamentous bacteria abundance. These tools support prediction of disturbances such as bulking and foaming and complement physicochemical monitoring. The growing role of digital image analysis and machine learning in automating microscopic assessment is highlighted as an emerging direction. The review concludes that biological indicators offer a cost-effective, sustainable complement to chemical methods, and identifies the need for standardized interpretation of indices and further automation of microscopic diagnostics.

Keywords: activated sludge, wastewater treatment, bioindication, biocenotic indices, biodiversity indices, sludge biotic index, sludge volume index, filamentous bacteria, microscopic image analysis, process diagnostics.

INTRODUCTION

According to a report by the Chief Inspectorate for Environmental Protection (GIOŚ, 2022), in 2021, Poland's annual water resources amounted to 1,400 m³ per capita. According to UN criteria (GUS, 2020), a country is considered at risk of water scarcity if its freshwater resources fall below 1,700 m³ per capita per year. In the face of global environmental challenges, such as drinking water scarcity and the growing number, as well as complexity of pollutants, effective and stable wastewater treatment becomes particularly important for protecting limited water resources (Łagód, 2017; Rauba, Brulińska, 2017; Bauer et al., 2020).

The quality of wastewater at treatment plants is primarily monitored using physicochemical methods based on the measurement of parameters such as BOD_5, COD, total suspended solids, and concentrations of nutrients (Dymaczewski et al., 2011; Yotova et al., 2020). These indicators provide information on the current quality of wastewater; however, they do not fully reflect the biological condition of the activated sludge, which is a key element of the treatment process (Fiałkowska et al., 2010; Dymaczewski et al., 2011; Łagód, 2017). Activated sludge is a dynamic ecosystem of microorganisms that responds sensitively to changes in load and the presence of toxic substances; therefore, its structure and diversity can serve as an indicator of process

stability (Fiałkowska et al., 2010; Zhang, et al., 2018; Johnston et al., 2019; Yang et al., 2020).

There is growing interest in biological methods for evaluating wastewater treatment processes that utilize the properties of activated sludge (Fiałkowska et al., 2010; Łagód, 2017; Yang et al., 2020; Staniszewski et al., 2024; Dziadosz et al., 2024), since traditional chemical methods – though widely used – require costly reagents and generate waste that burdens the environment (Jaromin et al., 2012). Therefore, bioindication methods serve as a valuable complement to physicochemical analyses (Leal et al., 2013), enabling the assessment of the state of the biocoenosis based on the presence and abundance of characteristic groups of microorganisms. They are relatively simple to use, do not require the production or disposal of chemical reagents, and at the same time allow for ongoing monitoring of wastewater treatment plant performance (Fiałkowska et al., 2010; Leal et al., 2013; Babko et al., 2014a; Babko et al., 2014b; Łagód, 2017). In the context of limited water resources and increasing environmental requirements, the development and refinement of bioindicator methods take on particular significance (Sowinska et al., 2017; Stoeck et al., 2018).

This article reviewed contemporary biological methods for assessing the properties of activated sludge and discussed the role of bioindicators in monitoring and diagnosing wastewater treatment processes. It also covered the current state of knowledge regarding the application of automated image analysis methods and their potential in the development of sustainable water quality monitoring systems. In addition to keyword-based searches in BazTech, Biblioteka Nauki, Web of Science, Google Scholar backward and forward snowballing was applied to key sources, following the procedure described by Wohlin (2014): reference lists of core publications were screened for additional relevant studies, and citation-tracking tools (Google Scholar, Scopus) were used to identify subsequent literature citing these sources.

BIOLOGICAL WASTEWATER TREATMENT AND CHARACTERISTICS OF ACTIVATED SLUDGE

Requirements under the Water Framework Directive and national regulations require wastewater treatment plants to maintain a high level of pollutant removal efficiency, which in turn requires the stable operation of biological processes (Dyrektywa 2000/60/WE; Dz.U. 2019 poz. 1311; Dz.U. 2025 poz. 647; Dz.U. 2025 poz. 960).

Modern treatment plants use sequential mechanical, biological, and – when necessary – chemical processes, with activated sludge typically playing a key role in the removal of organic compounds and nutrients (Fiałkowska et al., 2010; Dymaczewski et al., 2011; Naidoo i Olaniran, 2014; Tchobanoglous et al., 2014; Quach-Cu et al., 2018).

Biological wastewater treatment – process fundamentals

Biological wastewater treatment is a key technological stage the primary objective of which is to remove organic compounds and nutrients – primarily nitrogen and phosphorus – by utilizing the metabolic activity of microorganisms. This process is referred to as the secondary treatment stage and is based on the functioning of activated sludge, i.e., a suspension of flocculent aggregates of microorganisms that, under technologically controlled conditions, replicate the self-purification mechanisms found in natural aquatic ecosystems (Fiałkowska et al., 2010; Dymaczewski et al., 2011; Quach-Cu et al., 2018).

Unlike mechanical treatment, the biological stage enables the removal of dissolved and colloidal compounds through their biodegradation, including sorption and incorporation into biomass. The primary tasks of the biological stage include: the decomposition of dissolved and particulate organic pollutants into simple end products (CO_2, H_2O, biomass), and then it can be subsequently separated from the treated water. Key processes include the oxidation of organic matter by heterotrophic bacteria, nitrification, denitrification, and biological phosphorus removal (EBPR). Their progression depends on oxygen conditions, sludge age, loading rate, and the structure of the sludge biocenosis. Reduction of nitrogen and phosphorus concentrations is of particular importance, as excessive discharges of these substances lead to the eutrophication of surface waters (Zborowska et al., 2010; Dymaczewski et al., 2011; Chmielowski et al., 2015; Quach-Cu et al., 2018). This phenomenon is associated with disturbances in the trophic balance, oxygen deficiency, and a deterioration in the quality of

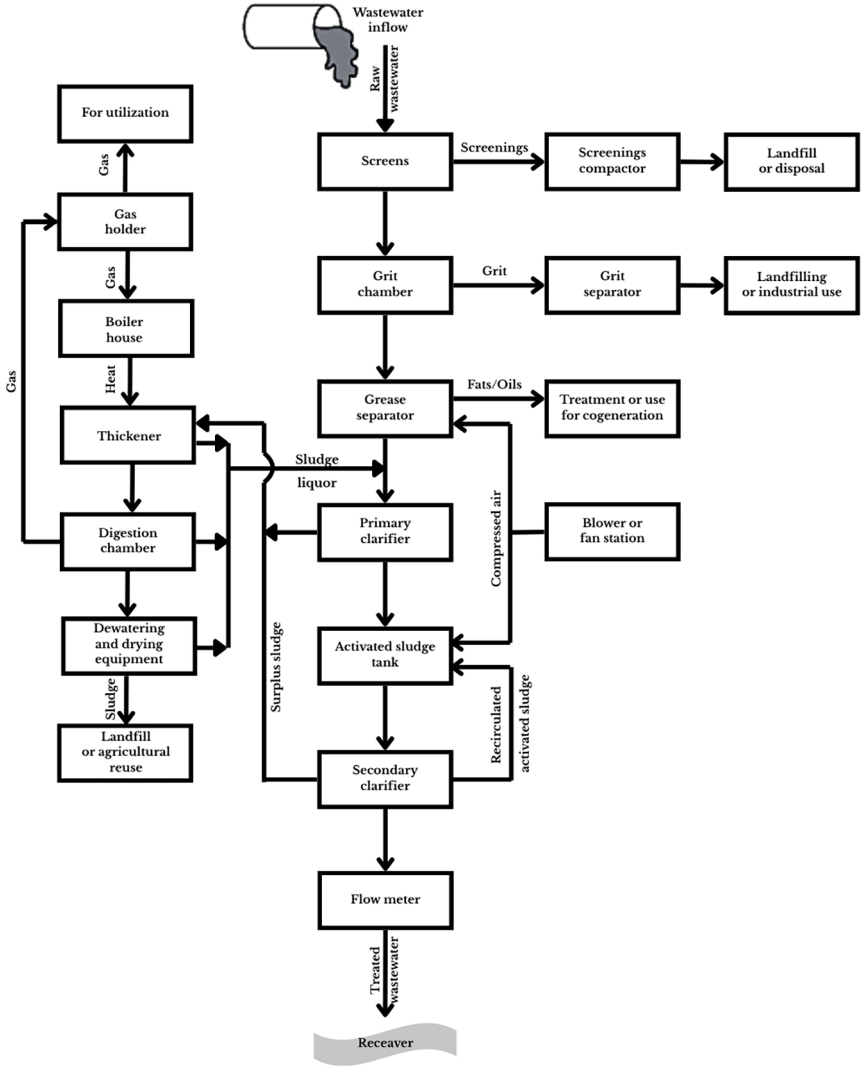

Figure 1. Process flow diagram of a wastewater treatment plant, showing mechanicaland biological processes as well as sludge management (based on: Zdebik, Korczak, 2010; Dymaczewski et al., 2011)

receiving waters. For this reason, the effectiveness of biological treatment at the wastewater treatment plant is a key element in the protection of the aquatic environment.

The efficiency of biological wastewater treatment is directly dependent on the structure and activity of the activated sludge biocenosis; therefore, monitoring it is a key tool for process control (Fiałkowska et al., 2008; Łagód, 2017)

The activated sludge process

Historical background and technological significance

The activated sludge process is the primary biological wastewater treatment technology used worldwide. Its origins date back to the early 20th century, when, in 1914, English engineers Edward Ardern and William T. Lockett described the phenomenon of biological oxidation of organic matter in wastewater using a suspensionof microorganisms under aerobic conditions, which they termed "activated sludge" (Ardern, Lockett, 1914).

Over the following decades, this technology underwent numerous modifications, ranging from the classic activated sludge process with sludge recirculation, through processes with extended aeration, to modern MBR (Membrane Bioreactor) and SBR (Sequencing Batch Reactor) systems, which are characterized by high operational flexibility and treatment efficiency (Tchobanoglous et al., 2014, Łagód et al., 2019; Rahman et al., 2023).

Today, the activated sludge method is widely used in both municipal and industrial wastewater treatment plants. It is often supplemented by auxiliary processes, such as coagulation, filtration, or biological treatment using biofilms (Michalska, Mrozik, 2018). The digitization of sludge monitoring processes is also gaining increasing importance, including automated image analysis, which enables early detection of anomalies and improves the stability of treatment processes (Staniszewski et al., 2024; Dziadosz et al., 2024).

Principle of operation of the activated sludge system

The activated sludge process is based on cultivating a mixed population of microorganisms in a biological reactor with sufficiently intense aeration and mixing. Under aerobic conditions, organic compounds undergo biodegradation, and nitrogen compounds undergo transformation (nitrification and – in appropriate zones – denitrification) (Groβmann et al., 1999; Jenkins et al., 2003; Pinther et al., 2022). The basic configuration of the system generally includes (Dymaczewski et al., 2011):

- an activated sludge chamber (biological reactor),
- a secondary clarifier,
- a sludge recirculation system.

After the biomass is separated in the secondary clarifier, a portion of the sludge is returned to the reactor (internal recirculation), while excess sludge is removed from the system to maintain the target sludge retention time (SRT). The stability of the process depends, among other factors, on the balance between the pollutant load and the activity and structure of the microbial community (Dymaczewski et al., 2011; Modin et al., 2016; Wilen et al., 2018).

The literature emphasizes that the effectiveness of the process is determined by both physicochemical parameters (DO, pH, temperature, F/M ratio) and the biological structure of the sludge (Wolfe et al., 2015; Wilen et al., 2018).

Microorganisms and the structure of activated sludge flocs

Activated sludge constitutes a complex, multi-species ecosystem of microorganisms that form aggregates called flocs (Groβmann et al., 1999; Fiałkowska et al., 2010; Łagód, 2017). This structure arises from the self-organization of bacterial cells and the production of extracellular polymeric substances (EPS), which serves as a structural matrix (Pell, Wörman, 2008; Cel, 2021; Mirghani et al., 2022; Zhao et al., 2023).

The biocenosis of activated sludge consists of (Fiałkowska et al., 2010; Babko et al., 2015; Dziadosz et al., 2024):

- heterotrophic bacteria – responsible for the decomposition of organic compounds,
- autotrophic (nitrifying) bacteria – including Nitrosomonas and Nitrobacter,
- filamentous bacteria – affecting structure and sedimentation,
- protozoa (especially ciliates),
- metazoans (rotifers, nematodes).

Properly formed flocs are characterized by a compact, porous structure that allows for effective diffusion of oxygen and substrates (Fiałkowska et al., 2010; Grady et al., 2011; van Loosdrecht et al., 2016). Disturbances in the biological balance (e.g., excessive growth of filamentous bacteria) lead to a deterioration in sedimentation properties (bulking) and destabilization of the process (Jenkins et al., 2003; Fiałkowska et al., 2010; van Loosdrecht et al., 2016; Cydzik-Kwiatkowska, Zielińska, 2016; Sam et al., 2022; Ma et al., 2024).

Floc structure, the proportion of indicator organisms, and EPS properties are currently analyzed using both classical microscopy and digital image analysis combined with artificial intelligence tools. This trend aligns with the development of the "smart wastewater treatment" concept, in which biological sludge parameters serve as inputs to decision-support systems (Satoh, H., et al., 2021; Staniszewski et al., 2024; Dziadosz et al., 2024; Li, H., et al., 2025).

Despite the widespread use of the activated sludge method, there is still a lack of standardized, quantitative tools for assessing the structure of the biocenosis that could be routinely used in process diagnostics.

Process parameters and properties of activated sludge

Process parameters are a key element in evaluating the performance of activated sludge systems, determining both the stability of the biocenosis and the efficiency of pollutant removal. Each of these parameters influences the course of biochemical processes, the microbiological balance, and the sedimentation properties of the sludge (Figure 2).

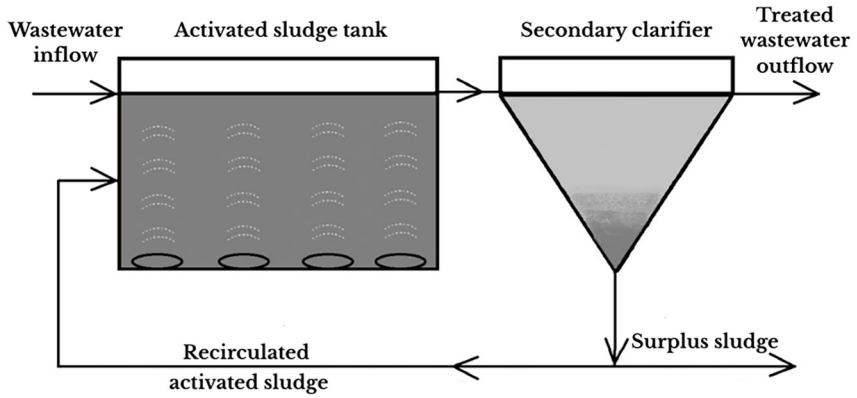

Figure 2. The concept of activated sludge (based on: Chrzanowska, et al., 2003; Zdebik, Korczak, 2010; Fiałkowska et al., 2010)

a) Sludge age (SA), defined as the average residence time of biomass in the system, is one of the fundamental parameters controlling the dynamics of biological treatment processes (Dymaczewski, et al., 2011; Drewnowski et al., 2019). Under operating conditions, the theoretical minimum sludge age at 20°C is assumed to be 3–5 days. In technical practice, SA is determined based on the desired treatment outcomes (Kalinowska et al., 2005):

- 3–7 days – used primarily in small treatment plants and in processes aimed at removing organic carbon,
- 7–20 days – most commonly used in municipal treatment plants where complete nitrogen removal is required.

This parameter directly determines the structure of the biocenosis. A long sludge age favors the growth of slow-growing organisms, including nitrifying bacteria; however, it can also promote excessive growth of filamentous bacteria, leading to sludge bulking. Conversely, a short retention time creates favorable conditions for polyphosphate and denitrifying bacteria, which can be utilized in the design of systems for biological phosphorus removal (Dymaczewski, et al., 2011; Drewnowski, et al., 2019; Pajura, Czarnota, 2021; Sam et al., 2022).

$$SA = \frac{X \times (V_K + V_{os})}{X_n \times Q_n + X_e \times Q} \; [d] \qquad (1)$$

where: SA – sludge age [d], X – sludge concentration in the activated sludge tank [mg/dm³], X_n – sludge concentration from the bottom of the clarifier hopper [mg/dm³], X_e – sludge concentration in the clarifier effluent [mg/dm³], V_K – volume of the activated sludge tank [m³], V_{OS} – volume of the clarifier [m³], Q_n – quantity of excess sludge discharged from the system [m³/d], Q – wastewater inflow [m³/d].

b) The pollutant load on activated sludge (SL) defines the amount of pollutants expressed as kg of BOD_5 per day per 1 kg of dry weight of activated sludge (Dymaczewski et al., 2011; Łobos-Moysa, 2016).

$$S_L = \frac{L_{inflow}^{BOD_5}}{V \times X_{AS}} = \frac{s_{BOD_5}^{inflow} \times Q_d}{V \times X_{AS}} \left[\frac{gBOD_5}{X_{d.o.m} \times d}\right] \qquad (2)$$

where: S_L – activated sludge organic loading rate [g BOD_5/($g_{d.o.m.}$·d)], $L_{inflow}\text{^}(BOD_5)$ – daily BOD_5 load [g BOD_5/d], V – aeration tank volume [m³], X_{AS} – activated sludge concentration in the reactor [$g_{d.o.m.}$/m³], $s_{BOD_5}\text{^}inflow$ – representative BOD_5 concentration in raw wastewater [g BOD_5/m³], Q_d – average daily wastewater flow rate [m³/d].

c) The active sludge growth (ΔX_{AS}) allows for the determination of the increase in sludge mass over a 24-hour period, which depends on the growth of both autotrophic and heterotrophic bacteria, as well as the mass of suspended solids within the floc structures (Łomotowski, Szpindor, 1999; Turovskiy, Mathai, 2006; Janus, 2008).

$$\Delta X_{AS} = \Delta X_B + \Delta S \left[\frac{g_{d.o.m}}{d}\right]$$
$$\Delta X_{AS} = \left[Y_{BOD_5}\left(s_{BOD_5}^{in} - s_{BOD_5}^{out}\right) \times Q_d - bX_B V\right] + \qquad (3)$$
$$+\left(X_{min}^{in} - X_{min}^{out}\right) \times Q_d + Z_k \left[\frac{g_{d.o.m}}{d}\right]$$

where: ΔX_{AS} – activated sludge growth [$g_{d.o.m.}$/d], ΔX_B – growth of autotrophic and heterotrophic bacteria [$g_{d.o.m.}$/d], ΔS – mass of

suspended solids in flocs [$g_{d.o.m.}$/d], Y_{BOD5} – biomass growth coefficient [$g_{d.o.m.}$/g BOD_5/d], $s^{in}_{BOD_5}$, $s^{out}_{BOD_5}$ – representative BOD_5 concentration in the influent to the aeration tank and the effluent from the secondary clarifier [g/m³], Q_d – average daily wastewater flow rate [m³/d], b – activated sludge lysis rate constant [d⁻¹], X_B – biomass concentration in the activated sludge [$g_{d.o.m.}$/m³], V – aeration tank volume [m³], X^{in}_{min}, X^{out}_{min} – representative concentration of mineral suspended solids in the inflow to the chamber and the outflow from the secondary clarifier [g/m³], Z_k – quantity of sludge generated through simultaneous coagulation [g/d].

d) Sludge concentration – a properly functioning system is characterized by a biomass concentration of 1.5–3.0 g/dm³, and in some systems even up to 5 g/dm³. High biomass concentrations promote nitrogen removal processes, especially in systems with extended retention times (Hartmann et al., 1999; Pajura, Czarnota, 2021).

e) Sludge volume index (SVI) is one of the most widely used parameters for evaluating the settling properties of activated sludge due to its simplicity and low cost of determination. The index represents the volume occupied by settled sludge after 30 min of settling per unit mass of suspended solids. However, SVI is an empirical indicator rather than a direct measure of solid–liquid separation processes, and its interpretation is limited by the variable relationship between suspended solids concentration and the obtained value. Dick and Vesilind (1969) demonstrated that SVI may change with increasing sludge concentration, particularly under conditions where compression effects influence settling behaviour. Therefore, SVI values should be interpreted together with complementary parameters, including microscopic observations, filamentous bacteria abundance, and operational characteristics of the activated sludge system. Moreover, methodological factors, such as mixing conditions during the settling test, may affect the measured SVI value and should be considered when comparing results between studies (Dick, Vesilind, 1969; APHA, 1980; Bye & Dold, 1998; Jenkins et al., 2003; Martins et al., 2004; Billery et al., 2025). Under normal operating conditions at a wastewater treatment plant, the SI value typically ranges from 80 to 120 ml/g dry weight. An increase in the index above optimal values is most often the result of excessive filamentous bacterial growth induced by unfavorable operating conditions (e.g., oxygen deficiency, load fluctuations, or nutrient deficiency) (Martins et al., 2004; Wacławek et al., 2015; Liu et al., 2016; Deepnarain et al., 2019; Deepnarain et al., 2020; Fan et al., 2018).

f) Shape and structure of flocs – the morphology of activated sludge flocs is a critical indicator of process quality and is directly related to the functioning of the biocenosis and the process conditions. Flocs typically take on a rounded shape, though they are never perfectly spherical. In cases of significant deviations from a spherical shape, they are described as irregular. Abnormal floc shapes may result from the dominance of filamentous bacteria, fungal growth, or an increase in hydraulic load or pollutant load. Weak flocs are characterized by low cell cohesion, an unstable core, and a blurred phase boundary. A significant amount of slowly floating cellular material is observed in their vicinity, which negatively affects sedimentation. Floc size is an important process parameter. Optimal sizes range from 150 to 300 μm, which ensures effective sedimentation and a proper metabolic rate for microorganisms. Flocs smaller than 100 μm prevent proper sedimentation, while those exceeding 500 μm are typically associated with filamentous bacterial overgrowth and reduced treatment efficiency (Jenkins et al., 2003; Bitton, 2005; Pell, Wörman, 2008; Fiałkowska et al., 2010; Dymaczewski et al., 2011; Łagód, 2017; Cel, 2021; Ledakowicz, Paździor, 2021).

g) pH – the optimal pH range for activated sludge operation is 6.5–8.5. Nitrification processes occur most efficiently at pH 7.5–8.5, although they can proceed at pH 6.5. Denitrification is most efficient at pH 7–9; however, the process is inhibited at pH values below 6 and above 8 (Dymaczewski et al., 2011; Pajura, Czarnota, 2021).

h) Temperature – in the range of 10–22 °C, there is an intensification of the biochemical processes responsible for the removal of nitrogen compounds through the classic processes of nitrification and denitrification. Above 35 °C, the growth of nitrifying bacteria is inhibited, while a drop in temperature below 11 °C leads

to a reduction in the rate of the second phase of nitrification. The denitrification process slows significantly at a temperature of approximately 8 °C, which is significant during the winter months (Dymaczewski et al., 2011; Smyk et al., 2016; Pajura, Czarnota, 2021).

Activated sludge bioindicators

The activated sludge biocenosis is a sensitive system that can provide information about the conditions prevailing in a biological reactor (Groβmann et al., 1999; Łagód, 2017; Fiałkowska et. al., 2010). The structure and abundance of individual groups of organisms reflect the system's loading level, oxygen availability, and process stability (Groβmann et al., 1999; Jaromin et al., 2012; Łagód, 2017; Fiałkowska et. al., 2010). The following are considered to be of particular diagnostic importance (Groβmann et al., 1999; Fiałkowska and Pajdak-Stoś, 2008; Fiałkowska et. al., 2010):

- free-swimming and sessile ciliates,
- rotifers,
- nematodes,
- filamentous bacteria.

Changes in the abundance or dominance of specific groups may indicate organic overload, hypoxia, or sediment that is too young or too old (Madoni, 1994; Fiałkowska et al., 2010). In contrast to temporary fluctuations in physicochemical parameters, the structure of the biocenosis reflects environmental conditions over a longer time horizon, making it a useful diagnostic tool (Kozłowska et al., 2023). However, classical microscopic analysis requires experience and is time-consuming. For this reason, quantitative methods – including digital image analysis and machine learning algorithms – are becoming increasingly important, as they enable the automated assessment of the structure of sediment microbial communities. However, the practical application of AI-based image analysis remains dependent on the quality and representativeness of image datasets, standardized image acquisition, and validation under different wastewater treatment conditions. Consequently, AI-based methods should currently be regarded as complementary tools supporting, rather than replacing, established microscopic assessment and expert interpretation. In the longer term, their integration with biological indicators, conventional process parameters, and online monitoring data may provide a basis for more comprehensive and timely diagnosis of activated sludge processes. The integration of biological methods (including those supported by AI-driven automated image analysis) with mathematical and statistical approaches is currently one of the main directions in the development of diagnostics for wastewater treatment plant process factors (Satoh, H., et al., 2021; Staniszewski et al., 2024; Dziadosz et al., 2024; Li et al., 2025).

In bioindication analyses of biological wastewater treatment systems, it is common practice to classify organisms into morphological groups that can be reliably identified in live specimens using optical microscopy (Abraham



Figure 3. Schematic structure of an activated sludge floc, composed of bacterial aggregates and organic as well as inorganic matter, colonized by bacteria-feeding ciliates (based on: Pell, Wörman, 2008; Pinther et al., 2022)

et al., 2019; Fiałkowska et. al., 2010). This approach minimizes the risk of taxonomic errors while allowing for an assessment of the state of the biocenosis in the context of the reactor's operating conditions (Madoni, 1994; Fiałkowska et al., 2008; Ouyang et al., 2019).

The list of bioindicator organisms (Table 1) includes both eukaryotic microfauna (ciliates, rotifers, nematodes) and groups of bacteria of technological importance (filamentous bacteria). The presence and dominance of specific taxa are linked to the characteristics of the biological process: favorable aerobic conditions and a balanced organic load promote the dominance of ciliates and rotifers, whereas substrate overload and hypoxia correlate with the growth of filamentous bacteria and sedimentation disturbances (bulking) (Madoni, 1994; Fiałkowska et. al., 2010; Foissner, 2016; Abraham et al., 2019; Ouyang et al., 2019; Gao et al., 2024).

The structure of the activated sludge biocenosis is strongly correlated with the operating parameters of the process. Under conditions of high oxygenation and moderate organic loading, groups indicative of process stability dominate. In contrast, under conditions of organic loading or anoxia, an increase in the proportion of filamentous bacteria and other organisms associated with sedimentation disturbances and floc integrity issues is observed (Jenkins et al., 2003; Foissner, 2016; Ouyang et al., 2019; Gao et al., 2024).

Among the operational disturbances that can be predicted from observations of the activated sludge biocenosis, bulking and foaming are particularly important (Jenkins et al., 2003; Bazeli, 2007). Both phenomena are closely related to changes in the composition of the microorganisms that make up the sludge and serve as an example of the practical application of bioindication in monitoring wastewater treatment processes. The dominance of filamentous bacteria is considered one of the main indicators of the risk of bulking, while an increased abundance of microorganisms with hydrophobic surface properties – especially radioles and certain filamentous bacteria – may indicate the development of foaming. Microscopic observations, therefore, allow not only for assessing the current state of the activated sludge but also for identifying symptoms of potential operational problems at an early stage of their development (Zhang et al., 2019; Szeląg et al., 2020; Nittami, Batinovic, 2022; Sam et al., 2022).

A review of the literature indicates that combining traditional microscopic analysis with quantitative methods and statistical tools can be an effective approach to monitoring the condition of activated sludge, especially in large and diverse municipal and industrial wastewater treatment plants. This approach enables the early detection of adverse biological trends and the identification of potential operational problems, supporting more informed process monitoring and management (Ouyang et al., 2019; Asgharnejad, Sarrafzadeh, 2020; Maal-Bared, 2020; Staniszewski et al., 2024; Dziadosz et al., 2024).

BIOCENOTIC INDICES FOR THE EVALUATION OF ACTIVATED SLUDGE

The importance of biocenotic indicators

The assessment of activated sludge performance has traditionally been based on physicochemical and operational parameters, such as the Sludge Volume Index (SVI), Sludge Age (Solids Retention Time – SRT), the substrate loading to biomass ratio (F/M), and dissolved oxygen concentration. These parameters provide information on the current state of the process but do not fully reflect the structural and trophic relationships occurring within the biocenosis of the biological reactor. In response to this gap, the concept of biocenotic indices was developed, which allows for a quantitative assessment of the microbial community structure and its relationship to treatment efficiency (Foissner, 2016; Abraham et al., 2019; Ouyang et al., 2019; Speirs et al., 2019).

Biocenotic indices assume that organisms inhabiting activated sludge occupy relatively stable ecological niches and respond in a predictable manner to changes in environmental conditions. Microfauna – particularly ciliates, rotifers, and nematodes – exhibit high sensitivity to changes in organic loading, oxygenation, and sludge age, and can therefore serve as biological indicators of environmental stress. Unlike point-in-time chemical measurements, the structure of the biocenosis reflects conditions over a longer time horizon, serving as a record of the system's operational history (Curds, 1968; Madoni, 2011; Foissner, 2016).

Despite its numerous advantages, the index-based approach is not without limitations. First, the accuracy of the indices depends on correct taxonomic identification, which, in the case of protists, requires a high level of specialized expertise. Second, the composition of the biocenosis

Table 1. Selected groups of bioindicator organisms in activated sludge and their technological interpretation

Group of organisms	Conditions favorable for growth	Technological significance	Source
Ciliates (Ciliophora)	Stable aerobic conditions; moderate F/M loading; appropriate sludge age	The dominance of sessile and creeping forms indicates mature, well-sedimenting sludge; a predominance of free-swimming forms may indicate system overload	(Madoni, 1994; Groβmann et al., 1999; Fiałkowska et. al., 2010; Foissner, 2016).
Heterotrophic flagellates	High organic load; early stabilization phase	Indicator of sludge overload; rapid response to an increase in the concentration of easily biodegradable matter	(Madoni, 1994; Groβmann et al., 1999; Fiałkowska et. al., 2010; Foissner, 2016).
Naked amoebae	Low oxygen concentration; high pollutant load	Signal of stressful conditions and hypoxia	
Testate amoebae (Testacea)	Stable operating conditions; nitrification/denitrification processes	Indicator of biological stability and sludge maturity	(Groβmann et al., 1999; Fiałkowska et. al., 2010; Foissner, 2016).
Rotifers (Rotifera)	Long sludge age (high SRT); low F/M	Indicator of mature sludge; contributes to the regulation of bacterial populations and floc structure free-swimming and attached forms	(Groβmann et al., 1999; Fiałkowska et. al., 2010; Foissner, 2016).
Nematodes (Nematoda)	High SRT; stable oxygen conditions	Improvement of floc structure through the formation of diffusion channels; biomass maturity index and indicator of mature sludge; role in regulating bacterial populations and floc structure	(Groβmann et al., 1999; Fiałkowska et. al., 2010; Foissner, 2016).
Oligochaetes (Oligochaeta)	Long biomass retention time	Excessive abundance may indicate very high sludge age	(Groβmann et al., 1999; Fiałkowska et. al., 2010; Foissner, 2016).
Autotrophic organisms (diatoms, green algae, cyanobacteria)	Light availability; presence of nutrients	Characteristic of biofilms and flocs; limited significance in evaluating the performance of bioreactors with conventional activated sludge	(Fiałkowska et. al., 2010; Foissner, 2016).
Filamentous fungi and yeasts	pH fluctuations; presence of poorly biodegradable compounds	May indicate a specific composition of industrial wastewater	(Groβmann et al., 1999; Fiałkowska et. al., 2010).
Filamentous bacteria	Low F/M ratio, oxygen deficiency, disturbances in wastewater composition	Excessive growth leads to sludge bulking	(Bazeli, 2007; Fiałkowska et. al., 2010; Kocwa-Haluch, Woźniakiewicz, 2011; Foissner, 2016).
Zoogleal and floc-forming bacteria	Stable organic loading	Responsible for the proper structure and sedimentation of flocs	(Groβmann et al., 1999; Fiałkowska et. al., 2020; Kocwa-Haluch , Woźniakiewicz , 2011; Foissner, 2016).

exhibits seasonal variability and sensitivity to local factors (the nature of the wastewater, reactor configuration), which may limit the universality of certain indices. Third, many indices were developed based on data from municipal wastewater treatmentplants with a conventional configuration, which raises questions about their suitability for modern multi-chamber systems, membrane systems, or high-load hybrid bioreactors (Madoni, 2011; Foissner, 2016; Sobczyk et al., 2021).

Biodiversity indices

Research on biodiversity, which began on a global scale, has also found application in the assessment of biological processes in wastewater treatment plants, including the quality of activated sludge and the biofilm (Burian et al., 2022; Godzieba et al., 2022). The structure of microbial communities can be quantified using appropriately selected indicators that are both practically useful and scientifically sound and sensitive to significant environmental changes (Haegeman et al., 2013; Sobczyk et al., 2021). Due to the complexity of biocenoses, there is no single universal measure for describing them; therefore, the selection of indices and indicators should always be tailored to the research objective (Haegeman et al., 2013; Sobczyk et al., 2021; Burian et al., 2022; Godzieba et al., 2022).

The structure of biological communities can be described using numerous quantitative

indicators, which differ in the scope of information they provide and in how they are interpreted . Individual measures are constructed based on measurable characteristics of the community, such as the number of distinct categories, the abundance of individuals, or the proportions between them. In research practice, indicators that combine methodological soundness with applicability in empirical analysis are particularly useful. This means that they should be based on numerically expressed parameters that are representative of the analyzed property (e.g., diversity) and, at the same time, can be reliably determined using standard sampling and data processing procedures (Haegeman et al., 2013; Morris et al., 2014; Kim et al., 2017; Mammola et al., 2021; Łagód, 2017).

There is no single, universal indicator capable of fully characterizing all aspects of a community's structure. Various components of this structure – such as taxonomic richness, evenness of abundance, or degree of dominance – require different analytical tools. Consequently, the choice of a measure should always be guided by the purpose of the study and the specific characteristics of the biological material being analyzed (Haegeman et al., 2013; Morris et al., 2014; Ricotta et al., 2020).

- Taxonomic richness (S) (Łagód, 2017; Kozłowska et al., 2025)

$$S^* = \sum_{i=1}^{S} s_i \tag{4}$$

where: S_i – groups of organisms taken under consideration.

The simplest characteristic of a community is the number of distinct categories (species, morphological groups), defined as taxonomic richness S. This index provides information only on how many systematic units were found in the sample; it does not take their abundance into account. For this reason, it provides limited information about the community's structure. Furthermore, its value depends on the sample size, the intensity of the research effort, and the spatial scale of the analysis, which can make comparisons between sites difficult (Finlay et al., 1998; Pliashechnyk et al., 2018; Szoszkiewicz et al., 2019).

Richness alone also does not reflect the internal diversity of a community. Two communities with an identical number of categories may differ significantly in the distribution of the abundance of individual groups. These limitations have served as the basis for the development of more complex indices that combine information on the number of categories with their quantitative share (Montusiewicz et al., 2006; Montusiewicz et al., 2010; Sienkiewicz, 2010; Haegeman et al., 2013; Morris et al., 2014; Chao, et al., 2014; Łagód, et al., 2016; Kim et al., 2017; Łagód, 2017; Ricotta et al., 2020).

- Shannon index (H)

$$H = -\sum_{i=1}^{s*} \Pi_i log\ (\Pi_i) \tag{5}$$

where: S – number of different species in the sample (taxonomic richness); p_i – proportion of the number of individuals of a given species (group) to the total number of individuals present in the sample.

One of the most commonly used measures of biological diversity is the Shannon index (H), derived from Claude Shannon's information theory (Babko et al., 2014; Babko et al., 2016; Guevara et al., 2016; Łagód, 2017; Kozłowska et al., 2025). Originally, this index was used to describe entropy, that is, the average amount of information per individual message (Shannon, Weaver, 1964; Gove et al.,1994). A similar approach was applied in ecology, based on the assumption that the structure of a community can be interpreted as a system with a certain degree of "uncertainty" or diversity (Babko et al., 2014; Babko et al., 2016; Guevara et al., 2016; Łagód, 2017; Kozłowska et al., 2025; Domingues, Pires, 2026).

The value of H depends both on the number of categories present in the sample and on the evenness of their proportions. An increase in the number of groups and a more even distribution of group sizes lead to an increase in the index value. This measure is particularly sensitive to the presence of rare categories, while it reacts to a lesser extent to changes in the sizes of dominant groups. It reaches its minimum value in a completely homogeneous community and its maximum when all categories are equally represented. Under natural conditions, H values most often range from approximately 1.5 to 3.5, with higher values indicating a more stable and balanced community structure (Shannon, 1948; Gove et al.,1994; Babko et al., 2014; Babko et al., 2016; Łagód, 2017; Kozłowska et al., 2025).

- Margalef index (Mg, D_{Mg})

$$Mg = \frac{S-1}{lo\,g(N)} \tag{6}$$

where: S – number of species (groups) in the sample, N – total number of organisms in the sample.

One of the simpler indices that takes into account both the number of categories and the total number of individuals is the Margalef index (Mg). This measure relates taxonomic richness to sample size, allowing for an assessment of the relative diversity of a community. It has no theoretically defined maximum value, but takes on a value of zero when all individuals belong to a single category. This index is relatively easy to interpret, although – like S itself – it focuses primarily on the aspect of richness (Margalef, 1958; Babko et al., 2014; Babko et al., 2016; Łagód, 2017; Domingues, Pires, 2026).

- MacArthur index (E)

$$E = z^{H} \tag{7}$$

where: H – fractional share of individuals counted in individual species, Shannon index, z – base of the logarithm used (in this paper, the base-2 logarithm).

Based on the Shannon index, the so-called effective number of elements – referred to as the MacArthur index (E) is determined. It is calculated as the exponent of the H value and is interpreted as the number of equally frequent categories that would yield the same diversity value. This transformation gives the index the property of additivity (doubling), which facilitates comparisons between communities. In the literature, it also corresponds to a measure known as Hill's N1. Unlike the raw H value, this index has a more intuitive quantitative interpretation (Jost, 2006; Jost, 2007; Głowacki, 2013; Łagód, 2017).

- Proportionality index (P)

$$P = \frac{E}{S^{*}} \cdot 100 \tag{8}$$

where: E – MacArthur proportionality index, S^{*} – species richness.

The proportionality index P (Montusiewicz et al., 2006; Chomczyńska et al., 2005; Łagód, 2017; Kozłowska et al., 2023; Kozłowska et al., 2025) is the quotient of the effective number of elements (E) and taxonomic richness (S). It reflects the degree to which the potential richness of a community is utilized, indicating the extent to which the observed categories are represented in a balanced manner. Low values may suggest strong dominance by certain groups.

- Pielou's evenness index (J)

$$J = \frac{H}{H_{max}} = \frac{H}{l\,n(S)} \tag{9}$$

where: H – Shannon diversity index, S – species (group) richness in the sample.

Based on the Shannon index value, Pielou's evenness index (J) can be calculated; this index determines the degree of uniformity in species abundances within a community. It ranges from 0 to 1, with values closer to unity indicating a more even distribution of abundance among species, while lower values indicate the dominance of specific taxa (Pielou, 1966; Bulmer and Pielou, 1976; Sienkiewicz, 2010; Domingues, Pires, 2026).

- Menhinick's index (M, D_{Mn})

$$M = \frac{S^{*}}{\sqrt{N}} \tag{10}$$

where: S^{*} – species richness, N – total number of individuals

Menhinick's Index assesses species richness, placing greater emphasis on the relationship between the number of species and the total number of individuals. Higher M values suggest greater diversity (Bis et al., 2013; Thukral, 2017; Domingues, Pires, 2026).

- The Berger–Parker index (d)

$$d = \frac{N_{max}}{N} \tag{11}$$

where: N_{max} is the number of individuals in the most abundant species.

A simple and intuitive measure of dominance is the Berger–Parker index (d), which determines the proportion of the most abundant species in the total number of individuals.

In summary, classical diversity and dominance indices form the basis for the quantitative assessment of biocenosis structure. In activated

sludge studies, their interpretation should be supplemented by an analysis of parameters specific to this system, such as the number of functional taxa or the morphological characteristics of flocs, which allows for a more comprehensive assessment of the stability of the technological process (Berger, 1970; Magurran, 2004; Domingues, Pires, 2026; Shahat et al., 2026).

- McIntosh index (Mc) (McIntosh, 1967; Ravera, 2001; Palaghianu, 2014; Łagód, 2017)

$$Mc = \frac{N - \sqrt{\sum_{i=1}^{S} n_i^2}}{N - \sqrt{N}} \quad (12)$$

where: $\sum_{i-1}^{s}$ – total number of organisms, S – total number of organism groups (group richness), n_i – abundances of individual organism groups.

The McIntosh index is based on a geometric interpretation of the community structure, drawing an analogy to distance measures in multidimensional space. It is particularly sensitive to the evenness of the distribution of individuals across categories and—like the Simpson index—responds clearly to the presence of dominant groups. Its values range from 0 to 1, with values close to 1 indicating a more balanced distribution of the analyzed categories (McIntosh, 1967; Ravera, 2001; Palaghianu, 2014; Łagód, 2017; Thukral, 2017; Kitikidou, et al., 2024).

- Brillouin index (HB)

$$HB = \frac{\ln(N!) - \sum_{i=1}^{S} l\, n(n_i!)}{N} \quad (13)$$

where: N – total number of organisms; n_i – abundance of individual organism groups.

In analyses where the assumption of random sampling is not met, or when a fully inventoried population is being studied, it is recommended to use the Brillouin index (HB) instead of the Shannon index (H), as it provides an adequate measure of information for complete samples. However, the Shannon index remains one of the fundamental measures of biological diversity, widely used in ecological and technological analyses, including studies of activated sludge biocenoses (Brillouin, 1956; Pielou, 1974; Magurran, 2004; Diaz, Malhi, 2022; Hatton et al., 2024; Domingues, Pires, 2026).

- Simpson's index (Si)

$$Si = \frac{\sum_{i=1}^{S} \times\, n_i(n_i - 1)}{N(N-1)} \quad (14)$$

where: S – total number of organism groups (group richness), N – total number of organisms, n_i – abundance of individual organism groups.

Simpson's index describes the probability that two individuals randomly selected from a sample will belong to the same category. In its basic form, it takes values ranging from 0 to 1. Unlike the Shannon index, this measure is more strongly dependent on the size of the dominant groups and thus places greater emphasis on dominance than on the presence of rare categories. For this reason, it is often classified as a dominance index, and various modifications of it appear in the literature, including the inverse of the basic form (Simpson, 1949; Gove et al.,1994; Guevara et al., 2016; Łagód, 2017; Priyadarshana et al., 2024).

- Simpson's equivalence index (E_D) (Simpson, 1949; O'Keeffe, 2004)

$$E_D = \frac{Si}{Si_{max}} = \frac{1}{\sum_{i=1}^{S} p_i^2} = \frac{1}{S} \quad (15)$$

where: Si – Simpson index, Si_{max} – maximum value of the Simpson index, S – total number of organism groups (group richness).

Dominance indices constitute an important group of measures, among which the Simpson Index (D) is widely used. It indicates the probability that two randomly selected individuals belong to the same category. In practice, both the classic form of D and its transformations (1 − D, 1/D), which facilitate interpretation, are used (Simpson, 1949; O'Keeffe, 2004; Domingues, Pires, 2026).

- Simpson's reciprocal index (D_O)

$$D_O = \frac{1}{Si} \quad (16)$$

The values of this index increase with increasing diversity, and the maximum value indicates the total number of species in the analyzed sample (Simpson, 1949; Sienkiewicz, 2010; Ławniczak, 2011; Okpiliya, 2012).

This methodological caution rests on more technical considerations of sampling and estimation. Haegeman et al. (2013) showed that the number of taxa actually present in a community cannot be reliably estimated from a finite sample without additional assumptions about the underlying abundance distribution, since rare categories routinely escape detection; as a result, richness-based estimators can even rank communities incorrectly when many rare taxa are present. Chao et al. (2014) addressed this limitation by framing richness, Shannon, and Simpson indices as members of a single family of Hill numbers, differing only in the weight given to rare versus dominant categories, and by proposing rarefaction and extrapolation procedures that allow samples of unequal size to be compared fairly. Morris et al. (2014) further showed, through empirical testing along an environmental gradient, that no single index consistently outperforms the others, with the most informative measure depending on the specific question asked. These considerations are directly relevant to activated sludge microscopy, where the number of organisms identified per sample is often limited by counting time and microfauna density on the slide. Taken together, these findings indicate that selecting and comparing biodiversity indices in activated sludge should account explicitly for sample size and the specific analytical objective of the study.

- Sludge biotic index

Unlike classical measures of diversity (e.g., Shannon or Simpson), active sludge-specific indices were developed specifically to meet the needs of wastewater treatment technology. They take into account the functional structure of microbial communities and their operational significance, thereby allowing not only a description of the biocenosis but also a diagnosis of the state of the biotechnological process.

One of the best-documented indices is the Sludge Biotic Index (SBI), developed by Giuseppe Madoni (Madoni, 1994). This index is based on the analysis of the presence and relative abundance of isolated functional groups of protozoa and selected multicellular organisms, without the need for detailed species identification. The method takes into account, among others, bacteriophagous ciliates (swimming, crawling, and sessile), predatory ciliates, -testate amoebae, large flagellates, and metazoans such as rotifers and nematodes. Specific point values have been assigned to individual groups, reflecting their significance as indicators and their sensitivity to environmental conditions. The SBI value (typically on a scale of 0–10) correlates with the biological condition of the sludge – higher values correspond to a well-developed, stable biocenosis and proper reactor operation, while low values indicate disturbances such as overload or hypoxia. Owning to its simplicity, reproducibility and strong relationship with treatment efficiency, the SBI remains one of the most widely applied microscopic indices for routine monitoging of activated sludge (Madoni, 1994a; Madoni, 1994b; Fiałkowska et al., 2010; Madoni, 2011; Hu et al., 2013; Hu, Qi, Yang, 2013; Arregui et al., 2013; Amanatidou et al., 2016; Amaral-Silva et al., 2016 Pedrazzani, et al., 2016; Łagód, 2017; Asgharnejad, Sarrafzadeh, 2020; Kepec et al., 2021; Bravo et al., 2024; Iurchenko, Tkachenko, 2024; Naranjo, Sandí, 2024; Popovici et al., 2024).

Assessment methods related to filamentous bacteria constitute a separate category, as excessive filament proliferation is closely associated with sludge bulking and foaming, two of the most common operational problems in activated sludge systems.(Eikelboom, 1999; Eikelboom, 2000; Jenkins et al., 2003). The classic method of morphological identification system developed by David H. Eikelboom is based on the analysis of microscopic characteristics of bacterial filaments, such as thickness, segmentation, branching, and staining reactions (Eikelboom, 1999; Eikelboom, 2000). This enables the identification of characteristic filament morphotypes that are commonly associated with specific operational conditions and process disturbances. (Eikelboom, 1999; Eikelboom, 2000; Jenkins et al., 2003; Bazeli, 2007; Speirs et al., 2019). In contrast, the approach developed by David Jenkins integrates microscopic evaluation with the analysis of operational parameters and includes a semi-quantitative filament index of filamentous form abundance (e.g., filament index), enabling the monitoring of changes over time (Eikelboom, 1999; Eikelboom, 2000; Jenkins et al., 2003; Fiałkowska et al., 2010; Speirs et al., 2019).

A review of the literature indicates that activated sludge-specific indices have high practical value, particularly in the operational monitoring of wastewater treatment plants. SBI index provides information on the structure of eukaryotic communities and the stability of aerobic conditions, SVI, on the other hand, provides information on the sedimentation properties of the sludge,

while methods for assessing filamentous bacteria enable the early identification of operational problems. Their complementary use increases the reliability of interpreting changes occurring in the activated sludge biocenosis and supports technological decision-making.

Despite their practical usefulness, SBI and other indices have limitations that should be considered when interpreting their results. SBI provides a relatively rapid and easily interpretable assessment of the biological condition of activated sludge; however, its value reflects the structure and condition of the microfaunal community rather than directly measuring treatment performance. Similarly, biodiversity indices such as the Shannon and Simpson indices describe aspects of community diversity, evenness, or dominance, but their interpretation in activated sludge should take into account the ecological and functional characteristics of the microbial community. Therefore, these indices are most appropriately used as complementary indicators and interpreted together with microscopic observations, operational parameters, and treatment performance data rather than as independent measures of process efficiency.

Application of indices in wastewater treatment plant diagnostics

A review of the literature indicates that biocenotic indices are an important tool for diagnosing biological wastewater treatment processes, particularly in activated sludge systems. Their importance stems from the fact that the structure of microbial communities reflects current environmental conditions in the biological reactor and responds to changes in operating parameters more quickly than many physicochemical indicators. (Fiałkowska et al., 2010; Łagód, 2017; Godzieba et al., 2022; Iurchenko, Tkachenko, 2024; Naranjo, Sandí, 2024; Domańska et al., 2026).

Predicting process disturbances

Numerous studies have shown that changes in the structure of the biocoenosis precede the onset of visible operational problems, such as impaired sedimentation or reduced efficiency in removing organic and nutrients. An increase in dominance indices (e.g., Simpson's) or a decrease in diversity (Shannon's) may signal a simplification of the community structure, often associated with sludge overload, oxygen deficiency, or the presence of toxic substances (Deepnarain et al., 2020; Karczmarczyk, Kowalik, 2022; Wang et al., 2023).

Particular diagnostic significance is attributed to indicators specific to activated sludge, such as the sludge biotic index developed by Giuseppe Madoni, a decrease in the value of which correlates with a deterioration in sedimentation properties and process instability. Similarly, semi-quantitative indices of filamentous bacteria, developed by David H. Eikelboom and David Jenkins, among others, enable the early detection of the risk of bulking and foaming (Fiałkowska et al., 2010; Deepnarain et al., 2020; Karczmarczyk, Kowalik, 2022; Wang et al., 2023; Iurchenko, Tkachenko, 2024; Naranjo, Sandí, 2024).

In light of the literature, monitoring of sludge microfauna can therefore serve as a predictive tool, allowing identification of adverse trends before the limit values of pollution indicators in the treatment plant effluent are exceeded (Fiałkowska et al., 2010; Łagód, 2017; Deepnarain et al., 2020; Karczmarczyk, Kowalik, 2022; Paździor, Bilińska, 2022; Wang et al., 2023; Iurchenko, Tkachenko, 2024; Naranjo, Sandí, 2024).

Assessment of the quality of aerobic and anaerobic processes

The composition of the microbial community is strongly dependent on redox conditions and oxygen availability. In aerobic reactors, the stable dominance of sessile and creeping ciliates and the presence of metazoans are interpreted as indicators of a mature, well-oxygenated sludge of appropriate age. Conversely, a predominance of flagellates and free-swimming ciliates is often associated with high sludge loading or unstable aerobic conditions. In systems with distinct anoxic and anaerobic zones, changes in the composition of the microfauna may indirectly indicate the efficiency of denitrification or nitrification processes. Although classical diversity indices are not specifically designed to assess anaerobic processes, their interpretation in the context of the functional structure of the community provides information on the degree of ecological niche diversity and the stability of oxygen gradients within the treatment system (Fiałkowska et al., 2010; Drzewicki, Kulikowska, 2011; Babko et al., 2014; Babko et al., 2014; Bernat et al., 2017; Xu et al., 2019; Kepec et al.. 2021).

Table 2. Activated sludge assessment method

Indices / method	Advantages	Limitations	Practical application
Taxonomic richness (S)	This index is very simple to calculate and makes it easy to compare different samples based on the number of taxa they contain.	It does not take the abundance of individual taxa into account, and its value depends on the sample size and the completeness of the sampling.	It is used to characterize and compare the taxonomic composition and richness of different biocenoses.
Shannon Index (H)	This index takes into account the overall structure of the community and is widely used for assessing biodiversity.	It does not indicate which particular taxa are responsible for differences between communities, and its value depends on the sampling and identification methods used.	It is used to compare changes in the structure and diversity of biocenoses.
Margalef Index (Mg, DMg)	This index is simple to calculate and partially accounts for differences in sample size when assessing taxonomic richness.	It does not take into account the evenness or dominance of the taxa within the community.	It is used to compare the taxonomic richness of different biocenoses.
MacArthur Index (E)	This index makes the interpretation of diversity easier by expressing it in terms of the effective number of taxa.	Its terminology and mathematical formula are not completely uniform across the scientific literature.	It is used to compare the structural characteristics of different communities.
Proportionality Index (P)	This index can be used to complement the Shannon Index and Taxonomic richness when describing community structure.	Its interpretation depends on the definition of the MacArthur Index used.	It is used to describe the structural characteristics and proportional relationships within a community.
Pielou's evenness index (J)	This index is simple and intuitive to interpret and provides useful complementary information alongside the Shannon Index.	It does not describe taxonomic richness independently because it focuses primarily on the evenness of the distribution of individuals among taxa.	It is used to assess the dominance and evenness of taxa within a biocenosis.
Menhinick's Index (M, DMn)	This index provides a simple measure of taxonomic richness and is relatively easy to calculate and interpret.	It does not take into account the frequency or distribution of individuals among the different taxa.	It is used to compare the taxonomic richness of different communities or samples.
The Berger–Parker Index (d)	This index is very simple to calculate and is particularly sensitive to changes in the dominance of the most abundant taxon.	Its value relies mainly on the abundance of a single, dominant taxon and therefore provides limited information about the rest of the community.	It is used to detect changes in taxonomic dominance within communities.
McIntosh Index (Mc)	This index takes into account the relative abundances of the different taxa present in the community.	It is less intuitive to interpret than some other indices and is used less frequently in ecological studies.	It is used to compare the structure of different communities.
Brillouin Index (HB)	This index is particularly suitable for samples in which the entire population is treated as a known or fully enumerated set.	Its calculation is more computationally demanding, which makes it less convenient for routine ecological monitoring.	It is used for ecological and comparative analyses of communities, particularly when complete population data are available.
Simpson's Index (Si)	This index is strongly influenced by the dominant taxa in a community, making it useful for describing dominance patterns.	Its sensitivity to rare taxa is relatively low, and the index name and formula may be defined differently in different sources.	It is used to assess the dominance structure of a community.
Simpson's Dominance Index (ED)	This index is simple to calculate and is particularly sensitive to differences in taxonomic dominance.	It is a transformation of Simpson's Index and therefore does not represent an independent measure of diversity.	It is used to assess the dominance structure of a community.
Simpson's reciprocal index (DO)	This index has an intuitive interpretation because higher values indicate greater diversity within the community.	It is a transformation of Simpson's Index and therefore does not constitute an independent diversity measure.	It is used to compare differences in dominance and diversity between communities.
sludge Biotic Index (SBI)	This index was developed specifically for activated sludge and provides a practical quality scale that facilitates the interpretation of biological conditions.	Its application requires microscopic identification of organisms and focuses on specific groups of microorganisms.	It is used to assess the biological condition of activated sludge and to monitor the operation of wastewater treatment plants.
Sludge Volume Index (SVI)	This index is a simple, rapid, and routinely used technological parameter for evaluating the settling characteristics of activated sludge.	It does not describe the structure of the biocenosis and does not directly indicate the specific cause of changes in sludge settling properties.	It is used to control sludge sedimentation properties and to evaluate the performance of secondary clarifiers in wastewater treatment plants.

Note: Simpson, 1949; Pielou, 1966; McIntosh, 1967; Bulmer and Pielou, 1976; O'Keeffe, 2004; Magurran, 2004; Bis et al., 2013; Babko et al., 2014; Babko et al., 2016; Łagód, 2017; Kozłowska et al., 2023; Kozłowska et al., 2025; Domingues, Pires, 2026.

Supporting biological process control

The literature emphasizes the growing importance of integrating biological indicators with operational parameters within the framework of so-called biological process control. Biocenotic indices can support decisions regarding sludge retention time, aeration intensity, or volumetric load (Leal et al., 2016; Malindzakova, 2020 ; Naranjo, Sandí, 2024).

In operational practice, observed trends in index changes (e.g., a systematic decline in the SBI or an increase in the dominance index) may serve as a basis for adjusting reactor operating parameters before the quality of treated wastewater deteriorates. Incorporating microbiological assessment into routine monitoring thus increases the system's resilience to disturbances and improves the long-term stability of the process (Madoni 1994a; Madoni, 1994b; Leal et al., 2013; Pedrazzani et al., 2016; Iurchenko, Tkachenko, 2024).

In summary, available studies indicate that biocenotic indices serve diagnostic, prognostic, and process-management support functions in wastewater treatment plants. Their effectiveness increases when combined with the analysis of physicochemical and technological parameters, which allows for a comprehensive assessment of the biological system's performance (Leal et al., 2013; Karczmarczyk, Kowalik, 2022; Iurchenko, Tkachenko, 2024; Naranjo, Sandí, 2024).

Such integration between biological and physicochemical monitoring can be tested rigorously using multivariate statistical tools such as principal component analysis (PCA) and redundancy analysis (RDA), which allow the structure of the microbial community to be related directly to measured operational and effluent-quality parameters. Sobczyk et al. (2021) applied this approach to protozoa and metazoa community composition in four full-scale municipal wastewater treatment plants monitored over one year. PCA showed that community composition was primarily shaped by the individual characteristics of each plant, while RDA identified reactor temperature as the strongest predictor of community variation among the parameters examined, with COD, BOD_5, suspended solids, and total nitrogen reduction rates contributing comparatively less. These findings demonstrate that multivariate analysis can successfully identify which operational factors most strongly influence biocenosis structure, offering a quantitative basis for interpreting biological indicators alongside process data. At the same time, they indicate that the strength of this relationship is plant-specific, reinforcing the need, noted elsewhere in this review, for index-based interpretation to be validated on a plant-by-plant basis.

CONCLUSIONS

A review of the literature confirms that biological assessment of activated sludge may be less expensive and less environmentally burdensome than chemical methods, while also providing information on the stability and condition of the biocenosis. Key factors in sludge assessment include: the functional structure of the microbial community, the diversity and dominance of organisms, and the presence of indicator species, such as filamentous bacteria.

The most valuable measures are those specific to activated sludge, such as the Sludge Biotic Index (SBI), indicators for assessing filamentous bacteria, and classic diversity indices, which allow for a quantitative description of changes in the structure of the biocenosis. Chemical methods alone do not allow for the early detection of disturbances or the diagnosis of the causes of problems in the process.

Further work is needed to describe the relationships between selected parameters of the activated sludge biocenosis structure and the course of the treatment process as well as the quality of the treated wastewater. Similarly, research is needed on the automation of microscopic analyses – both regarding the standardized preparation and acquisition of microscopic images and methods for automatic image analysis (e.g., using machine learning techniques) – the integration of biological indicators into monitoring and control systems, as well as the standardization of result interpretation for different types of wastewater treatment plants.

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