The impact of microbiological consortia of autochthonous and allochthonous microorganisms on the parameters of wastewater treatment from car washes

Wpływ biomieszanek mikroorganizmów auto oraz allochtonicznych na parametry oczyszczania ścieków z myjni samochodowych

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Microbiological biopreparations as biotechnological methods support wastewater treatment processes. Unfortunately, in conditions with a short hydraulic retention time (up to 22 hours) in wastewater treatment plants, biological treatment processes are not able to effectively reduce the concentrations of COD, BOD_5, and nutrients in the sewage. The authors tested both biopreparations with consortia of autochthonous and allochthonous microorganisms, and in both cases, no significantly significant differences in treatment efficiency were found compared to the control group (without the addition of biopreparations).
Keywords: industrial wastewater treatment, biopreparations, biological treatment, car wash wastewater (CWW)

Biopreparaty mikrobiologiczne jako metody biotechnologiczne wspomagają procesy oczyszczania. Niestety w warunkach oczyszczalni z krótkim HRT (do 22 godzin), procesy biologicznego oczyszczania nie są w stanie efektywnie redukować stężeń ChZT, BZT_5, oraz biogenów w ściekach. Autorzy testowali zarówno biopreparaty z konsorcjami mikroorganizmów autochtonicznych oraz allochtonicznych, w obu przypadkach nie wykazano znacząco istotnych różnic w efektywności oczyszczania w porównaniu z grupą kontrolną (bez dodatku biopreparatów).
Słowa kluczowe: oczyszczanie ścieków przemysłowych, biopreparaty, oczyszczanie biologiczne, ścieki z myjni samochodowej (CWW)

Introduction

The use of microbiological biopreparations has shown significant growth in the last decade. They are applied in many areas of the economy, including industry, agriculture, environmental protection, food processing, medicine, and more. Access to specialized consortia of microorganisms is also available to private individuals, as the market eagerly absorbs new formulations and offers them to all interested customers. What are biopreparations and can they be applied in so many areas of our lives?

The technology of effective microorganisms (EM) and their consortia was developed in the 1970s at the University of Ryukyus in Okinawa, Japan [1, 2, 3].

Therefore, biopreparations are an achievement of biotechnology, which utilizes specific strains of microorganisms (bacteria, viruses, fungi) cultivated under appropriate conditions and in specific types of microbiological bioreactors. The product of such cultivation may be a colony of a single species of microorganism or a multi-species consortium living in a network of micro-ecosystem relationships along with the nutrient medium in which these microorganisms exist. Biopreparations also include extracted enzymes and metabolites of cultivated microbes, as well as other bioproducts obtained through advanced biotechnological and biochemical methods. In various fields of our economy, they have revolutionized classical methods of treating many diseases, agricultural practices, production of various materials and food products, as well as obtaining vaccines capable of decomposing diverse and toxic pollutants in water, air, soil, etc.. [2, 3].

The specific biopreparations are also used to support treatment processes in both

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industrial wastewater treatment plants and small domestic installations. In scientific and industry literature, there is a lot of information about the impact of various biomixtures on stabilizing wastewater sludge and the effectiveness of wastewater treatment in large and small treatment plants. [3, 4].

The impact of various biomixtures on the stabilization of sewage sludge and treatment of domestic-municipal wastewater has been studied by many scientists in the fields of civil engineering and biotechnology. The results of conducted tests indicate a positive influence of such biopreparations on the reduction of various forms of pollutants as well as improvement of technological parameters in different types of bioreactors used in wastewater treatment plants. [1, 3, 4, 5]. Safwat and Matta, in their review article, also confirm the beneficial effects of using various types of EM group biopreparations on the treatment processes and organic biodegradation in sewage sludge [3, 6, 7]. Most of the investigated treatment plants for sewage purification were characterized by a classical bioreactor technology scheme with the selection of biological carriers and a sufficiently long reaction time. No information was found on the study of car wash wastewater treatment processes under a 24-hour time regime.

The authors conducted research on the impact of both allochthonous and autochthonous biopreparations on the treatment process of car wash wastewater (CWW), in which the treatment time regime had to be appropriately short and could not exceed 24 hours. The key task of the CWW treatment plant was to minimize the volume of wastewater tanks and treatment chambers. This condition forced the maximum shortening of the retention time of the wastewater in the treatment chamber.

Description of the research station:

The research was conducted using 4 laboratory-scale MBBR bioreactor models with a height of 1000 mm and an internal diameter of 194 mm (Fig. 1). The aeration system consisted of 150 mm disc diffusers, with fine bubble aeration at an air flow rate of 1 dm^3/min. The main biological carrier used was Mutag BioChip 30™ with an active surface area of 5500 m^2/m^3 (made of PE material) with an established biofilm. Additional media were made up of 3 types of biopreparations. Two hybrid biomixtures were developed based on autochthonous strains and biocenosis of strains that best suited the composition of the raw sewage and organic pollutants present in it, in the biotechnology laboratory of the Department of Biochemistry at the University of Agriculture in Krakow. A commercial biopreparation from Fermentica, selected for the parameters of the raw sewage, was also used – BIO (a biopreparation from the EM group with allochthonous strains).

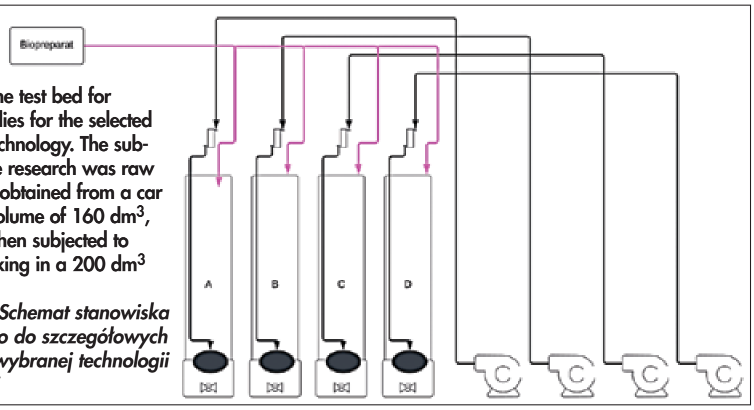


Figure 1.
Scheme of the test bed for detailed studies for the selected biological technology. The substrate for the research was raw wastewater obtained from a car wash in a volume of 160 dm^3, which was then subjected to dynamic mixing in a 200 dm^3 tank
Rysunek 1. Schemat stanowiska badawczego do szczegółowych badań dla wybranej technologii biologicznej

Parameters of the treatment process:

In each bioreactor, 30% of the filling level was composed of Mutag BioChip 30™ carriers in the total volume of treated wastewater (Fig. 2). The volume of raw wastewater in each test group was V = 25 dm^3. Biopreparations were dosed by peristaltic pumps at a rate of approximately 0.5 dm^3 per bioreactor in which the purification process was carried out.

Experimental groups:

- Control group A – no biopreparation was introduced,
- Experimental group B – BIO biomixture from Fermentica was introduced,
- Experimental group C – ERM2 biomixture from UR Krakow was introduced,
- Experimental group D – ERM4 biomixture from UR Krakow was introduced.

The aeration process was carried out in the regime of 1.5 h of aeration and 0.5 h of anaerobic phase. The filling with medium accounted for 30% of the bioreactor's volume.

Samples were collected for laboratory tests after early mixing (2 minutes after the start of the process), and then at 2 hours, 6 hours, and 22 hours thereafter.

Figure 2.
Bioreactors filled with biocarriers at 30% of chamber volume
Rysunek 2. Bioreaktory wypełnione kształtkami w ilości 30% objętości komory

Characteristics of the applied biopreparations:

Hybrid biopreparations:

In the process of isolation and preparation of biomixtures for technological tests, liquid cultures of pure strains were established on a nutrient broth enriched for bacterial cultivation SNB (Standard Nutrient Broth) consisting of peptone (15 g · dm^{-3}, Biocorp), yeast extract (3 g · dm^{-3}, Biocorp) and NaCl (6 g · dm^{-3}). After 2 days of high-density culture, i.e. above 5 107 cfu · cm^{-3}, the cultures were centrifuged (3600 rpm/10 min), the supernatant was decanted, and the cell biomass was suspended in minimal Bushnell – Haas Broth (BH, Fluka Analytical) to an optical density of OD600 = 1.8. Then, the appropriate mixed cultures were decanted into 25 cm^3 volumes. Previously sterilized car wash wastewater was added to the mixed cultures in a 1:1 ratio. The growth of bacteria in the consortium was stimulated by the addition of appropriate micro and macroelements, as well as vitamins.

Produced consortia in biopreparations

- ERM2 – a consortium formed by mixing 8 pure autochthonous strains isolated from wastewater samples collected during different periods of operation of car washes, showing the highest tolerance to detergents, oils, WWTP, and other xenobiotics present in the wastewater during the selection process.
- ERM4 – a consortium produced from 28 environmental strains originating from active biological sludge from wastewater treatment plants, soil contaminated with pesticides, soil contaminated with petroleum derivatives, extremely phenol and WWTP-contaminated water, soils accompanying brown coal deposits, and soils contaminated with heavy metals.Biopreparat BIO – w typie EM.
- They contain a consortium of probiotic microorganisms (various species of *Saccharomyces* sp. and *Lactobacillus* sp., and others according to the Fermentica company's formula). The liquid solution of the biomass mixture contains high

concentrations of Paraguayan molasses, as well as macro and microelement additives and vitamins that stimulate their growth during the adaptation phase.

Analytical methodology:

In raw and treated wastewater, a series of physicochemical parameters were determined according to the following methodology:

- Chemical oxygen demand (COD) was determined according to PN-74/C-04578/03 by dichromate method at a dilution of 1:1. A 10 cm^3 sample was taken for analysis, to which 0.22 g to 0.62 g of mercury (II) sulfate, 10 cm^3 of 0.25 M potassium dichromate and 40 cm^3 of concentrated sulfuric acid containing 0.4 g of silver sulfate were added. The solution was boiled and kept at this state for 10 minutes. After cooling and rinsing with 50 cm^3 of distilled water, it was titrated with 0.125 M iron-ammonium sulfate solution.
- Biochemical oxygen demand after 5 days (BOD_5) was determined according to PN-EN 1899-1 by dilution and seeding with the addition of allylthiourea. The sample of wastewater was diluted with water to a dilution of 1:100. 10 cm^3 was added to one dm^3 of treated wastewater solution from wastewater treatment plants, with 2 cm^3 per dm^3 of allylthiourea solution at a concentration of 1 g per dm^3. It was incubated for 5 days at a temperature of 20°C. Oxygen was determined according to PN-EN25813 (PN-ISO 5813) by iodometric method.
- Total phosphorus was determined according to PN-EN ISO 6678 by spectrophotometric method with ammonium molybdate after oxidation with potassium persulfate. For analysis, 1 or 2 cm^3 of sample was taken, diluted with 20 cm^3 of re-distilled water, and 2 cm^3 of potassium persulfate with a concentration of 50 g/dm^3 was added. It was gently boiled for 30 minutes. After cooling, the samples were quantitatively transferred to measuring flasks of 25 cm^3 and 0.5 cm^3 of ascorbic acid with a concentration of 100 g/dm^3 and 1 cm^3 of ammonium molybdate solution (molybdic acid solution II) were added, and the absorbance was measured after 30 minutes.
- Total nitrogen was determined by a modified Kjeldahl method according to PN-ISO 11261 on the KielFlex K-390 apparatus. 10 cm^3 of the sample was taken and 0.5 g of sodium thiosulfate, 1.1 g of a mixture of potassium sulfate and copper sulfate, and 10 cm^3 of a mixture of sulfuric and salicylic acid were added. It was mineralized for 8 hours at a temperature of 380°C in the VELP DK mineralizer. Then, it was distilled in the BÜCHI distiller with the addition of 50 cm^3 of distilled water,

Figure 3.
A photo of the microscope stand
Rysunek 3. Fotografia stanowiska mikroskopowego

100 cm^3 of 10 M sodium hydroxide and 50 cm^3 of boric acid. It was titrated potentiometrically in the TITROLINE 500 titrator with 0.05 M sulfuric acid.

- pH was determined according to PN-EN ISO 10523:2012 by the ERH 11 electrode of Elmetron with the CP-411 apparatus of Elmetron.
- Specific electrical conductivity (SEC) was determined according to PN-EN 27888:1999 by the EPS-2z electrode of Eurosensor with the CC-315 apparatus of Elmetron.
- The color was determined spectrophotometrically using the CECIL CE 2021 instrument according to PN-81/ C-04534.01.
- Turbidity was measured by the nephelometric method on the CECIL CE 2021 spectrophotometer.

Microscopic analyses were performed using a Nikon ECLIPSE E200 microscope with the following objectives (Fig. 3):

- CFI E P-Achromat 4X/0.10/30.00
- CFI E P-Achromat 10X/0.25/7.00
- CFI E P-Achromat 40X/0.65/0.65
- CFI E P-Achromat 100X oil/1.25/0.23

Images were recorded using a Nikon D5100 camera connected directly to the visual path.

To determine the number of aerobic microorganisms, microbiological cultures were prepared using the modified method of Koch's serial dilutions on agar growth media. The plates were incubated at 22°C for 3 days. The bacterial count in the sample was determined by counting the number of colonies formed (CFU – colony forming units) and calculated per unit volume of the bacterial suspension (cm^3). Each measurement was performed in at least two independent replicates, and the result was given as the averaged sum of microorganism counts per 1 cm^3.

Results:

The effects of wastewater treatment conducted in 4 experimental groups and 3 replications are presented in graphs 1-8.

A significant reduction of organic pollutants (measured by COD and BOD_5 values) was observed in all experimental groups (fig. 4 and 5). The COD values of raw wastewater ranged from 1100 to 1500 mg $O_2 \cdot dm^{-3}$, and the BOD5 level was around 250 $O_2 \cdot dm^{-3}$. The BOD5/COD ratio ranged from 0.16 to 0.32, indicating that the substrate was difficult to biodegrade [16, 17]. The addition of hybrid biomixtures ERM2 and ERM4 did not increase the COD and BOD_5 values compared to raw wastewater to which they were introduced. The BIO biopreparation, due to the significant amount of molasses in the nutrient medium, increased the COD and BOD_5 values significantly when added to raw wastewater and in treated wastewater (fig. 4 and 5). During the 22-hour purification process, the bed was unable to reduce the organic matter to

Figure 4.
Changes in COD values in the tested wastewater samples treated in the MBBR bioreactor.
A – Control, B – BIO, C – ERM2, D – ERM4
Rysunek 4. Zmiany wartości ChZT w badanych próbkach ścieków oczyszczanych w bioreaktorze MBBR.
A – Kontrola, B – BIO, C – ERM2, D – ERM4

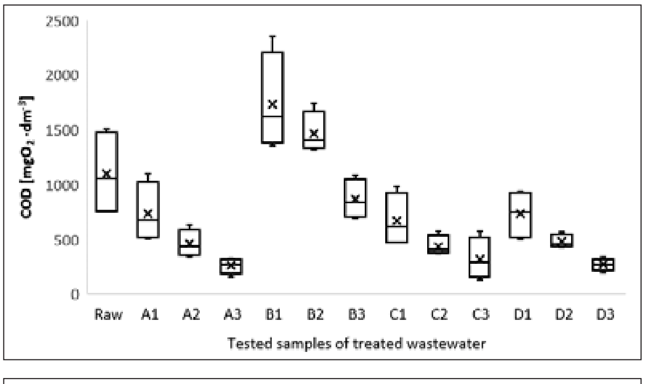
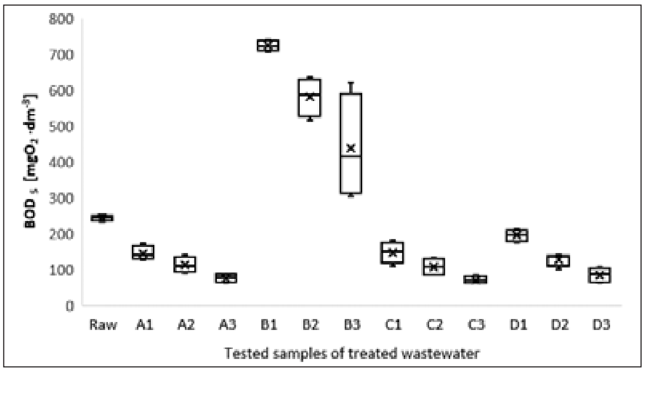


Figure 5.
Changes in BOD_5 values in the tested wastewater samples treated in the MBBR bioreactor.
A – Control, B – BIO, C – ERM2, D – ERM4
Rysunek 5. Zmiany wartości BZT_5 w badanych próbkach ścieków oczyszczanych w bioreaktorze MBBR.
A – Kontrola, B – BIO, C – ERM2, D – ERM4



a level lower than the initial level of raw wastewater. In wastewater with ERM2 and ERM4 biopreparations, the process of reducing organic pollutants was comparable to the control groups (fig. 4 and 5).

No significant increase in total nitrogen was observed in any of the experimental groups with the addition of the applied types of biopreparations. The process of TN reduction, despite the visible changes, did not differ statistically significantly compared to the control group in any of the experimental groups (Fig. 6). The MBBR reactors were configured with a 1.5-hour nitrification phase and a 0.5-hour denitrification phase, which could significantly affect the removal of total nitrogen from the treated wastewater solution.

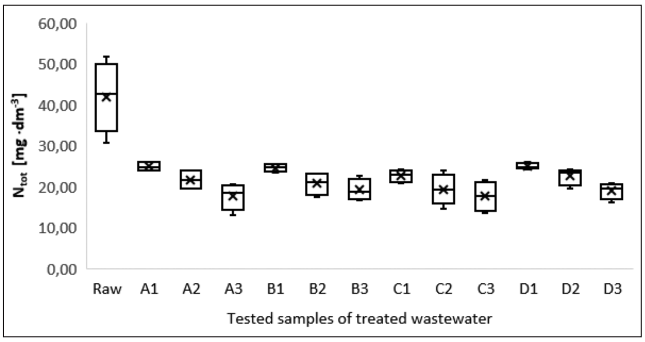


Figure 6.
Changes in N_{tot} values in the tested wastewater samples treated in the MBBR bioreactor. A – Control, B – BIO, C – ERM2, D – ERM4
Rysunek 6. Zmiany wartości $N_{og.}$ w badanych próbkach ścieków oczyszczanych w bioreaktorze MBBR. A – Kontrola, B – BIO, C – ERM2, D – ERM4

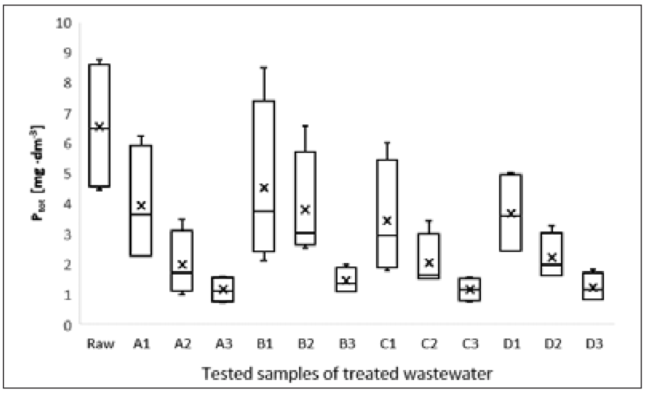


Figure 7.
Changes in P_{tot} values in the tested wastewater samples treated in the MBBR bioreactor. A – Control, B – BIO, C – ERM2, D – ERM4
Rysunek 7. Zmiany wartości $P_{og.}$ w badanych próbkach ścieków oczyszczanych w bioreaktorze MBBR. A – Kontrola, B – BIO, C – ERM2, D – ERM4

Changes in the balance of total phosphorus also did not show that the applied biopreparations increased the level of total phosphorus relative to raw sewage (Fig. 7).

A significant reduction in the concentration of total phosphorus was noted during the 22-hour period in all experimental groups. Unfortunately, no significant differences were found between the experimental groups and the control group (Fig. 7). A interesting trend was the very high reduction of total phosphorus, which strongly correlated with the process of reducing the turbidity of treated wastewater. It can be assumed that the main character of the decrease in Ptot. concentration was due to the removal of suspended solids from the solution of treated wastewater.

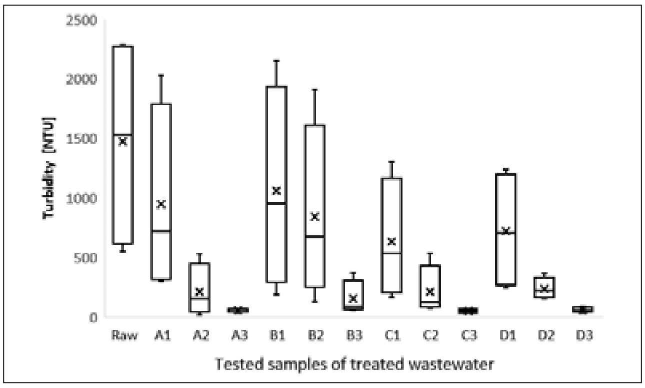


Figure 8.
Changes in turbidity values in the tested wastewater samples treated in the MBBR bioreactor. A – Control, B – BIO, C – ERM2, D – ERM4
Rysunek 8. Zmiany wartości mętności w badanych próbkach ścieków oczyszczanych w bioreaktorze MBBR. A – Kontrola, B – BIO, C – ERM2, D – ERM4

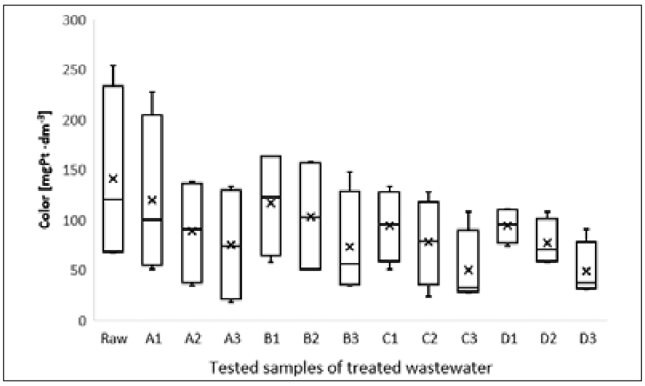


Figure 9.
Changes in color values in the tested wastewater samples treated in the MBBR bioreactor. A – Control, B – BIO, C – ERM2, D – ERM4
Rysunek 9. Zmiany wartości barwy w badanych próbkach ścieków oczyszczanych w bioreaktorze MBBR. A – Kontrola, B – BIO, C – ERM2, D – ERM4

Turbidity, like the above-described parameters, also shows a very similar character of reduction. No differences were found between the experimental groups and the control, however, the clarification process in all reactors was very significant during the 22-hour period (Fig. 8). In the experimental group with the BIO mixture, a significant spread of results was noted in the final stage (Fig. 8).

An intensive reduction in colour was recorded in all experimental groups, with the greatest difference observed in the experimental group with ERM4 bio-mixture (Fig. 9). The worst results were shown in the control group and the group with the addition of the BIO biopreparation (Fig. 9).

The results of the conducted research do not allow to indicate a positive impact of any of the tested biopreparations on the process of treating raw car wash wastewater. The only parameter in which the ERM4 biopreparation performed favourably was the reduction of colour compared to raw wastewater.

Microbial counts of aerobic bacteria abundance were also conducted on the biopreparations used in treating the tested wastewater (Table 1).

Table 1. Aerobic bacteria abundance in the tested biopreparations
Tabela 1. Liczebność bakterii tlenowych w badanych biopreparatach

Biopreparation type	Bacterial abundance [jtk/cm^3]
BIO	$8{,}20\ 10^{3}$
ERM2	$5{,}95\ 10^{7}$
ERM4	$4{,}72\ 10^{8}$

The results indicate that ERM2 and ERM4 biomixtures contain significantly more aerobic microorganisms compared to the BIO biopreparation (Table 1). The applied purification process was based on aerobic processes, therefore, the ERM2 and ERM3 biopreparations would be activated more quickly. Biopreparations of the EM type (BIO) are rather intended for processes in which no additional aeration is used. Despite the significantly higher density of proper aerobes in hybrid biopreparations, their impact on purification efficiency during the short duration of the process was not significantly different from that of the biological bed (Mutag BioChip 30™ biofilm carriers used).

It cannot be ruled out that, in the case of extending the purification time, additional favorable effects from the use of the applied biomixtures are possible. However, the main assumptions of the purification process require the fastest possible reduction of pollutants and small installation capacity. Biopreparations are unable to meet these requirements, so their use in fast biological purification processes is not effective.

Discussion of the results:

The dominant form of biopreparations used in purification processes is the utilization of Effective Microorganisms (EM), which do not require aerobic stimulation in water reservoirs [8, 9, 10]. In wastewater treatment processes, the use of biopreparations is mainly limited to supporting selected stages or conditioning of wastewater sludge [11]. They are particularly widely used in on-site treatment plants (including hydrophytic beds), due to the long HRT period [12]. Due to biotechnological limitations, it is difficult to adapt such biomixtures to fast purification systems on biological beds, as confirmed by research results on the biodegradation of organic compounds (fig. 4 and 5) and reduction of nutrients from raw wastewater (fig.. 6 and 7). The application of specialized biopreparations in the disposal and treatment of toxic waste and wastewater always requires a long period for biodegradation processes, which ensures satisfactory purification results [13]. Sowińska and Makowska in their research noted problems in the course of wastewater treatment processes by activated sludge in wastewater treatment plants where EM biopreparations were added in an uncontrolled manner [14]. In the research on the wastewater treatment processes from car washes in MBBR reactors, no additional negative effects were observed, however, the applied biomixtures did not increase the efficiency of biodegradation of organic pollutants and nutrients, which was characterized by the working biological bed (on Mutag BioChip 30™ type carriers). This would confirm the increased stability of moving beds in the MBBR system compared to the activated sludge method. Jakubas et al. also conducted research on the effect of Eco Tabs biopreparations (lyophilized) on the processes of municipal wastewater treatment in treatment plants. Statistical analyses also did not show significant effects on the quality of purification [15]. In this case, the length of HRT also plays a significant role. The research by Jakubas et al. confirms the limitations of biopreparations, regardless of whether they are in liquid or solid form.

The conducted studies exclude the application of specialized biopreparations and EM types in fast processes of industrial wastewater treatment, and studies by other authors confirm these trends in relation to municipal wastewater. The negative research result does not exclude their advantages in other areas of environmental protection and liquid and solid waste disposal, provided that the appropriate conditions characteristic of biochemical processes, which are characteristic of consortia of microorganisms in such biomixtures, are met.

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