

Some effects of drilling fluids on communities of selected eukaryotic organisms in activated sludge from sequencing batch reactors

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ABSTRACT

Laboratory-scale sequencing batch reactors (SBRs) with activated sludge were used for co-treatment of municipal wastewater and two drilling fluids the polymer-potassium (DF1) and polymer (DF2) types. The influence of two doses of each type of drilling fluids, 1% and 3% of total added wastewater volume, on the wastewater treatment processes and community of eukaryotes in activated sludge was examined. During 6 cycles, the response of ciliate (distinguishing bacterivorous and predatory ciliated protozoa), naked amoeba and total eukaryote was observed. At 1% concentration, DF2 negatively affects the eukaryotic community, however DF1 does not reliably affect the structure of the activated sludge eukaryotic population. In parallel, the increase in DF1 concentration in the sixth cycle induces significant structural changes in the eukaryotic population, similar to the effects of DF2.

Keywords: drilling fluids, wastewater treatment, sequencing batch reactor, activated sludge community, eukaryote.

INTRODUCTION

The activated sludge process is a widely used method for municipal and industrial wastewater treatment (Tran et al., 2022; Zhang, 2020) applied in both conventional activated sludge (CAS) systems and sequencing batch reactors (SBRs) (Gu et al., 2023). Unlike CAS, the SBR method relies on temporal, rather than spatial, separation of processes—activated sludge operations are conducted with intermittent aeration (Song et al., 2023). Nevertheless, the fundamental principle underlying both technologies is the biochemical activity of activated sludge, which consists of a complex consortium of microorganisms from various taxonomic groups – mainly bacteria, fungi, and protozoa – suspended in the treated wastewater (Abu Shmeis, 2018). The biochemical reactions

they carry out, coupled in time and space, lead to the biodegradation of organic matter and the removal of nitrogen and phosphorus, resulting in the formation of new microbial biomass, CO₂, water, and mineral compounds (Jaromin-Gleń et al., 2020). As microorganisms proliferate, they form aggregates (flocs) that tend to adhere to each other, facilitating sedimentation by allowing these structures to settle more easily at the bottom of the treatment tank. Filamentous bacteria play a crucial role in the structural formation of these aggregates; their abundance significantly influences the ability of sludge to thicken and resist bulking; it should be maintained at an optimal level. The presence of cations, such as calcium, contributes to the stabilization of sludge flocs (Whittaker, 2007). Cations also influence sludge dewaterability. Studies have shown that trivalent

cations, such as Al^{3+} and Fe^{3+} , reduce the energy of Lewis acid-base and electrostatic interactions, lowering the energy barrier of sludge flocs, which may lead to a decrease in bound water content and improved sludge dewatering characteristics. In contrast, monovalent cations did not exhibit such effects (Yu et al., 2021). The overall structure of sludge flocs is thus the result of physicochemical interactions among microorganisms, extracellular polymeric substances (EPS) produced by them, and other substances originating from the wastewater (Pechaud et al., 2021).

The quantity and composition of microorganisms forming the flocs are key factors determining the removal of dissolved contaminants from wastewater, and hence the efficiency of the treatment process (Tran et al., 2022). However, an equally important parameter affecting the final outcome of wastewater treatment is the effectiveness of solid-liquid separation, which depends on several factors, including suspended solids concentration and sludge age (Yetis & Tarlan, 2002), as well as the shape and density of the flocs (Nakaya et al., 2024). Increasing the weight of suspended solids enhances their sedimentation rate and facilitates the attainment of effluent clarity. One method of increasing the weight of the suspension is the use of a ballast agent (with high specific gravity), which, by binding with sludge particles, enables their easier gravitational removal from the suspension. At the same time, there is the issue of protecting the environment from the waste generated during drilling operations. One type of such waste is drilling fluid (Hossain, 2017; Ismail et al., 2017; Pereira et al., 2022). The addition of drilling fluids to municipal wastewater may have implications for the microorganisms responsible for biological wastewater treatment. The dosage of such added substances must not inhibit the metabolic activity of the activated sludge microorganisms—let alone lead to their mortality. Therefore, continuous monitoring of activated sludge quality is a crucial condition to prevent deterioration in wastewater treatment performance. Determining the characteristics of wastewater before treatment is an essential task that requires preliminary laboratory studies to assess the tolerance of the activated sludge microbial community to various contaminants. An important aspect in the process of wastewater treatment is their preparation for treatment, bringing the level of organic pollution and other impurities, including toxic substances and suspended matter, to a concentration that does not lead to the suppression of

the functional activity of the activated sludge community. Establishing such parameters of the composition of wastewater before the start of treatment is a very important task that requires preliminary laboratory studies of the tolerance of the activated sludge community to various pollutants.

An important component of activated sludge are eukaryotic organisms (Arregui et al., 2010; Foissner, 2016; Madoni, 1994). Among them, the most abundant are bacteriophagous protozoa, which exert a direct influence on bacterial populations – the primary agents responsible for the degradation of organic pollutants. These protozoa contribute to the effectiveness of biological wastewater treatment by filtering water during their life processes and by grazing on bacterial biomass (Bloem et al., 1988, 1989; Jürgens and Matz, 2002; Madoni, 2003).

Bacteriovorous protozoa, in particular crawling ciliates, naked amoebae and testate amoebae, consume not only the biomass of bacteria dispersed in the liquid phase of activated sludge, but also the bacteria associated with activated sludge particles, thereby affecting their properties. Carnivorous protozoa participate in clarification and removal of parasites (Arregui et al., 2010; Heck et al., 2023). Rotifers also affect the removal of solid particles and improve the process of their sedimentation (Chen et al., 2004; Fiałkowska et al., 2016; Lapinski and Tunnacliffe, 2003).

The composition, abundance, and physiological condition of these eukaryotic organisms are sensitive to variations in the organic loading of the activated sludge, the presence of toxic substances in the influent, and the concentration of suspended solids (Babko et al., 2016, 2017; Curds and Cockburn, 1970; Esteban et al., 1991; Salvado et al., 1995). This responsiveness enables the use of these organisms as reliable bioindicators for assessing the functional status and performance of activated sludge systems (Dziadosz et al., 2024; Foissner, 2016; Madoni, 1994, 2011; Staniszewski et al., 2024).

The aim of the study was to assess the possible impact of the addition of a loading agent (DF1 and DF2), at 2 doses (1% and 3% of the volume of incoming wastewater), on the composition and abundance of activated sludge organisms and the efficiency of the SBR wastewater treatment processes. Evaluation of the impact on activated sludge was carried out based on the structure of the activated sludge community.

MATERIAL AND METHODS

In this study, activated sludge and wastewater were obtained from the municipal wastewater treatment plant (WWTP) located in Lublin, southeastern Poland. The average daily flow rate at the facility was approximately $65,000 \text{ m}^3 \cdot \text{d}^{-1}$ (Jaromin-Gleń et al., 2013). Raw wastewater samples were collected from the outflow of the secondary sedimentation tank (Table 1), while activated sludge was taken from the external recirculation channel. The activated sludge was characterized by the following parameters: mixed liquor suspended solids (MLSS) concentration of $5.43 \text{ g} \cdot \text{L}^{-1}$, mixed liquor volatile suspended solids (MLVSS) concentration of $4.13 \text{ g} \cdot \text{L}^{-1}$, and a sludge volume index (SVI) of $178 \text{ mL} \cdot \text{g}^{-1}$. The sludge retention time (SRT) in the WWTP bioreactor was 14.9 days. The properties of the drilling fluids used in the experiment are presented in Table 2.

Experimental protocol

The experimental study was carried out using three sequencing batch reactors (SBRs) operated simultaneously, and consisting of 2 stages. The first stage involved the addition of drilling fluid 1 (DF1), second stage included drilling fluid 2 (DF2). The setup comprised three reactors: SBR1 served as the control (no drilling fluid added), SBR2 received 1% volumes corresponding to the raw wastewater volume (v/v) of

drilling fluid, and SBR3 received 3% (v/v). Each reactor had a total capacity of 1 L, with an effective working volume of 0.9 L. The SBR reactors were operated in 12-hour cycles per day, according to the phase sequence: filling (10 minutes), mixing (180 minutes), aeration (420 minutes), settling (90 minutes), decanting (10 minutes), and an idle phase (10 minutes). The experiments consisted of seven operational cycles, each performed in triplicate. Drilling fluids were mixed with the incoming wastewater during each cycle. The reactors were maintained at a constant temperature of $20 \pm 1 \text{ }^\circ\text{C}$ using a thermostatic system. Dissolved oxygen concentration was kept at approximately 2 mg L^{-1} , monitored and regulated using Hach LDO probes connected to an SC1000 control unit.

Counting and identification of organisms

To assess changes in protozoan abundance and species composition, microscopic analyses were conducted on samples collected from all bioreactors at the onset of the experiment and subsequently at 24-hour intervals. The samples were taken from a depth of 10 cm approximately five minutes after the initiation of the aeration phase, at which point the contents of the bioreactor were thoroughly homogenized. Microscopic observations were performed *in vivo*. Identification of species and enumeration of protozoa were carried out using a transmitted light optical microscope (CX41; Olympus). Phase contrast or dark-field microscopy techniques were employed when necessary to enhance visibility. The samples were counted in 25 ml subsamples under an $18 \times 18 \text{ mm}$ cover slip immediately after sampling (Madoni, 1994). Organisms were counted in 5 subsamples, and the results of the counts were averaged. Several taxonomic reference books have been used to identify ciliate species (Foissner et al., 1994; Kahl, 1930; Serrano et al., 2008).

Table 1. Average parameters concentrations of the wastewater

Parameter	Wastewaters
Chemical oxygen demand, COD (mg L^{-1})	599.38 ± 50.31
Total suspended solids, TSS (mg L^{-1})	203.42 ± 20.83
Total nitrogen, TN (mg L^{-1})	102.79 ± 7.67
Ammonia nitrogen, N-NH_3 (mg L^{-1})	93.25 ± 2.27
Turbidity (NTU)	81.28 ± 8.42

Table 2. Average parameters concentrations of drilling fluids

Parameter	Polymer-potassium drilling mud - I	Polymer drilling mud – II
Chemical oxygen demand, COD (mg L^{-1})	18.250 ± 30	35.205 ± 780
Total solids (mg L^{-1})	289.544 ± 434	262.340 ± 890
Total volatile solids (mg L^{-1})	36.193 ± 522	25.106 ± 734
Total volatile solids (% of dry weight)	12.50 ± 0.18	9.57 ± 0.28
Total nitrogen, TKN (mg L^{-1})	137 ± 68	313 ± 12

Statistical analysis

The data were analyzed using STATISTICA 13.3. The normality of distributions was tested using the Shapiro-Wilk test. The statistical significance of differences between the proposed pre-treatment procedure variants and the sludge sampling points was analyzed using Kruskal-Wallis rank ANOVA and post-hoc Dunn tests $p < 0.05$ (multiple comparisons of average ranks).

RESULTS

The effects of drilling fluids adding on eukaryotic organisms in activated sludge

The community of eukaryotic organisms in the activated sludge was represented by ciliated protozoa, testate and naked amoebae, heterotrophic flagellates, and rare metazoans – rotifers and nematodes. In this work, the authors focused on the reactions of ciliates (distinguishing bacterivorous and predatory ciliated protozoa), naked amoeba and eukaryotic organisms as a total population structure. The total number of eukaryotic organisms under the control SBR conditions (without adding drilling fluids) did not change significantly (Figure 1). In the SBR with 1% DF1, the total density of eukaryotes also changed little until the 6th cycle, and with an increase in the

DF1 concentration to 3%, a threefold decrease in the total density of organisms was observed by the end of the experiment (Figure 1A). In the SBR with 1% DF2, the density of eukaryotes decreased twofold by the 6th cycle, and in the SBR with 3% DF2, the density of eukaryotes decreased fourfold by the end of the experiment (Figure 1B).

The majority of the organisms in the activated sludge in both experiments were ciliated protozoa and naked amoebae (Figure 2). In the control SBRs, the ratio of naked amoebae to ciliated protozoa remained stable (Figure 2A and 2B). In the experiments with the addition of 1% DF 1 and DF 2, a tendency toward a decrease in the proportion of naked amoebae and an increase in the proportion of ciliated protozoa in the total number was observed (Figure 2C and 2D). In the experiments with addition of 3% DF 1 and DF 2, the decrease in the proportion of naked amoebae and the increase in the proportion of ciliated protozoa in the total number were more noticeable (Figure 2E and 2F).

Changes in the density of the main groups of organisms - naked amoebae and ciliated protozoa during the experiment generally repeated the trends in changes in the overall density of the eukaryotic community of activated sludge, namely, small changes in densities in the control SBR and a significant decrease in the density of both groups of organisms by the end of the experiment at a 3% concentration of both additives (Figure

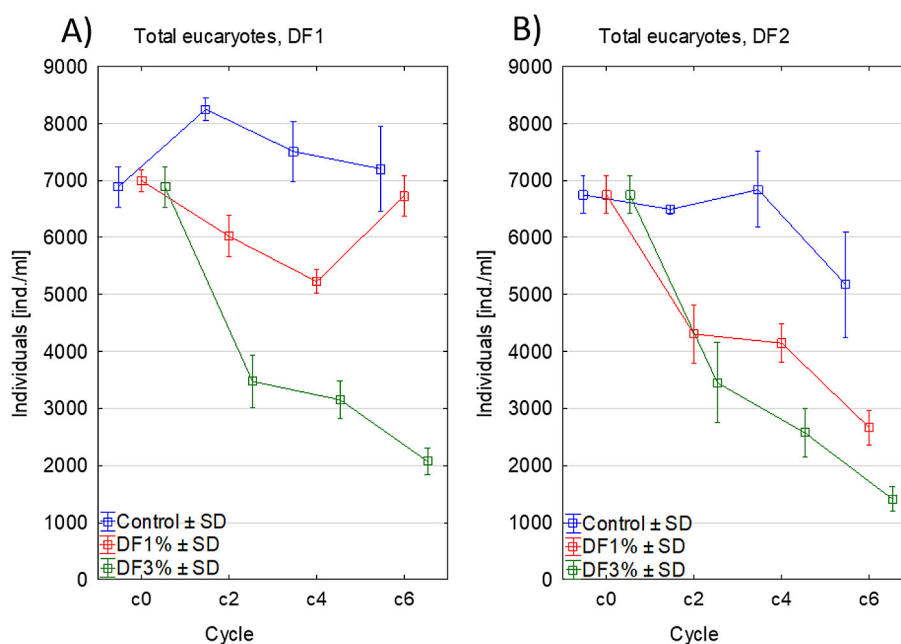


Figure 1. Changes in eukaryotic organisms density in the community of activated sludge in the SBRs with the addition of: A) DF 1 and B) DF 2 at concentrations of 1% and 3% and control

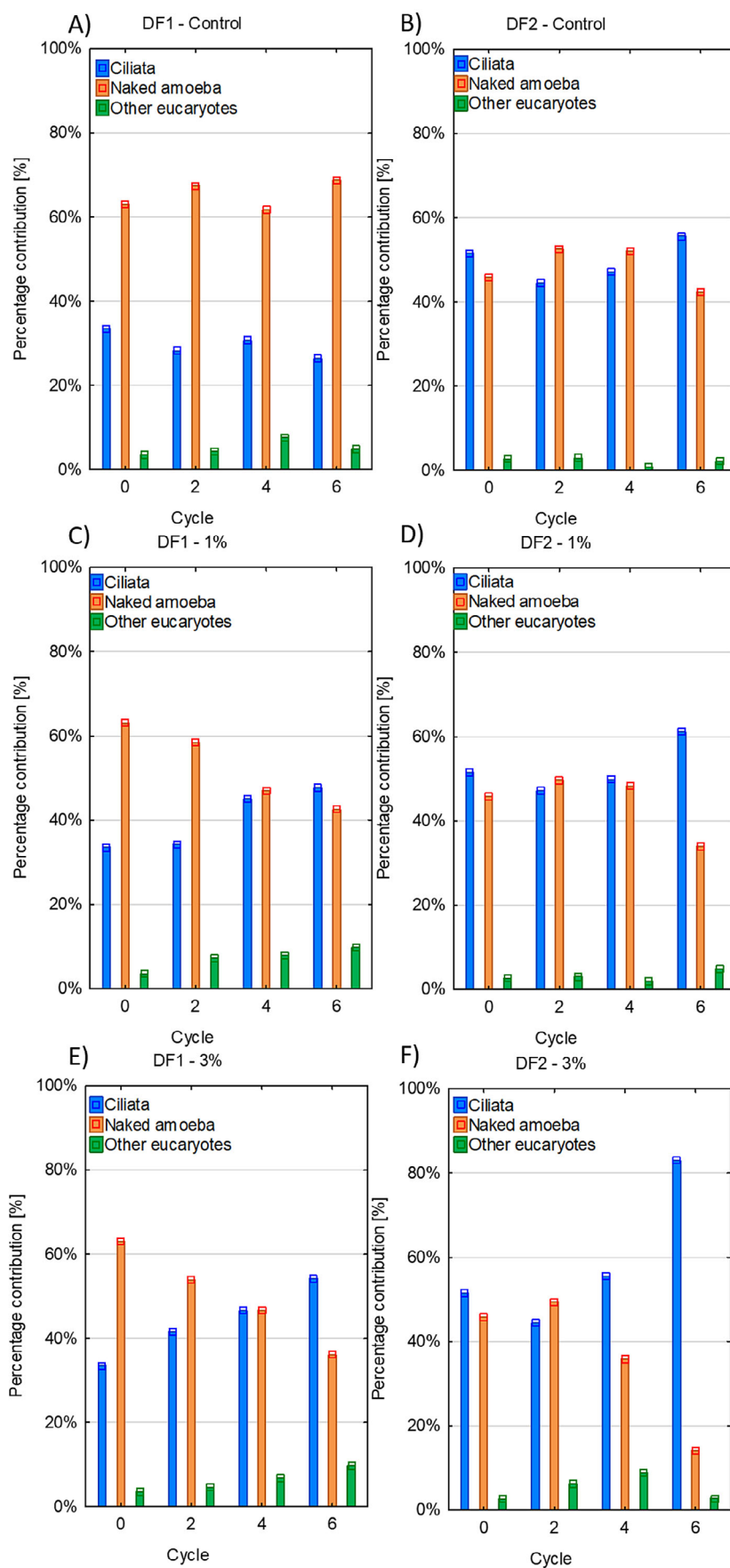


Figure 2. Ratio of abundance of different groups of eukaryotic organisms in the activated sludge community in SBRs with: A, C, E) DF1 in control and concentrations of 1% and 3% and B,D,F) DF2 in control and concentrations of 1% and 3%

3). Despite starting the experiment at different levels of organism density, the naked amoebas reacted (with both DF additions in the same way) – a sudden decrease (Figure 3A and 3B). However, differences were observed in the reaction of ciliates to the addition of different additives. Adding 1% DF 1 did not lead to a decrease in the density of ciliates. On the contrary, their density increased by the end of the experiment (Figure 3C). Adding 1% DF 2 led to a sharp decrease in

the abundance of ciliates already in cycle 2 at the beginning of the experiment (Figure 3D).

At the same time, as the analysis of quantitative data showed, ciliated protozoa – representatives of different trophic groups reacted differently to the addition of drilling solutions. The ciliated protozoa feeding on bacteria reacted negatively to the addition of drilling fluids DF 1 3%, DF 2 1% and DF 2 3% (Figure 4A and 4B). Predatory ciliated protozoa not only did not show a decrease in density in response to the addition of drilling

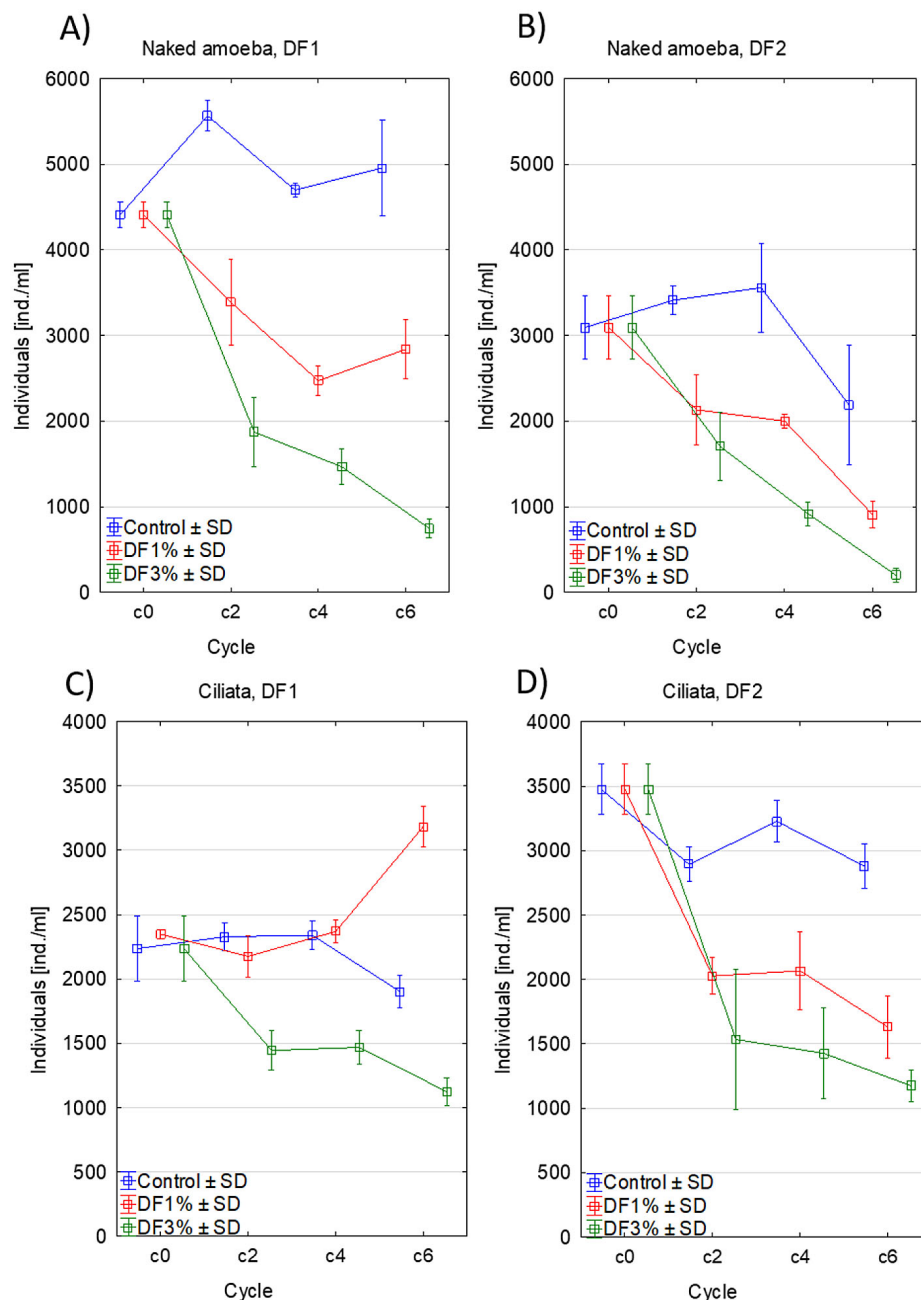


Figure 3. Changes in the density of A) naked amoebae with the addition of DF 1, B) naked amoebae with the addition of DF2; C) ciliated protozoa with the addition of DF1; D) ciliated protozoa with the addition of DF2 in SBRs at concentrations of 1% and 3% and control

fluids, but on the contrary, in the SBR with the addition of both DF 1 and D 2, some increase in the density of predatory ciliated protozoa was observed (Figure 4C and 4D).

DISCUSSION

Initially, it can be assumed that the addition of drilling fluids can have a negative impact on activated sludge organisms due to an increase in

the suspended solids content and as a result of the toxic effect. The effect of death or suppression of the vital activity of organisms due to the toxic effect was not observed in the conducted experiments. Moreover, individual populations demonstrated an increase in density by the end of the experiment Figure 4 and this is in line with earlier studies (Babko et al., 2017). The suspended solids content is a factor of concern for aquatic organisms (Bilotta and Brazier, 2008; Zhou et al., 2008). Thus, a negative effect of TSS concentration in

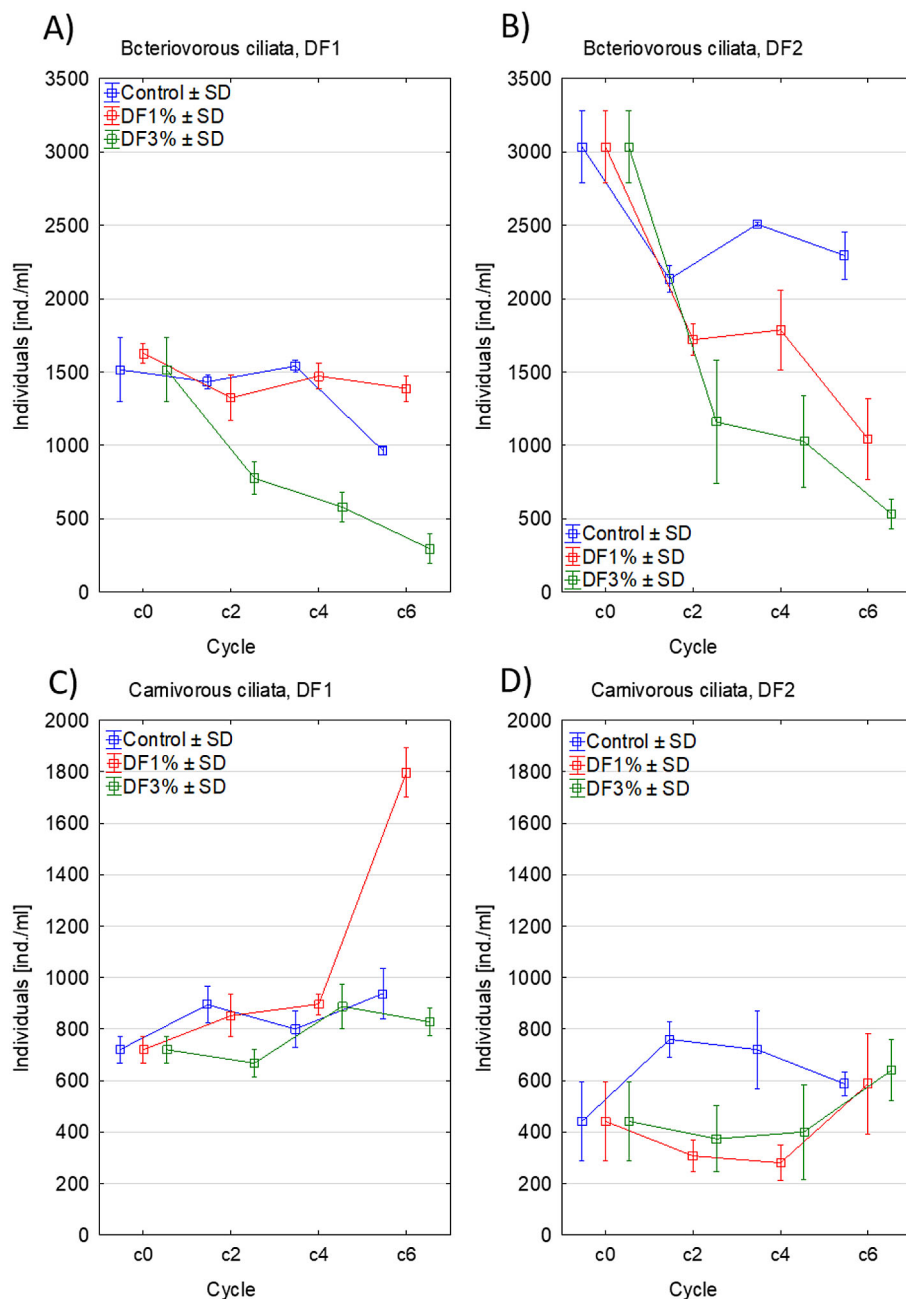


Figure 4. Changes in the density of: A) bacterivorous with the addition of DF1; B) bacterivorous with the addition of DF2; C) predatory ciliated protozoa with the addition of DF1 and D) C) predatory ciliated protozoa with the addition of DF in SBRs at concentrations of 1% and 3% and control

activated sludge on crawling ciliates and testate amoebae was shown (Zhou et al., 2008).

Comparison of changes in the number of activated sludge organisms between the initial value and the value at the end of the experiment (6th cycle) showed that the changes in the control SBR were insignificant in both experiments, which

allows drawing conclusions about the effect of drilling fluids on activated sludge organisms (Figure 5). The analysis shows that the addition of both types of drilling fluids led to a significant decrease in the number of activated sludge organisms at a concentration of 1% and to an even greater decrease in their number at a drilling fluid

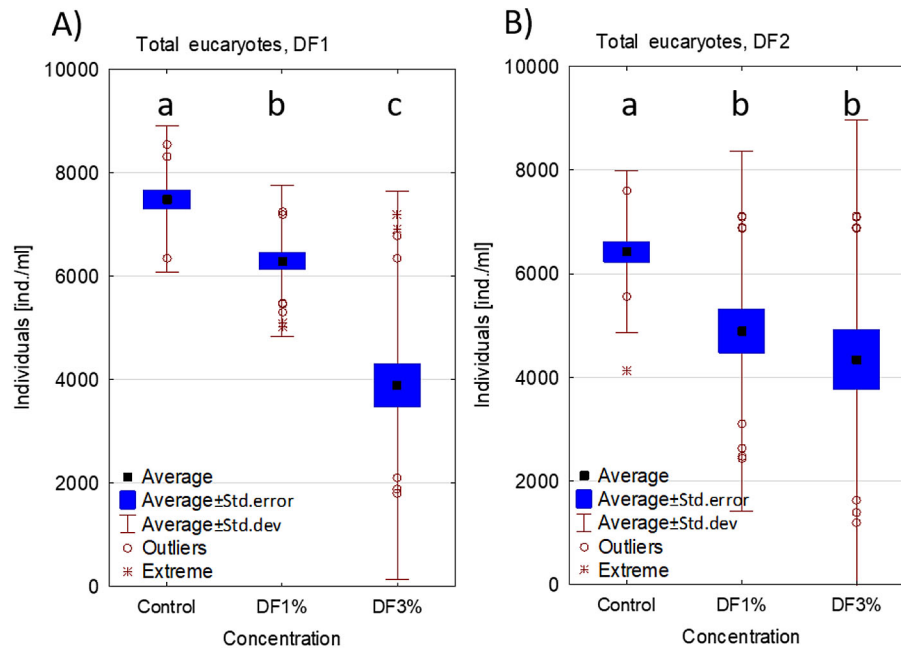


Figure 5. Average total eukaryote density at different level of additions: A) DF1 and B) DF2.). Small letters indicate statistically significant differences; Post-hoc Dunn test $p < 0.05$

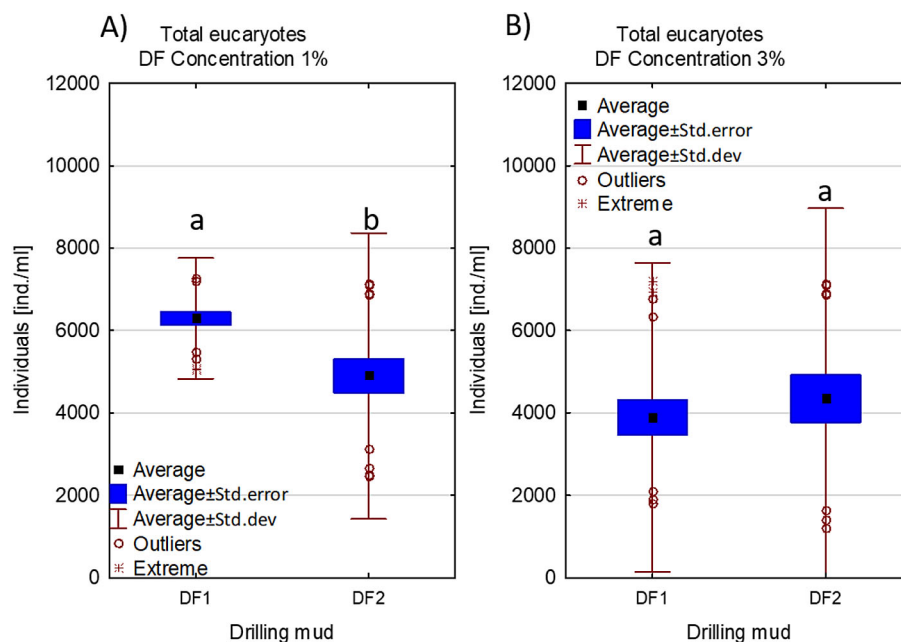


Figure 6. Comparison of average total eukaryotic density for additions (DF1 and DF2) in different concentration: A) 1% and B) 3%.). Small letters indicate statistically significant differences; Post-hoc Dunn test $p < 0.05$

concentration of 3% (significant differences at 95%, $p < 0.01$) (Figure 5).

Despite the presence of a general trend regarding the effect of drilling fluid on the density of eukaryotic organisms, differences were also revealed between the effect of DF1 and DF2. For DF1, there is a reliable difference in the effect of the drilling fluid both when compared with the control and when comparing the results of experiments with 1% and 3% concentrations. At the same time, in the case of adding DF2, the difference between the effect of adding 1% and 3% is statistically insignificant. The absence of a difference between the effect of DF2 at a concentration of 1% and 3% gives grounds to assert that DF2 had a significant negative effect already when adding 1%, and an increase in concentration to 3% did not change the situation significantly.

The statistically significant differences in the changes in the eukaryotic community composition between DF1 and DF2 at the addition of 1% were at the level of 0.005, whereas no statistically significant difference was found between the structures of the eukaryotic communities in the experiments with the addition of drilling fluids at a concentration of 3%. This shows that DF 2 has a significant negative effect on the eukaryotic community already at a concentration of 1%. At the same time, DF 1 had a negative effect only when the concentration was increased to 3%. Moreover, the addition of 1% DF 1 did not lead to a decrease in the number of naked amoebae, but stimulated an increase in the number of ciliated protozoa (Figure 6).

CONCLUSIONS

The effects of drilling fluid addition on activated sludge organisms varied depending on the drilling fluid type and concentration.

The addition of DF 1 at a concentration of 1% throughout the experiment did not have a significant negative effect on the structural characteristics of the activated sludge community: a decrease in the population density of naked amoebae, no significant changes in the population density of bacterivorous ciliates, and an increase in the population density of carnivorous ciliated protozoa were observed.

The addition of DF2 at a concentration of 1% resulted in a significant decrease in the total density of eukaryotes due to a decrease in the population density of dominant organisms,

namely, naked amoebae and bacterivorous ciliates. Carnivorous ciliates showed no decrease in density when both types of drilling fluids were added at 1% and 3% concentrations.

Both types of drilling fluids at a concentration of 3% had a significant negative impact on the total density of activated sludge organisms through a sharp decrease in the population density of bacterivorous eukaryotic organisms – naked amoebae and ciliates.

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