

doi: 10.1080/19443994.2015.1033137

#### 57 (2016) 1490–1498 January



# Effect of drilling mud addition on activated sludge and processes in sequencing batch reactors

## Roman Babko<sup>a</sup>, Katarzyna Jaromin-Gleń<sup>b,\*</sup>, Grzegorz Łagód<sup>c</sup>, Małgorzata Pawłowska<sup>c</sup>, Artur Pawłowski<sup>c</sup>

<sup>a</sup>Schmalhausen Institute of Zoology NAS of Ukraine, B. Khmelnitskyego 15, 01601 Kiev, Ukraine <sup>b</sup>Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland, Tel. +48 817445061; email: k.jaromin-glen@ipan.lublin.pl

<sup>c</sup>Environmental Engineering Faculty, Lublin University of Technology, Nadbystrzycka 40B, 20-618 Lublin, Poland, Tel. +48 712 010 46 51; email: m.pawlowska@pollub.pl (M. Pawłowska)

Received 22 September 2014; Accepted 24 February 2015

#### ABSTRACT

Laboratory-scale sequencing batch reactors (SBR) with activated sludge were used for co-treatment of drilling mud and municipal wastewater. The influence of two doses of drilling mud, 1% (SBR2) and 3% (SBR3) of total added wastewater volume, on wastewater treatment efficiency and community of eukaryotes in activated sludge was examined. Addition of drilling mud significantly decreased the efficiency of TSS removal in SBR2 and SBR3 (from 97% in control bioreactor SBR1 to 75% and 49%, respectively), COD removal (from 93% to 57% and 48%, respectively) and N-NH<sup>+</sup><sub>4</sub> removal (from 93% to 56% and 48%, respectively). Values of TSS concentration in outflow from SBR2 and SBR3 greatly exceeded the limit value appointed for the wastewater discharged into the environment. Drilling mud addition led to decrease in population and diversity of eukaryotes' groups in activated sludge, which was reflected by changes in sludge biotic index (SBI). It amounted to 10 in all the SBRs at the beginning of the experiment, and in SBR1, it remained at this same high level throughout. After 1% addition of drilling mud, a slight decrease in SBI (to 8) was observed, but 3% addition lowered the SBI to 4, reflecting serious deterioration of activated sludge quality.

*Keywords:* Drilling mud; Wastewater treatment; Activated sludge; Sequencing batch reactor; Protozoa; Sludge biotic index (SBI)

#### 1. Introduction

A large number of drillings are made while searching for shale gas, leading to the creation of huge amount of drilling waste. This waste may contain high concentrations of substances hazardous for the environment, i.e. heavy metals, mainly, arsenic, barium, chromium, copper, lead, nickel and zinc [1], as well as complex organic compounds, i.e. aliphatic and aromatic hydrocarbons. One method of managing drilling mud is separating the spent drilling from drill cuttings. The latter are usually stored in deposits, introduced to soil in the form of land spreading or land farming [2], or solidified and processed into materials used in reclamation or as the foundation for road construction. However, neutralization of the spent drilling mud remains troublesome, as it includes

<sup>\*</sup>Corresponding author.

<sup>1944-3994/1944-3986 © 2015</sup> Balaban Desalination Publications. All rights reserved.

highly concentrated liquids containing both heavy metals and complex organic compounds in the amounts hazardous for the natural environment. Part of the spent drilling mud is reused for the production of drilling liquid, but this method is not always employed. One possible way of treating the liquid phase of drilling waste is introducing them to the stream of municipal sewage and co-treatment in mechanical-biological municipal wastewater treatment plant. However, it raises concerns that the chemical substances found in drilling mud may disrupt the wastewater treatment process, causing deterioration of physicochemical properties of the treated sewage. This issue has not been researched thus far.

One of the methods for the assessment of processes occurring during biological sewage treatment is the analysis of changes in composition and abundance of activated sludge organisms. Protozoa are an important group of consumers that decide about the properties of activated sludge flocs. By consuming a part of the accumulating bacteria biomass, they maintain their population on a certain and adequate level, thus stabilizing the activated sludge system [3-6]. Protozoa present in activated sludge can be successfully used as bio-indicative organisms that show the conditions in bioreactors or treatment process [7]. It was noticed that protozoa—especially ciliates—are highly vulnerable to any changes in environmental conditions, including the ones in bioreactors with activated sludge, and react to them with species composition and the size of each representative population [8,9]. Protozoa are sensitive to changes in environmental conditions makes them important bio-indicators which enable controlling the environment quality in bioreactors and the efficiency of wastewater treatment processes [7]. These organisms are especially vulnerable to the distortions in aerobic conditions and significant fluctuations in the nutrient amount, as well as the influx of toxic substances [9–11]. They also react to the changes of physical parameters, such as the change in size of nutrient particles and changes in the amount of solid particles (often expressed as suspension and turbidity). The latter two indicators are rarely studied in relation to their impact on protozoa. In the case of drilling mud, which is characterized by high concentration of slowly settling suspension, the analysis of the impact of solid particles concentration seems to be justified.

From the practical point of view, determining permissible dose of drilling mud is crucial for proper functioning of the treatment plant, which would not impede the homeostasis of the environment in activated sludge chamber and have no effect on the efficiency of pollutant removal from sewage. Chemical compositions as well as the physical properties of drilling mud suggest that dose is an essential parameter, which may alter physical and chemical parameters of the bioreactor environment, causing changes in the composition of microflora and microfauna inhabiting it. In order to minimize the potential negative impact, it is important to establish safe or optimal concentrations which do not create stress conditions of activated sludge and do not cause a visible drop in treatment process efficiency.

The results of preliminary research on the impact of drilling mud addition on the course and efficiency of municipal wastewater treatment process were presented in this paper. The results of the analysis concerning both physical and chemical parameters of the treated wastewater and observations of variations in species composition and abundance of eukaryotic bio-indicators constituted the basis for evaluation.

#### 2. Materials and methods

### 2.1. Characteristics of raw wastewater, activated sludge and drilling mud

Wastewater, used as the substrate for conducting an experiment, was taken from primary settling tank of the Hajdów Wastewater Treatment Plant (Lublin, South-Eastern Poland). This is a mechanical–biological plant, which employs modified Bardenpho method. Its daily wastewater volume amounts to approximately 65,000 m<sup>3</sup>. Basic parameters of wastewater are presented in Table 1. The activated sludge used in the experiments for inoculating the bioreactor chambers was taken from the external recirculation channel in the same plant.

The drilling mud utilized in research was classified by the producer as polymer–potassium mud. Its main physicochemical properties are presented in Table 2.

#### 2.2. Experimental set-up and procedure

Experiments regarding the influence of drilling mud on the organisms in activated sludge were conducted in sequencing batch reactors (SBR). Three SBRs were employed in the experiment, each with the

Table 1

Presettled wastewater composition (the mean value and standard deviation are given)

Parameter	Value
Chemical oxygen demand (COD), mg/L	$599.38 \pm 50.31$
Total suspended solids (TSS), mg/L	$203.42 \pm 20.83$
Total nitrogen (TKN), mg/L	$102.79 \pm 7.67$
Ammonia nitrogen (N-NH <sub>4</sub> <sup>+</sup> ), mg/L	$93.25 \pm 2.27$
Turbidity, NTU	$81.28 \pm 8.42$

1492

Table 2 Properties of drilling mud used in the experiment

Parameter	Value
Bulk density, g/mL	$1.7 \pm 0.09$
Loss of drying, wt.%	$42 \pm 2.1$
Loss of calcination, dry wt.%	$21.6 \pm 0.9$
Total suspended inorganic solids (TSS <sub>in</sub> ), g/mL	$770 \pm 44$
Chemical oxygen demand (COD), mg/L	$18,250 \pm 330$
Total nitrogen (TKN), wt.%	$0.04 \pm 0.02$

total volume of 1 L and with active volume of 0.9 L. SBR1 was the control reactor in which no drilling mud was added. Drilling mud was added to the SBR2 and SBR3 in the amount of 1 and 3% of the total volume of wastewater dose, respectively. Bioreactors were equipped with thermostat, which enabled to maintain the temperature inside them on the stable level of 20 °C.

The experiment was conducted in seven 12-h long cycles, each of them was divided into six phases with respective length: filling: 10 min, mixing: 180 min, aeration: 420 min, settling: 90 min, decanting: 10 min, pause: 10 min. The length of each phase was adjusted on the basis of the results of earlier research [12], in order to achieve highest wastewater treatment efficiency. It was important to match the settling time to enable adequate settling rate of solid phase and production of sufficient amount of water over the precipitation, discharged from the bioreactor as treated sewage. On the other hand, the settling time could not be too long, in order to prevent secondary flotation and delamination of activated sludge.

A short pause was made following the filling and thorough mixing of biofilter content in order to collect clear liquid from above the surface of precipitate. This liquid was treated as the initial charge of the bioreactor, which constituted the habitat of activated sludge organisms. The mixing, as well as other remaining phases, was resumed after the samples had been collected. Aeration of bioreactors was made by means of compressed air, dosed to achieve the concentration of dissolved oxygen close to 2 g/m<sup>3</sup>. Following the settling, the supernatant-treated wastewater-was collected (decanting phase). This sewage was subjected to physicochemical analyses concerning selected pollution indicators, the values of which are limited by Polish legal regulations [13].

#### 2.3. Analytical protocol

#### 2.3.1. Microscopy analysis

The composition and abundance of organisms of the activated sludge were determined with a subsampling technique. A 25-µL drop of sludge was taken with a micropipette, placed on a microscope glass slide and examined under  $18 \times 18$  mm cover glass slip. The following organisms were found: ciliates, naked amoebae, testate amoebae, ciliates, flagellates, rotifers and nematoda. Among these groups, ciliates and amoebae were classified up to the rank of identified at species level. Calculating average total number of organisms and the sizes of individual populations was done on the basis of the results from counting procedure, which was repeated five times. The obtained data were calculated per 1 ml of activated sludge. Counting and identification of species carried out "in vivo" took place with in situ method under the Olympus CX41 optical microscope in transmitted light and bright field illumination, with dark field illumination or phase contrast used when necessary.

Sludge biotic index (SBI), so-called Madoni index was calculated in order to assess biological performance of activated sludge. SBI is calculated on the base of differences in the reaction of main protozoa groups (ciliates, amoebae, flagellates) and invertebrates (rotifers and nematodes), as well as on the changes in their abundances in activated sludge, according to method described by Madoni [7].

#### 2.3.2. Efficiency of wastewater treatment

The efficiency of pollutants removal was assessed on the basis of suspension COD,  $N_{tot}$  and  $N-NH_4^+$ research of raw sewage, mixtures obtained in the reactor chamber after the introduction of raw sewage and drilling mud (taken during the mixing phase) as well as treated sewage. The analyses of suspension, COD,  $N_{tot}$  and  $N-NH_4^+$  were carried out by means of DR 3600 spectrophotometer made by Hach-Lange, complying with the instructions given by the producer. Mineralization of COD and  $N_{tot}$  tests was necessary prior to the analysis, which was conducted with the use of HT 200S high-temperature thermostat made by Hach-Lange. The analysis of turbidity was carried out my means of TN-100 turbidity indicator by EUTECH INSTRUMENTS, in accordance with the instructions provided by the producer.

The efficiency of pollutants removal was calculated with two methods. The first method—standard one, enabled to calculate the technological efficiency, understood as the relation of pollutant removed in the process to its concentration in raw wastewater (Eq. (1)):

Efficiency of pollutant removal ( $\eta T_{rem}$ ):

$$\eta T_{\rm rem} = \frac{S^{\rm in} - S^{\rm out}}{S^{\rm in}} \times 100\% \tag{1}$$

where  $\eta T_{\text{rem}}$ —efficiency of the pollutant removal (%);  $S^{\text{in}}$ —concentration of the pollutant in the bioreactor inflow (mg/L);  $S^{\text{out}}$ —concentration of the pollutant in the bioreactor outflow (mg/L).

However, mixing of polluted substances introduced to SBR with activated sludge and remaining supernatant drastically lowers their actual concentrations. Therefore, the organisms live and function in the averaged concentration conditions, rather than in the conditions characterizing sewage introduced to an SBR. The method of calculating efficiency on the basis of averaged concentration (measured in the samples taken from reactors directly after filling and mixing wastewater with activated sludge) is represented by Eq. (2):

Customized efficiency of pollutant removal ( $\eta B_{rem}$ ):

$$\eta B_{\rm rem} = \frac{M^{\rm in} - S^{\rm out}}{M^{\rm in}} \times 100\%$$
 (2)

where  $\eta B_{\text{rem}}$ —customized efficiency of the pollutant removal (%);  $M^{\text{in}}$ —concentration of the pollutant in the bioreactor feedstock (mg/L).

Comparing the results of two types of efficiency is interesting both from the practical point of view of using the gathered information while interpreting the laboratory experiments results, as well as from the scientific point of view due to an approach to the conditions and parameters of wastewater treatment by means of activated sludge differing from technological one.

#### 3. Results and discussion

#### 3.1. Removal of total suspended solids

SBR bioreactor operating in standard conditions, similarly to a treatment plant, ensures removal of organic substances and suspensions which can be expressed with suitable physical–chemical indicators such as COD, TSS and turbidity. It is widely known that significant increase in suspension and turbidity in water bodies negatively impacts the majority of hydrobionts. Protozoa are no exception, especially the ones from the settling group, which constitute a substantial ingredient of activated sludge in bioreactors of wastewater treatment plant.

From the point of view of legal regulations pertaining to the quality of sewage discharged to water or land [13], it is required to remove at least 90% of their introduced amount or to achieve the concentration in outflow no higher than 35 mg/L in case of TSS. According to our research, TSS is removed efficiently only in SBR1 (control bioreactor). During the experiment, average percentage of TSS removal in this bioreactor amounted to 97.1% (Fig. 1). In the case of this bioreactor, maximum permissible value of TSS in outflow was not exceeded. Average amount of suspension in outflow calculated from all the cycles equalled  $6.1 \pm 2.8$  mg/L. Simultaneously, in SBR2 (1% of drilling mud added), the amount of suspension in outflow exceeded the maximum value 12 times on average already in the first cycle, while in SBR3 (3% of drilling mud added)-50 times. Similarly, the requirements concerning the 90% reduction of this pollution indicator were not observed in SBR2 and SBR3. Average efficiency of suspension removal in the reactor containing 1% of drilling mud equalled 74.7 ± 7.7%, whereas in the reactor with 3% addition—only  $48.7 \pm$ 19% (Fig. 1(a)).

In the case of control reactor (SBR1) a slight increase in suspension removal efficiency was observed in the following cycles, while in the case of bioreactors with drilling mud added the efficiency dropped significantly, from 89% (after the first cycle) to 63% (after the seventh cycle) in SBR2 and from 83% (after the first cycle) to 22% (after the seventh cycle) in SBR3.

Taking into account that, in fact, the activated sludge organisms are not exposed to concentrated wastewater for prolonged period of time, but they inhabit the mixture of treated and raw wastewater, the efficiency was calculated on the basis of Eq. (2). This parameter reflects actual conditions of sludge operation more truly. It was observed that the customized efficiency of TTS removal in control reactor did not vary much from the technological efficiency and amounted to  $97.1 \pm 1.1\%$  on average (Fig. 1(b)). In comparison to the two other reactors, it was the highest value. A slight increase was noticed in the following operation cycles. On the other hand, the customized efficiency of TSS removal in SBR2 (with 1% of drilling mud added) was approximately 2.5 times lower than mean technical efficiency

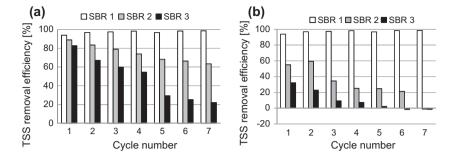


Fig. 1. Efficiency of removal of total suspended solids in SBRs calculated on the base of values measured (a) in inflow and outflow, and (b) in mixture inside the bioreactor and outflow.

(equalled  $30.5 \pm 16.4\%$  on average) and decreased in following cycles from 59.4% (in the first cycle) to below 0% (in the seventh cycle). Moreover, after the final cycle of the experiment, the concentration of suspension in bioreactor effluent increased in relation to its amount in the influent. Hence, it can be supposed that adding 1% of drilling mud causes accumulation of suspended substance in a bioreactor.

Even greater accumulation of suspended substance was observed in SBR3 (3% of drilling mud added). In comparison to SBR2, the efficiency of suspension removal decreased three times, while the mean customized efficiency of TSS removal in SBR3 equalled  $10.1 \pm 9.97\%$ . Maximum efficiency in SBR3, amounting to 32.2% was noted after the first cycle, while the maximum customized efficiency in the case of SBR2, equalled 59.5% and was observed after the second cycle (Fig. 1(b)).

#### 3.2. Removal of COD and ammonium

One of the vital chemical parameters characterizing sewage is COD. This indicator is used for the evaluation of wastewater treatment process efficiency and also reflects the physiological activity and activated sludge status. According to the legal regulations in Poland [13], the efficiency of COD removal (calculated on the basis of Eq. (1)) should equal at least 75% or the COD of treated sewage should not be higher than 125 mg/L.

On the basis of the above-mentioned requirements regarding COD, it was noticed that they were met only by the wastewater treated in SBR1. Technological efficiency of COD removal was highest and most stable in relation to the other bioreactors. It amounted to  $93 \pm 0.7\%$  on average. On the other hand, in SBR2 and SBR3, this efficiency equalled  $56.7 \pm 11.8$  and  $47.7 \pm 10.2\%$ , respectively, in most cases and exhibited significant downward trend during the experiment (Fig. 2). In contrary to our observation, Mostafa [14]

did not notice inhibiting effect of enhanced TSS concentration on the quality of treated wastewater. He added a cement kiln dust to activated sludge in concentration of 1 g/L and achieved very high efficiency of BOD<sub>5</sub>, COD, TSS and *P* total removal equal to 93.5, 93.3, 93.6, and 87%, respectively. Moreover, addition of cement kiln dust accelerated the rate of activated sludge settling and inhibited its swelling and bulking. A similar inhibition effect was observed in SBR2 and SBR3 when the drilling mud was added.

Starting with the second cycle, the COD value in SBR2 outflow equalled 350 mg/L and reached 711 mg/L after the sixth cycle. Upward trend of COD value was also observed in SBR2. COD value increased from 850 mg/L after the second cycle to 1,400 mg/L after the sixth cycle.

Apart from COD, the concentration of ammonium ions is another important parameter taken into consideration while evaluating the quality of treated wastewater The efficiency of ammonium ions removal (calculated according to Eq. (1)) was high and stable only in the case of SBR1 (Fig. 3(a)). In SBR2 and SBR3,

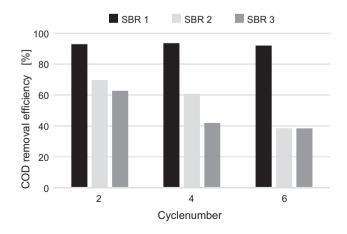


Fig. 2. Efficiency of removal of COD in SBRs in accordance with the amount of added drilling muds.

a significant drop in ammonia nitrogen oxidation was noticed, from roughly 60–70% (after the second cycle) to approximately 39% at the end of experiment. Mean values amounted to  $56.4 \pm 8.9\%$  in SBR2 and  $47.7 \pm 7.5\%$  in SBR3 (Fig. 3(a)).

Such prominent changes in COD and ammonia nitrogen removal efficiency in time mean that adding drilling mud to sewage on bioreactor input impacts not only physical parameters of treated sewage quality, mainly by TSS, but also the biochemical activity of activated sludge organisms.

In the case of ammonia removal, huge differences in efficiency calculated on the basis of Eqs. (1) and (2) were noticed. These differences also concerned SBR1, in which mean technological efficiency maintained 93  $\pm 0.3\%$ , while when it was calculated in relation to the averaged input composition, it was lower and equalled 79.2  $\pm 0.7\%$ . However, the ammonia nitrogen oxidation process in bioreactor was stable throughout the experiment (Fig. 3(b)).

In the case of SBR2 and SBR3, the differences between the values of the parameters analysed in the samples were also visible, but decreased throughout the experiment. The technological efficiency in SBR reached 70% after the second cycle, whereas the customized efficiency achieved approximately 50%. After the sixth cycle, these values came at 39 and 43%, respectively. Similarly, in SBR3, the values of both types of customized efficiencies after the second cycle amounted to 63 and 39%, and after the sixth cycle—38.5 and 33%.

## 3.3. Changes of activated sludge community during the experiment

The qualitative analysis of protozoa found in activated sludge allowed for the identification of 32 species of ciliates, 3 species of testate amoebae and 2 species of naked amoebae. Other eukaryotes present in the sludge, include small flagellates and Metazoa rotifer and nematodes, were counted but the aforementioned groups were not identified at species level.

In the group of eukaryotes inhabiting activated sludge taken from wastewater treatment plant, ciliates and naked amoebae constituted over 90% of total organisms. At the beginning of the experiment, the dominant ciliates in activated sludge were *Aspidisca cicada* and *Epistyliscoronata*, subdominant—*Aspidisca-lynceus* and *Vorticella infusionum*. Among other eukaryotic groups, only naked amoeba *Cochliopodium bilimbosum* was numerous. The testate amoebae, flagellates, nematodes and rotifers were present in low numbers throughout the experiment. Changes of

organisms' abundance in the activated sludge are shown Fig. 4.

The total number organisms dropped during the experiment in all SBRs. This drop was most prominent in SBR3 (equalled 79%). The decrease in SBR2 amounted to 60%, whereas in control reactor (SBR1), it did not exceed 20%. Thus, the addition of drilling mud caused three- and four-times lower drop in the abundance of eukaryotic organisms in comparison to the control test.

During the experiment, the number of ciliates constituted almost 50% of the total eukaryotic organisms found in activated sludge. Ciliates are the most diverse group in respect to the number of species, and numerous protozoa group in activated sludge [7,15,16]. They have an important role in the functioning of activated sludge, especially as an element of its trophic network [3–6], which influences the processes of pollutants biodegradation [17–20]. Changes in the abundances of these organisms in bioreactors are presented in Fig. 5.

The abundance of ciliates in control group during the experiment does not change much and the deviations are within the margin of error. Moreover, it was noted that in SBR1 despite decreased number of species, their total abundance was compensated with increased number of other populations. On the other hand, in SBRs 2 and 3, a significant drop in the abundance of ciliates was observed (Fig. 5).

The changes in trophic (Fig. 6(a)) and morphological (Fig. 6(b)) structure of ciliate groups were analysed in three SBRs during the experiment in relation to the initial parameters of activated sludge (taken from treatment plant).When the drilling mud was added with the dose of 3%, decrease in attached and increase in swimming ciliates were observed.

Reaction of different morphological and functional groups of ciliates and others organisms inhabiting activated sludge on TSS concentration in bioreactor were examined by Zhou et al. [21]. They showed slight significant negative correlation between crawling ciliates and testate amoeba number and TSS concentration in activated sludge ( $R^2 = -0.283$ , -0.281, P = 0.05). Insignificant negative correlation was observed in the case of attached ciliates and naked amoebae. However, the number of free-swimming bacterivorous ciliates, carnivorous ciliates and flagellates were insignificantly positively correlated with TSS concentration.

As the quality activated sludge is reflected by the diversity and number of bacterivorous organisms, their behaviour under the influence of drilling mud addition is an important indicator of its impact on activated sludge. Changes in the groups of bacterivorous

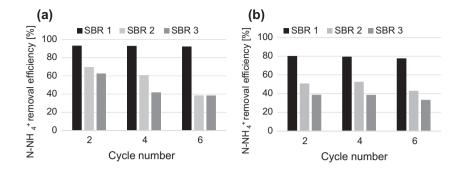


Fig. 3. Efficiency of removal of  $N-NH_4^+$  in SBRs calculated on the base of values measured (a) in inflow and outflow, and (b) in mixture inside the bioreactor and outflow.

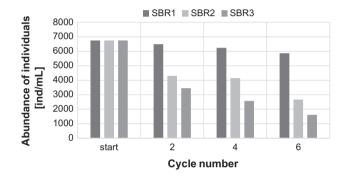


Fig. 4. Total abundance of eukaryotic organisms in the activated sludge in following cycles of the experiment.

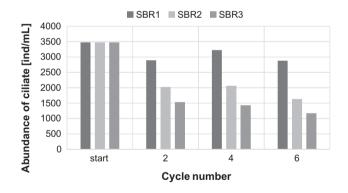


Fig. 5. Abundances of ciliates in the activated sludge in following cycles of the experiment.

organisms in SBRs were presented in Fig. 7. It was noticed that the addition of 1 and 3% of drilling mud negatively lowers the population of bacterivorous organisms, and this drop is greater in the higher amount of drilling mud.

On the basis of SBI (Madoni index) variations in sludge, it can be concluded that the influence of drilling mud on the activated sludge is visible in relation to its value in the control reactor (SBR1), where it maintained the level of 10 (Fig. 8).

High Madoni index in SBR1 means that the sludge is well colonized by eukaryotic organisms and stable, while exhibiting very good biological activity in the process of wastewater treatment-which is confirmed by the high efficiency of removing the analysed pollution indicators described earlier. In SBR2 with 1% of drilling mud addition, the SBI equals 8 from the second to sixth cycle, which means slight decrease in the quality of activated sludge in the considered bioreactor. Visible deterioration of sludge status was observed in SBR3, where SBI equalled 4 already from the second cycle (Fig. 8), which means that the sludge is weak and has low efficiency that is tantamount to dissatisfactory efficiency of biological sewage treatment. The above-mentioned statement also confirms the results of physicochemical tests discussed earlier. It is interesting that the changes in SBI occur right after drilling mud is added to SBR2 and SBR3 and maintain stable level despite continuous introduction of new portions in each bioreactor cycle.

According to the data presented, a drop in the number of eukaryotic organisms was observed in all the SBRs in the consecutive cycles of bioreactor operation. The occurrence of this phenomenon also in the control bioreactor (SBR1) can be explained by not subjecting the sludge taken from the treatment plant to a long process of adaptation to laboratory conditions. More detailed description and the importance of sludge adaptation is given by Babko et al. [12]. Hence, in the controlled bioreactors, there is a visible slight drop in the total number of eukaryotic organisms' species that occurs when shifting from the technical to laboratory scale. This drop is usually connected with the decreasing diversity of groups. However, the changes observed in SBR1 are much lower than the ones in the other two reactors. For example, during the experiment, the number of ciliate species dropped

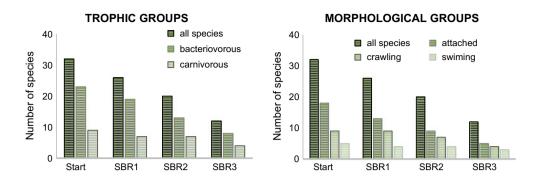


Fig. 6. Changes in the (a) trophic and (b) morphological groups of ciliate assemblages during the experiment.

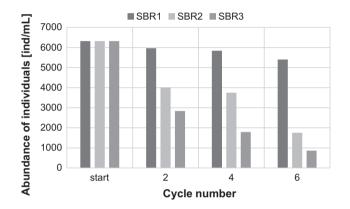


Fig. 7. Abundances of bacterivorous organisms in the activated sludge in following cycles of the experiment.

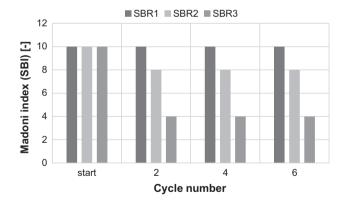


Fig. 8. Changes in Madoni index (SBI) in following cycles of the experiment.

from the initial 32 to 26 in control reactor (SBR1), 19 in SBR2 and 12 in SBR3. A conclusion can be drawn that relatively small decrease in SBR1 is the result of the adaptation of activated sludge from the treatment plant to the operating conditions of SBRs. Simultaneously, it must be noticed that the efficiency of treatment in SBR1 calculated on the basis removal of the analysed pollution indicators increased (Fig. 1(a) and (b)), or remained on relatively stable level (Figs. 2 and 3(a) and (b)) which can also be considered as the effect of the sludge adaptation to the conditions of laboratory scale. The precise control over the temperature, oxygen concentration and full mixing of the bioreactor content has impact on the process in question and, in turn, usually causes an increase in the efficiency of activated sludge operation. Therefore, with SBR1, as a point of reference, it can be observed how fast and in what way the parameters change in the other two reactors to which drilling mud was introduced along with municipal sewage.

#### 4. Summary and conclusions

Nowadays, wastewater treatment plants often face the issue of increasing concentrations of toxic compounds in the inflowing sewage. Numerous treatment plants are designed and operated using special methods for enhancing the efficiency of wastewater treatment. Chemicals are used for nutrient removal, sludge conditioning and promotion of better sedimentation and flotation. Thus far, few works on the impact of elevated TSS concentration (especially organic suspension and dispersed oily substances) on activated sludge flocs and eukaryotic organisms. As it was mentioned earlier, eukaryotes constitute a vital element of trophic network of activated sludge and possess large bio-indicative potential, which may be employed in the assessment of sludge parameters and the evaluation of the course and efficiency of treatment process. Moreover, eukaryotes—especially ciliates—play а major role in the removal of suspension and clearing of sewage. Concentration of suspension is one of the most important markers of treated wastewater quality. The results of our experiment obtained in the standard conditions of SBR1 showed that introducing drilling mud with high concentration of suspension significantly lowers the efficiency of TSS, COD and N-NH<sub>4</sub><sup>+</sup> removal. The efficiency of TSS removal in the control reactor equalled 97.11%, whereas in the reactors with 1 and 3% addition of drilling mud, it reached 63.38 and 22.14%, respectively, while the concentrations in the outflow greatly surpassed the limit values for discharging sewage to water or ground.

While summing up the analysis of bio-indicative research results, it can be stated that the drilling mud addition in the amount of 1 and 3% of volume is not toxic to the considered activated sludge organisms, i.e. no acute toxic effects were observed. It was reflected both in the abundance of eukaryotic organisms, as well as in the functioning of bacteria communities, which did not lose the capacity of COD removal capacity ammonium ions oxidation, although the efficiency of these processes dropped noticeably. Lack of toxic effect of drilling mud is also confirmed by stable SBI in SBR2 and SBR3, which did not change throughout the experiment.

The conducted experiments show the necessity of initial preparation of drilling mud prior to its introduction to treatment plant reactors. The most efficient removal of mineral suspension is advised. Further research should focus on devising the dosing method of drilling mud, determining its concentration in the inflow, as well as on the frequency of drilling mud addition to the bioreactor with activated sludge.

#### Acknowledgements

The study was supported by Polish Ministry of Science and Higher Education, Research Project No. BLUE GAS-BG1/SOIL/2013, carried out as a part of joint programme between the National Centre for Research and Development and the Industrial Development Agency JSC.

#### References

- M.L. McFarland, S.E. Feagley, T.L. Provin, Land application of drilling fluids: Landowner considerations, AgriLife Extension SCS 8 (2009) 1–5.
- [2] A.K. Wojtanowicz, Oilfield waste disposal control, in: S.T. Orszulik (Ed.), Environmental Technology in the Oil Industry, second ed., Springer, Hampshire, 2008, pp. 123–154.
- [3] J. Bloem, F. Ellenbroek, M.J.B. Bär-Gilissen, T.E. Cappenberg, Protozoan grazing and bacterial production in stratified Lake Vechten estimated with fluorescently labeled bacteria and by thymidine incorporation, Appl. Environ. Microb. 55 (1989) 1787–1795.
- [4] J. Bloem, M. Starink, M.J.B. Bär-Gilissen, T.E. Cappenberg, Protozoan grazing, bacterial activity, and mineralization in two-stage continuous cultures, Appl. Environ. Microb. 54 (1988) 3113–3121.

- [5] K. Jürgens, C. Matz, Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria, Antonie van Leeuwenhoek 81 (2002) 413–434.
- [6] P. Madoni, Protozoa as indicators of wastewater treatment efficiency, in: D. Mara, N. Horan (Eds.), The Handbook of Water and Wastewater Microbiology, Academic Press, London, 2003, pp. 361–371.
- [7] P. Madoni, A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis, Water Res. 28 (1994) 67–75.
- [8] G. Esteban, C. Tellez, The influence of detergents on the development of ciliate communities in activated sludge, Water Air Soil Poll. 61 (1992) 185–190.
- [9] G. Esteban, C. Téllez, L.M. Bautista, Effect of habitat quality on ciliated protozoa communities in sewage treatment plants, Water Res. 25 (1991) 967–972.
- [10] C.R. Curds, A. Cockburn, Protozoa in biological sewage treatment processes—II. Protozoa as indicators in the activated sludge process, Water Res. 4 (1970) 237–249.
- [11] H. Salvado, M.P. Gracia, J.M. Amigó, Capability of ciliated protozoa as indicators of effluent quality in activated sludge plants, Water Res. 29 (1995) 1041–1050.
- [12] R. Babko, T. Kuzmina, K. Jaromin-Gleń, A. Bieganowski, Bioindication assessment of activated sludge adaptation in a lab-scale experiment, Ecol. Chem. Eng. S. 21(4) (2014) 605–616.
- [13] Regulation of the Polish Minister of the Environment concerning the conditions to be met by water discharged into surface waterways or the soil, and on substances particularly harmful to the aquatic environment, Dz.U. 2014 item 1800 (in Polish).
- [14] A.H. Mostafa, Effect of cement kiln dust addition on activated sludge process without primary settling for reuse applications, House. Build. Nat. Res. Cent. J. 8 (2012) 14–25.
- [15] P. Madoni, Quantitative importance of ciliated protozoa in activated sludge and biofilm, Bioresour Technol. 48 (1994) 245–249.
- [16] P. Madoni, P.F. Ghetti, The structure of ciliated protozoa communities in biological sewage-treatment plants, Hydrobiologia 83(2) (1981) 207–215.
- [17] C.R. Curds, A theoretical study of factors influencing the microbial population dynamics of the activated sludge process—I. The effects of diurnal variations of sewage and carnivorous ciliated protozoa, Water Res. 7(9) (1973) 1269–1284.
- [18] G. Esteban, C. Téllez, L.M. Bautista, Dynamics of ciliated protozoa communities in activated-sludge process, Water Res. 25(8) (1991) 967–972.
- [19] A. Pajdak-Stós, E. Fiałkowska, J. Fyda, R. Babko, Resistance of nitrifiers inhabiting activated sludge to ciliate grazing, Water Sci. Technol. 61 (2010) 573–580.
- [20] P. Petropoulos, K.A. Gilbride, Nitrification in activated sludge batch reactors is linked to protozoan grazing of the bacterial population, Can. J. Microbiol. 51 (2005) 791–799.
- [21] K. Zhou, M. Xu, B. Liu, H. Cao, Characteristics of microfauna and their relationships with the performance of an activated sludge plant in China, J. Environ. Sci. 20 (2008) 482–486.