EFFECT OF MAZUT ON STRUCTURAL AND FUNCTIONAL INDICATORS OF ACTIVATED SLUDGE IN SOFIA WASTEWATER TREATMENT PLANT "KUBRATOVO"

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Abstract: The effect of inflow of 30 t mazut in Sofia Wastewater Treatment Plant (WWTP) "Kubratovo" on structural and functional parameters of activated sludge was investigated in this paper. Sludge volume index and filamentous index were analyzed in parallel with microscopic examination of activated sludge (AS) with a goal to ascertain eventual deformations in its structure. The metabolic activity of the biological system was investigated by measurement of the total aerobic dehydrogenase activity. The samples were taken from the aeration zone in biobasin.

The obtained data show that the activated sludge structure was deformed. A bulking activated sludge was ascertained because the sludge volume index was higher than 150 ml/g. The filamentous index was over 1.10^7µm/mg, which was an indicator for a filamentous bulking. The used correlation analysis and the obtained correlation coefficients among these two indexes and the key chemical indicators (BOD_5/COD and hydrocarbon index of petroleum products) showed that the structural deformations are due to the concentration of toxic pollutant and in less degree to the lower percentage of biodegradable organics. The initial effect of mazut on activated sludge structure was also related with disappearance of rotifers and nematodes. The total density of organisms from micro- and metafauna was $2,86.10^6$ ind./l on the 6^{th} day after mazut inflow and it increased to $8,77.10^6$ ind./l during the next studied period (from the 12^{th} to the 25^{th} day after the shock load with mazut). Also, appearance of rotifers and nematodes during this period clearly showed the improvement of the conditions in the aeration tank and decrease of negative effect on the activated sludge. The aerobic dehydrogenase activity was two times lower on the 6^{th} day after shock load with mazut ($0,75.10^{-4}$ µMH^+/min.mgPr) in comparison to data before risk event (control situation: $1,34.10^{-4}$ µMH^+/min.mgPr) which was an indicator for inhibition of activated sludge on a functional level. Although the registered deformations, a full recovery of aerobic dehydrogenase activity ($2,16.10^{-4}$ µMH^+/min.mgPr) was established during the period from the 12^{th} to the 25^{th} day after shock load with mazut.

Key words: activated sludge, biological wastewater treatment, mazut, structural and functional deformations

INTRODUCTION

The accidental discharge of toxic pollutants in the urban sewage and its inflow in the municipal wastewater treatment plants is one of the critical points in the technological cycle of water, which can lead to inhibition of activated sludge (AS), unsatisfactory treatment and pollution of the receiving water bodies [14]. One of the risk pollutants which still has a high probability of entry in natural water bodies as well as in urban sewage and in municipal wastewater treatment plants is the mazut. It is a mixture of different heavy hydrocarbons as asphaltenes, petroleum resins, paraffins, unsaturated, cyclic and polycyclic hydrocarbons, etc. [10]. Besides the hydrocarbon component of the mazut, it contains various other elements and compounds such as sulfides and heavy metals. Main locations where it is stored and used are large enterprises from heavy industry, oil bases and thermal power plants (TPPs). Examples of risk situations, in addition to large oil spills in the Gulf of Mexico and other marine water areas [18] were: pollution of Vaya Lake with petroleum products on March 15, 2011 [19]; the inflow more than 40 t mazut in WWTP "Balchik" on March 05, 2011 due to leakage of fuel oil from "Papas Olio" JSC [20], as well as the inflow of 30 t mazut in WWTP „Kubratovo" on November 05, 2014 as a result from accident in TPP "Sofia-Iztok" [21]. It is important to determine what are the consequences of such risky environmental disasters.

The effect of accidental inflow of xenobiotics (isopropyl benzene, chlorophenols, nitrophenols) in biobasins was investigated on biological wastewater treatment under model conditions. It was related to change of activated sludge structure and functioning due to accumulation of the toxic pollutants in biomass and increase of its inhibitory effect [8, 15, 16, 17]. The changes in the structure of AS were most often associated with: decrease of flocs size and their disintegration; increase of quantity of suspended microbial cells in water; increase of filamentous microorganisms; loss of biodiversity of microorganisms, protozoan and metazoan organisms, which led to settleability problems in secondary clarifiers [11, 17]. At shock load of AS with pentachlorophenol was ascertained that the quantity of flagellates and free-swimming ciliates was increased in comparison to the reduction of other groups of protozoan and metazoan organisms [17]. At shock load with isopropyl benzene (70 mg/l) Topalova et al. reported that *Paramecium aurelia*, *Vorticella microstoma* and *Euglipha laevis* showed highest resistance, while for the total number of individuals was ascertained reduction with 75% on the 24th hour and with 81% on the 144th hour after the shock load [16]. In the same time, the rotifers and stalked ciliates completely disappeared. In other study, the effect of 2,4,5-trichlorophenol and 3,4-dichlorophenol in a concentration of 20 mg/l was investigated and three times reduction of the total quantity of protozoan and metazoan organisms was registered. The changes in activated sludge on a functional level were related to: initial decrease of enzyme activities included in biodegradation of conventional and toxic pollutants. The central metabolic pathways and the synthesis of ATP were inhibited in the presence of xenobiotics [14]. The biodegradation effectiveness of trivial and of toxic pollutants was reduced. The increase of the detoxification activity was reported after initial adaptation, which was related to change in microbial community and predominant groups of microorganisms (increasing the bacteria from g. *Pseudomonas* and g. *Acinetobacter*) [17].

Repeatedly registered cases of risky entering the mazut in municipal wastewater treatment plants required to determine what is the effect of this pollutant on the structure and on the activity of the AS, what time is required to restore the activity of the AS and to what extent it is recovered. The aim of this article is to investigate the effect of inflow of 30 t mazut in Sofia Wastewater Treatment Plant "Kubratovo" on the structural and functional parameters of activated sludge after occurrence of the risky event.

MATERIALS AND METHODS

The analyzed samples in the present study were taken from the nitrification zone in the biobasins of WWTP "Kubratovo". That plant treats domestic wastewater from Sofia City as well as industrial wastewater from certain enterprises with which the station has a contract. Three control points (CPs) in time were studied and they were related to three main situations in the wastewater treatment practice. The first control point (CP1) serves as a control in the study, providing information about the characteristics of the activated sludge during normal operation of the WWTP. The sampling was done before the emergence of mazut in WWTP (March 28, 2014). The second control point (CP2) provides information for the effect of shock loading of the activated sludge with fuel oil, as sampling was carried out on the 6th day after the risk event, which took place on November 05, 2014. The third control point (CP3) includes information for the period between the 12th and the 25th day after the mazut inflow in WWTP (from November 17, 2014 to November 30, 2014) giving information about the long-term effect of mazut on AS and about the restoration of the sludge after the occurrence of the risky event.

The article includes data for the analyzed chemical, technological and enzymological indicators. The data for the chemical indicators, such as the hydrocarbon index (HCI), biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) was kindly provided by the Laboratory Testing Complex "Sofiyska voda" JSC. The hydrocarbon index was used as an indicator of the concentration of oil products, including fuel oil. It was examined by gas chromatography. BOD_5 and COD were determined of a mixed sample from the aeration tank (including AS and water) according to standard methods [1]. The indicator BOD_5/COD was used in the article, which provides information about the share of biodegradable organic matter to the total quantity of organics, measured as COD.

Technological indicators such as sludge volume index (SVI), filamentous index (FI) and sludge biotic index (SBI) were analyzed. The technological indicators were used to assess the long-term effect of mazut on the structure and biodiversity of the sludge, and the occurrence of possible deformations. Gravimetric method for the determination of the settleability of activated sludge was used for determination of SVI [2]. Filamentous index was analyzed according to the method described by Sezgin et al. [13]. It included microscopic examination of the total length of filaments in activated sludge to biomass quantity measured as mixed liquor suspended solids (MLSS). MLSS in activated sludge samples for calculation of SVI and FI were analyzed according to APHA [1]. Sludge biotic index was analyzed by microscopic method based on counting the total quantity individuals in unit volume, total number taxa, total number of small flagellates and determination of dominating groups of protozoan organisms in activated sludge [9]. The quantity of protozoan (naked and testate amoebae, flagellates, free-swimming ciliates, creeping ciliates, stalked ciliates, suctorians) and metazoan (rotifers and nematodes) fauna was analyzed by microscopic examination [4].

The enzymological indicator, total aerobic dehydrogenase activity (DHA) provides information about the total metabolic activity of AS. It was measured according to the spectrophotometric method described by Gabbita and Huang by the use of 2,3,5-triphenyltetrazolium chloride [5]. DHA was presented as μMol/min.mg protein and protein concentration in the cell extracts was measured according to the method described by Kochetov [7].

The correlation analysis was used to determine the relationship between the deformation of the activated sludge structure and the concentration of key pollutants in the aeration tanks of the WWTP. The correlation coefficient of Pearson (r) was studied for this purpose. The value for the significance level (P) was determined to be equal to or less than 0.05. The coefficient of determination (r^2) was interpreted as the proportion of the variance in the dependent variable that is predictable from the independent variable.

RESULTS AND DISCUSSION

Structural deformations of the activated sludge and reasons:

The data for technological indicators are shown on Fig. 1. The sludge volume index for normal functioning sludge ranges between 70 and 120 ml/g [2]. SVI was over 120 ml/g at the three investigated control points (Fig. 1a), which is typical for bulking activated sludge. In order to determine whether the bulking is due to the development of filamentous microorganisms or of increased excretion of exopolysaccharides was investigated and the filamentous index of the activated sludge. In CP1 and CP3 was ascertained filamentous bulking of the activated sludge because FI was over 1.10^7 μm/mg (Fig. 1b). Data for CP2 is not available, but the photographs from microscopic analysis confirmed the presence of filamentous bulking (Fig. 2b). An interesting fact was that in the control situation (during normal operation of the station) SVI was higher than in CP2 and CP3 (after entering the mazut in WWTP). Causer of filamentous bulking was *Microthrix parvicella* as we found in our previous study [12]. This information, together with comparison of our data about the technological parameters of the process, led to the assumption that the bulking in the control situation was primarily related to the low concentration of biodegradable organics in influent (BOD_5 below 200 mgO_2/l) and to the low ratio C:N [12].

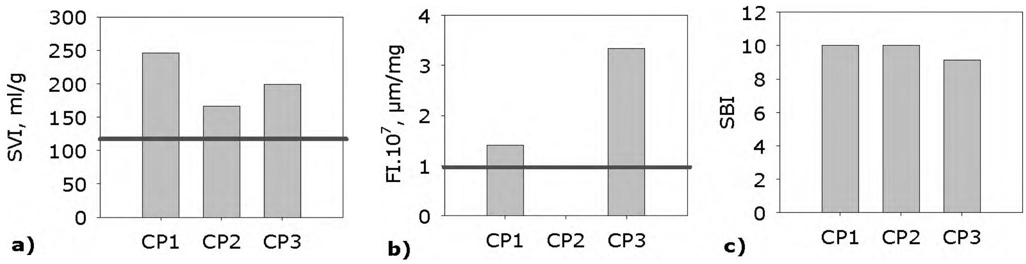

Fig. 1. Data for: a) Sludge volume index (SVI); b) Filamentous index (FI); c) Sludge biotic index (SBI) in three control points: CP1 – control situation (before accidental inflow of mazut); CP2 – at initial shock load with mazut (on the 6^{th} day after it accidental inflow); CP3 – during prolonged rehabilitation of activated sludge (between the 12^{th} and the 25^{th} day after shock load with mazut). The excess of threshold values (marked with red line) are indicator for presence of deformations in AS structure.

The bulking in CP2 and CP3 was caused by the presence of fuel oil, which was absorbed in flocs of AS (Fig. 2b and Fig. 2c). The comparison of the microscopic photographs showed that changes in the structure of the AS in the aeration zone occurred after the mazut entering. They were associated with decrease of the size of flocs, disintegrating the flocs and the development of filamentous microorganisms (Fig. 2b). Fig. 1c presents information about the sludge biotic index, which ranges between 0 and 10. The higher value of the index is an indicator for high enough biodiversity of protozoan organisms and for the significant quantities of creeping and stalked ciliates (indicators for good state of the activated sludge and more effective purification process). It was found that during normal operation of the station SBI was 10, which was an indicator for normally functioning AS with well presented biodiversity. The same result (SBI=10) was found for CP2 (on the 6^{th} days after the mazut inflowing). Probably this xenobiotic in the aeration tank was in high concentration, but not in a concentration which can lead to a sharp death of protozoan organisms that are more resistant to toxic pollutants in comparison to the higher trophic levels (rotifers and nematodes). The adaptive response of AS was shifted in time and SBI decreased to 9.14 at the CP3. This low deterioration of the AS structure during CP3 was related to the increased number of small flagellates. Madoni reported that its massive presence in a mature activated sludge is associated to bad performance of the biological purification and some of the possible reasons for that are: a shock load with toxic pollutants, low concentration of dissolved oxygen in aeration tank, overloading, and higher concentration of fermentation products [9]. In a normally-functioning activated sludge these protozoan organisms compete for bacteria with the free-swimming and stalked ciliates and they are strongly affected by predaceous activity of other protozoa. That`s why its presence in the normally-functioning AS is limited to few individuals (below 10 individuals counted along the diagonal in the Fuchs-Rosenthal chamber). In the case of the operation problems of the plant, their number can increase (over 10 counted individuals) and consequently, this indicates a decrease in the plant performance [9]. In our research, the counted small flagellates varied between 10 and 25 individuals during CP3. Also, the number of *Vorticella microstoma* was increased at CP3. This species indices presence of toxic pollutants and low concentration of dissolved oxygen [9]. The reason for decreased SBI during CP3, as can be seen from Fig. 2, was that the mazut concentration in the aeration tank was limited (by the applied physical methods to remove fuel oil in mechanical pretreatment), but still part of it going into bioreactors. It was accumulated in and on the biomass of the AS and the concentration in the flocs increased gradually, resulting in a later establishment of the negative effect on the SBI at the CP3. The residual concentration of mazut led to disturbance in mass balance and limited access to dissolved oxygen. However, the difference between SBI values for CP2 and CP3 was not significant, which could be related to the long restoration period of the AS.

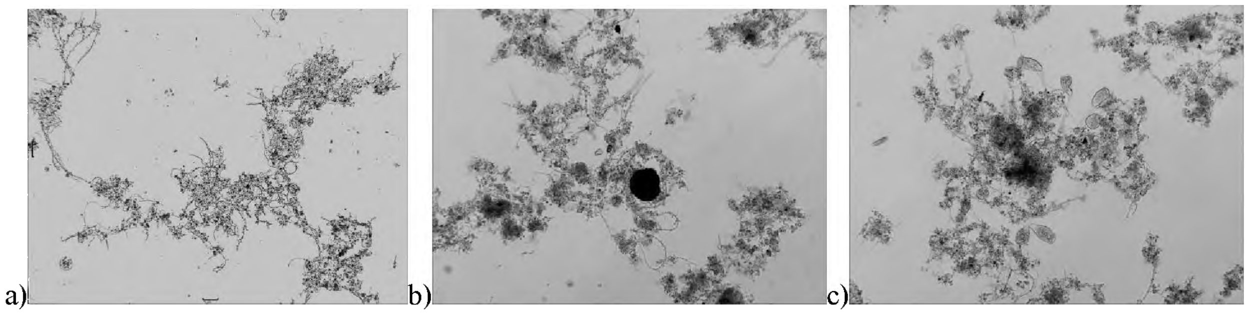

Fig. 2. Microscopic photographs (Light Microscope at magnification 100X) of activated sludge from: a) CP1 – control situation (before accidental inflow of mazut); b) CP2 – at initial shock load with mazut (on the 6^{th} day after it accidental inflow); CP3 – during prolonged rehabilitation of activated sludge (between the 12^{th} and the 25^{th} day after shock load with mazut)

Data for the density of microfauna and metafauna in the AS for CP2 and CP3 are presented on Fig. 3. The density of protozoan organisms was $2,86.10^6$ ind./l at CP2 (on the 6^{th} day after the inflow of fuel oil) and $8,73.10^6$ ind./l at the CP3 (Fig. 3a). An increased quantity of all groups of organisms of microfauna was registered. The increased amount of organisms during CP3 and especially of creeping and stalked ciliates (indicators of well-functioning AS) was a proof for the started process of restoration of activated sludge structure. At the shock loading with fuel oil (CP2) was ascertained that rotifers and nematodes completely lack (Fig. 3b), which was an indicator for deteriorating conditions in the aeration tank and of the negative effect of toxic pollutants on these two groups of organisms. Similar results for an inhibitory effect of the toxic pollutant (isopropyl benzene) on rotifers in AS were reported in other research [16]. The total density of organisms of microfauna and metafauna from $2,86.10^6$ ind./l at the CP2 was increased to $8,77.10^6$ ind./l at the CP3. That and the appearance of rotifers and nematodes during CP3 showed an improvement of the conditions in the aeration tank and restoration of biodiversity of the activated sludge after the first twelve days from mazut inflowing.

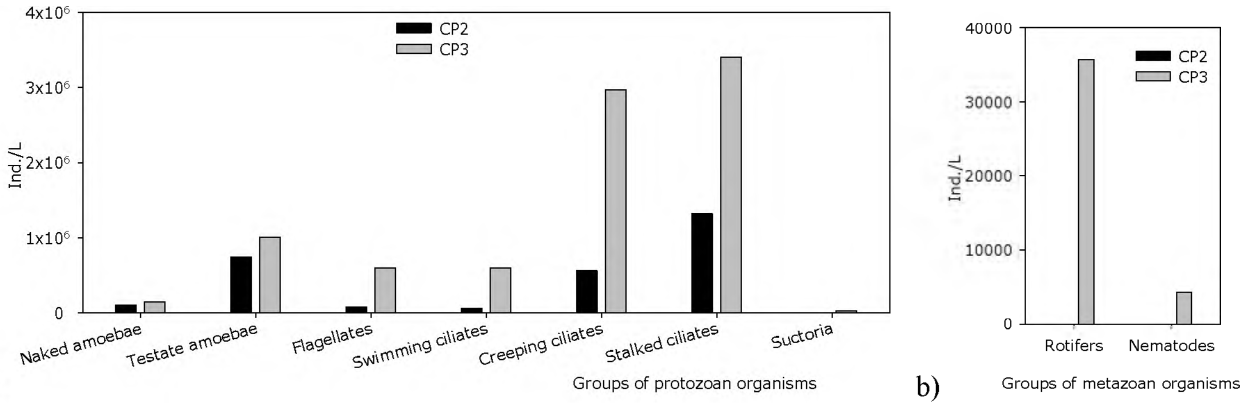

Fig. 3. Dynamics of different groups organisms from microfauna (a) and metafauna (b) in activated sludge at: a) CP2 – at initial shock load with mazut (on the 6th day after it accidental inflow); b) CP3 – during prolonged rehabilitation of activated sludge (between the 12th and the 25th day after shock load with mazut)

Data for the restructuring of microfauna and metafauna in the AS for CP2 and CP3 are presented on Fig.4. Predominant groups of organisms were these of stalked ciliates (46.15%), testate amoebae (25.87%) and creeping ciliates (19.58%). Each of the remaining groups holds less than 4%. Changes in percentage shares of different groups of organisms were ascertained during CP3 (Fig. 4b). The percentage of stalked ciliates decreased at the expense of increasing the creeping ciliates with 13% and the free-swimming ciliates with 4.5%, which was probably related to the initial disintegration of the flocs and more difficult survival of the attached forms. The increased percentage of the creeping ciliates was probably related to increasing the free area of the flocs (more flocs with smaller size), which provided more food substrate in the form of easily detachable particles from flocs. The proportion of flagellates, which are more resistant to toxic pollutants, was also increased by 4%. The decreased amount of amoebae, especially testate amoebae (14%) indicated a reduction of the toxic effect on biomass [15] and start-up of the restoration process of floccular structure of the AS, due to decrease of concentration of suspended in water particulate matter [6]. Despite the changes in percentage shares it can be said that the dominant groups remained stalked and creeping ciliates, and testate amoebae. The availability of rotifers and nematodes (less than 1%) at the CP3 clearly showed the improvement of the conditions in the aeration tank and structure of the AS, although slow restoration was ascertained. Moreover, the total share of creeping and stalked ciliates (indicators of well-functioning AS) was increased by 7% at CP3 (72.66%) in comparison to CP2 (65.73%).

Table 1 shows the correlation coefficients among SVI, HCI, BOD_5/COD and filamentous index in the aeration tank. More significant correlations are marked with gray in the table. A relatively high linear correlation between SVI and HCI (r = 0,698) was ascertained and the dependence was proportional, which indicated that with an increase of HCI was increased and the SVI. The increasing the concentration of xenobiotic led to increase of the deformations in the AS structure. The coefficient of determination showed that 49% of change of HCI led to a change in the sludge volume index. Statistically this percentage was not very high suggesting that on the activated sludge bulking and on the settleability influenced not only the xenobiotics, but also a complex of other factors. The study of the correlation coefficient between SVI and BOD_5/COD showed inverse relationship between both indicators. A decrease in the biodegradable organic matter led to increase in structural deformations that may be due to competition between the filamentous and floc-forming microorganisms for food substrates. In general, however, the coefficient of determination was low (only about 7% of changes of BOD_5/COD led to a change of SVI). The deformation in the AS structure defined by SVI depended to a greater extent on the mazut concentration in comparison to the concentration of biodegradable organics. This is logical bearing in mind that the xenobiotic pollutant has a toxic effect on the organisms.

Pearson`s correlation coefficient between HCI and FI was relatively low, as the relationship between indicators was proportional. About 5% of the change in the HCI led to a change in the quantity of filamentous microorganisms.

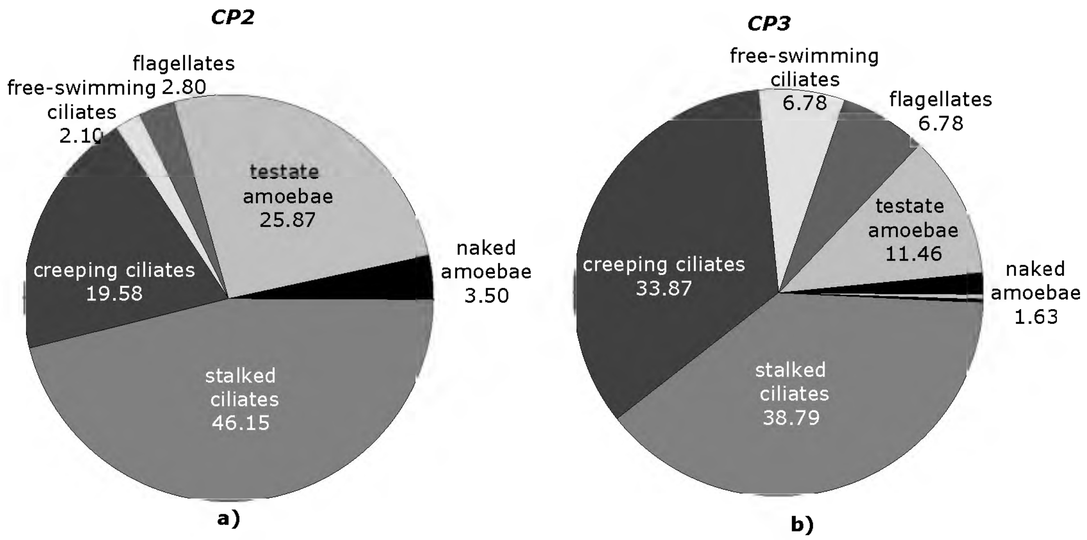

Fig. 4. Different groups organisms from micro- and metafauna (in %) in AS at: a) CP2 – at initial shock load with mazut (on the 6th day after it accidental inflow); b) CP3 – during prolonged rehabilitation of activated sludge (between the 12th and the 25th day after shock load with mazut)

Table 1. Coefficients of correlation and determination between key parameters

Coefficients of:	**SVI and HCI**	**SVI and BOD_5/COD**	**FI and HCI**	**FI and BOD_5/COD**	**SVI and FI**
correlation (r)	0.698	-0.264	0.232	-0.543	0.459
determination (r^2)	0.487	0.069	0.054	0.295	0.210

The lack of a statistically significant correlation between both indexes may be due to the fact that the filamentous microorganisms are slow growing and changing the values of the filament index was ascertained at a later point of time until the values of HCI changed dynamically over time. The correlation coefficient between BOD_5/COD and filamentous index showed an inverse relationship between indicators. The decrease of the biodegradable organic matter led to an increase in the growth of microorganisms with filamentous growth. The coefficient of determination showed that about 30% of changes of BOD_5/COD led to change of the filament index.

The correlation coefficient between SVI and FI was relatively high (r= 0,459) and relationship was proportional. It was found that about 21% of the increase of the amount of filamentous microorganisms led to difficulty in the settleability of the AS.

The occurred correlation analysis showed that the most significant in the aeration tank were relationships between HCI and SVI, and between FI and BOD_5/COD. The decrease in the biodegradable organics in the tank led to an increase in filamentous microorganisms because of their lower half saturation constant [3]. The increases of HCI intensified the deformations in the structure of the biological system and difficult settling was ascertained (rising SVI). Among other examined indicators revealed no significant correlations, suggesting that many different factors affecting the relevant deformation were registered.

Changes of activated sludge on functional level:

Data from enzymological analysis showed that during normal operation of the station (CP1) the total aerobic dehydrogenase activity was $1,34.10^{-4}$ $\mu MH^{+}/min.mgPr$ (Fig. 5). On the 6th day after penetration of the mazut DHA decreased twice (CP2), which was indication for inhibition of the synthesis of enzymes and their activity. Data show that in addition to negative interference on the biodegradation of toxic pollutants were inhibited central metabolic pathways taking place with the participation of oxido-reductases, and in particular of dehydrogenases.

After a two-week period was ascertained that AS restored its activity (during CP3) and reached an average value of about $2{,}16.10^{-4}$ μMH^{+}/min.mgPr. This value was about 1.5 times higher than the control situation. AS except that restored its metabolic potential, showed an increased activity. The used functional indicator (DHA) responded much faster to changes in the conditions of the aeration tank and showed reactivation of AS during the restoration period.

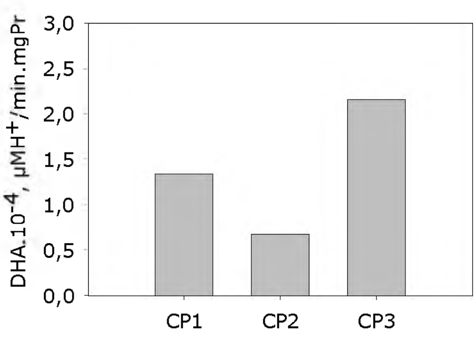

Fig. 5. Aerobic dehydrogenase activity (DHA) in three control points: CP1 – control situation (before accidental inflow of mazut); CP2 – at initial shock load with mazut (on the 6^{th} day after it accidental inflow); CP3 – during prolonged rehabilitation of activated sludge (between the 12^{th} and the 25^{th} day after shock load with mazut)

CONCLUSIONS

The discharge of 30 t mazut in municipal sewage and its influx in WWTP "Kubratovo" led to structural and functional deformation of activated sludge. The deformations on structural level were related to: initial disintegration of flocs and decrease of its size; difficulty in settleability of AS (SVI over 150 ml/g) due to increase of filamentous microorganisms (FI over 1.10^{7} μm/mg); disappearance of more sensitive organisms of higher trophic levels (rotifers and nematodes) and an increased share of testate amoebae. These changes were determined by the accumulation of fuel oil in and on flocs of AS as the adaptation was directed towards survival of more resistant organisms (protozoan organisms and filamentous microorganisms that are resistant to low concentration of biodegradable organics and to the presence of toxic pollutants). Deteriorating settling was determined by the mazut concentration and the development of filamentous microorganisms from low BOD_5/COD. The deformation of AS on the functional level was expressed in double decrease of total aerobic dehydrogenase activity on the 6^{th} day after mazut influx. However, full recovery of enzyme activity was registered after a three-week period from the occurrence of the risk situation. Investigated parameters (SVI, FI, SBI, qualitative and quantitative characteristics of micro- and metafauna, DHA) can be used as quick and adequate indicators for an evaluation and control of biological systems and processes during the analyzed risk event in municipal wastewater treatment plant.

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ЕФЕКТ НА МАЗУТА ВЪРХУ СТРУКТУРНИТЕ И ФУНКЦИОНАЛНИ ПОКАЗАТЕЛИ НА АКТИВНАТА УТАЙКА В СПСОВ „КУБРАТОВО”

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Резюме: Настоящето изследване проследява ефектът от навлизането на 30 тона мазут в СПСОВ „Кубратово” върху структурните и функционални характеристики на активната утайка (АУ). Показателите обемен индекс на утайката и филаментен индекс бяха анализирани паралелно с микроскопския контрол с цел да се установи има ли деформации в структурата на АУ. Сумарната аеробна дехидрогеназна активност бе изследвана, за да се определи дали метаболитната активност на утайката е инхибирана. Пробите бяха взети от аеробната зона на биобасейна.
Получените данни показват, че структурата на АУ е деформирана, а деформацията е от типа на разбухналата утайка, т.к. обемният индекс е над 150 ml/g. От данните за филаментния индекс (над 1.10^7 µm/mg) се установява, че е налице филаментозно разбухване. Проведеният корелационнен анализ и корелационните коефициенти между двата индекса и между ключовите химични показатели (БПК$_5$/ХПК и въглеводорден индекс за нефтопродукти) показват, че деформациите в структурата на биологичната система се определят по-значимо от концентрацията на токсичния замърсител и в по-ниска степен от по-ниския процентен дял на биоразградимата органика. Първоначалният ефект на мазута върху структурата на АУ е свързан с изчезване на ротиферите и нематодите. Общата численост на организмите от микро- и метафауната беше $2,86.10^6$ ind./l на шестият ден след навлизането на мазута и се повиши до $8,77.10^6$ ind./l през следващия изследван период (от 12 до 25 ден след появата на рисковото събитие). Освен това през този период бе отчетена и появата на ротифери и нематоди, което ясно показа подобряване на условията в биобасейна и снижаване на негативния ефект върху АУ. Данните за сумарната дехидрогеназна активност показват, че на шестият ден след навлизането на мазута, активната утайка е инхибирана и активността ѝ ($0,75.10^{-4}$ µMH$^+$/min.mgPr) се снижава два пъти спрямо данните преди настъпването на рисковата ситуация ($1,34.10^{-4}$ µMH$^+$/min.mgPr). Въпреки установените деформации през изследвания период между 12 и 25 ден от настъпване на рисковата ситуация се установява пълно възстановяване на активността на сумарната аеробна дехидрогеназа ($2,16.10^{-4}$ µMH$^+$/min.mgPr).

Ключови думи: активна утайка, биологично водопречистване, мазут, структурни и функционални деформации

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