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Adaptation of activated sludge to treatment of landfill leachate during model process

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Landfill leachate is generated from the waste degradation in landfill sites and rainwater infiltrates. Its treatment includes more often biological methods combined with physical and chemical methods. The availability of polycyclic aromatic carbohydrates, phenols, polychlorinated phenols, pesticides, heavy metals, and refractory organics in landfill leachate remains a critical technological problem during biological treatment. The effect of these toxic pollutants on activated sludge (AS) processes is related to deformations of AS structure /bulking or pin-point flocs/ and inhibition of biodegradation activity. One of the most economic and effective possibilities for problem solving is application of adaptation as a biological approach. The aim of the study is to assess the activated sludge from wastewater treatment plant to Municipal enterprise for waste treatment of Sofia City during model adaptation process with landfill leachate. The duration of adaptation process was 21 days. The results confirm that the leachate diluted 25X contains toxic xenobiotics, which concentration is close to the critical one for the development of adaptive potential of AS in the concrete experimental conditions. This dilution of the leachate and the ratio COD:BOD_5 are appropriate for accomplishment of wastewater treatment process.

Key words: landfill leachate; activated sludge; adaptation; biodegradation activity

INTRODUCTION

The enacted Directive 1999/31/EC aims a decrease in the consumption of natural resources and raw materials and a bigger decrease of the quantity of landfilled waste as in 2018 in Europe were generated 2317 million tons of waste [1]. It has been registered that for a year one landfill for non-hazardous waste in the early to the middle phase of its exploitation generates between 10000 and 20000 m^3 of leachate [2]. Its quantitative and qualitative composition is defined also by the quantity of the rainfalls and the snowmelt as well as by the type of the landfilled waste. The leachate from the landfills for non-hazardous waste might contain high concentrations of xenobiotics such as aromatic hydrocarbons, pesticides and heavy metals such as cadmium, copper, chrome, lead, nickel, zinc [2, 3]. In Table 1 are represented some of the pollutants in the leachate as well as their concentrations.

In these cases, the leachate is characterized with a high toxicity. Different approaches for treatment of the leachate from non-hazardous waste landfills exist. Main significance for the choice of the approach to be implemented have the parameters BOD_5, COD, nitrogen and halogen organic compounds. Often the equipment for the leachate treatment is designed in a combination to the conventional treatment systems [4]. For example, the landfill for non-hazardous waste of Sofia City, part of Municipal enterprise for waste treatment, on the "Sadinata" site works on the principle of a biological co-treatment of domestic wastewater and leachate.

The biological treatment of a leachate with xenobiotics has environmental risks that are related to the ineffective removal of the toxic compounds [5, 6]. They are related to a deformation of the structure of the activated sludge (AS) and an inhibition of the biodegrading and biodetoxifying processes [7]. The most common deformations in the AS structure are related to the formation of pin-point flocs or to a filamentous or non-filamentous bulking [8-10]. It is registered that the presence of xenobiotics leads to a restructuring of the communities of the microorganisms and the fauna in the AS [9, 10]. For example, in the bacterial complex start to dominate the representatives of the genera *Pseudomonas* and *Acinetobacter* [10-12], and in the complexes of the micro- and metafauna is registered a reduction of the attached and crawling ciliates and an increase of the part of the flagellates and the free-swimming ciliates [9, 10,

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13]. On a functional level the AS reacts with an increase of the activities of key for the biodegradation enzymes such as the catechol-1,2-dioxygenase, catechol-2,3-dioxygenase, protocatechuate-3,4-dioxygenase and others [10].

Table 1. Concentrations of some pollutants in a landfill leachate [2,3].

Indicator	Concentration
BOD_5 /biochemical oxygen demand/	2000-68000 mgO_2/L
COD /chemical oxygen demand/	2700-152000 mgO_2/L
TN /total nitrogen/	225 mg/L
TP /total phosphorus/	30 mg/L
Ca	270 6240 mg/L
Mg	1.4-164 mg/L
Na	474-2400 mg/L
K	350-3100 mg/L
Fe	48-2300 mg/L
Cl^-	659-4670 mg/L
SO_4^{2-}	5-1560 mg/L
Ni	0.03-1.87 mg/L
Cu	0.02-1.10 mg/L
Zn	0.09-140 mg/L
Cd	0.01-0.1 mg/L
Pb	0.04-0.65 mg/L
As	0.001-0.148 mg/L
Hg	0.0001-0.0015 mg/L
Polychlorinated biphenyls (PCB)	below 0.0007 mg/L
Dioxins	below 0.32 mg/L
Benzene	0.2-1630 μg/L
Toluene	1-12300 μg/L
Xylene	0.8-3500 μg/L
Ethylbenzene	0.2-2329 μg/L
Naphthalene	0.1-260 μg/L
Chlorobenzene	0.1-110 μg/L
1,1,1-trichloroethane	0.1-3810 μg/L
Trichloroethylene	0.5-750 μg/L
Phenol	0.6-1200 μg/L
Cresols	1-2100 μg/L

The activated sludge possesses a huge potential for biodegradation of pollutants based on a complex microbial consortium [14, 15]. The mechanism changing the structure and the function of the biological system is called an adaptation and the flexibility of the biological system depends on it [16]. That is why the adaptation of the AS is one of the possible solutions in the presence of biological treatment in the landfills for non-hazardous waste. One of the main factors for an effective adaptation of the microorganisms in the AS is the concentration of the xenobiotics in the leachate [17-19]. These concentrations should be optimal for the sustainment of the enzyme activities of the bacterial consortium in the activated sludge [20, 21]. Reaching or exceeding the critical concentration for each xenobiotic and for the specific biological system would lead to a switching from a biodegradation to a resistance and inhibition [22-26]. To be established and be derived the rules how the AS to be adapted in an industrial scale it is necessary to be conducted model lab studies which have to be subsequently verified [27-29].

The aim of the article is to assess if the activated sludge from WWTP "Sadinata" could be adapted step-by-step to the pollutants in the leachate from the landfill for non-hazardous waste "Sadinata" in Yana village. The assessment of the activated sludge is made on the basis of the changes in its structure. The changes of the technological and microbiological parameters are analyzed in the simulated adaptation process with a step-by-step increase of the concentration of the pollutants in the leachate. The results are important regarding the solution of the real problems in the plants treating leachate from non-hazardous waste landfills through the implementation of adapting algorithms.

MATERIALS AND METHODS

Experimental design

The assessment of the AS adaption to a leachate has conducted in a model process. During the experiment it has been constructed a model bioreactor – type "aerobic biobasin", presented on Fig. 1. The choice of an aerobic bioreactor has been made because the aerobic level is really important in the presence of highly toxic xenobiotics with an aromatic structure. The reactor was sequencing batch reactor (SBR) with five phases: 1/ fill phase, 2/ react phase, 3/ settle phase, 4/ decant phase and 5/ idle phase. The biological wastewater treatment was accomplished during the second phase, under aerobic conditions. The duration of the aerobic phase was between 46 and 70 hours. After aeration, the SBR enter the settle phase, where for a period from 2 hours under quiescent conditions (without aeration and mixing) activated sludge settles in the reactor. The biological system in the experiment has been the activated sludge from WWTP "Sadinata" to which periodically has been added a leachate from the non-hazardous waste landfill, part of Municipal enterprise for waste treatment of Sofia City. The activated sludge was taken from sequencing batch reactor which operated under aerobic conditions. The quantity of the activated sludge was 3.39 g/L at the beginning of the experiment. The treatment process has started with a diluted quantity of leachate (50X) as the concentration of pollutants in the leachate has increased each week and at the same time has been added co-substrate glycerol with a concentration 0.79 g/L. The glycerol has been used as a source of easily assimilating organic compound. This compound was used in the real WWTP "Sadinata" for increase of concentration of biodegradable organic matter (measured as BOD_5). The ratio $COD:BOD_5$ in WWTP "Sadinata" and in our experiment was 3:1. The experiment has continued for three weeks or twenty one days.

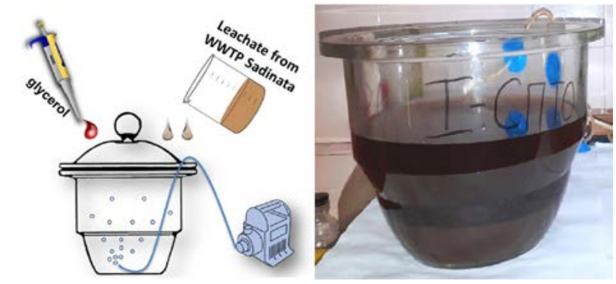

Fig. 1. Model aerobic bioreactor for simulation of adaptation processes with activated sludge to complex pollutants in landfill leachate.

The aim is to be achieved a step-by-step adaptation of the activated sludge towards toxic and hardly degradable pollutants through a step-by-step increase of the leachate concentration as on the 7th day of the start of the experiment has been added a 25X diluted leachate and on the 14th day – an undiluted leachate. In the adaptation of the biological systems, including the activated sludge, for wastewaters treatment of toxic and sustainable of biodegradation xenobiotics firstly, it is important to be defined what the critical concentration is according to the Haldane equation [30-32]. It depends mainly on the chemical structure of the compounds and on the adapting means of the biological system depending on their structure, enzyme potential and other their biological features [16]. The exceeding of the critical concentration would lead to a significant decrease of the growth speed of the microorganisms and their transition to a death phase and thus their biodegrading activity is inhibited. So the treatment process is strongly deformed and cannot be performed [33]. The challenge in the biological treatment of leachate is that in it is contained a complex of pollutants including inorganic and organic xenobiotics that mutually modulate the biodegrading process. In a complex they define the adaptive potential of the activated sludge. In a different manner they influence its components – various types of microorganisms and representatives of the micro- and meta-fauna. Data for the choice of dilution and the definition of the critical concentration of the pollutants complex in the leachate till this moment miss in the scientific literature. It is well known that for the extraction of an algorithm for an adaptation of the activated sludge to the pollutants in the leachate in real conditions it is necessary to be switched consecutively over the modelling processes in lab conditions (lab scale). After the specification of all the elements of the adaptation algorithm in a small lab scale it could be proceeded to a step by step scaling of the processes so to be guaranteed that the extracted dependencies and rules will work effectively in a pilot scale and in a full scale [33]. That is why in the present experiment we started the modelling of the process in a lab scale with a 50X diluted leachate that does not manifest a toxicity for the activated sludge. The studied steps of dilution are comparatively large because in the first stage of the experiment the aim is to be found this dilution that is closest to the critical concentration but does not exceed it. In the log phase of the adaptive potential development this step could be comparatively large. In this phase the microbial adaptive potential is more plastic. In this stage at the same time are turning on the different microbial mechanisms of detoxification – a level of synthesis and activation of the constitutive and induced enzymes and a level of interactions of microorganisms and representatives of the micro- and meta-fauna.

The dilution from 50 to 25 times is comparatively large and it is applied regarding an approach to the critical concentration of the complex of toxic pollutants in the leachate. By the obtained results from the first and second week of the experiment it was registered that we still did not reached the critical concentration and in this composition of the pollutants the adaptive and biodegrading potential function. However, this composition is acceptable enough for the realization of the treatment process in the specific model equipment. In the third stage, it was also applied such a large step of dilution from 25X to an input of undiluted leachate. This concentration of the toxic pollutants exceeded the critical one and a treatment process occurred in unacceptable for the modelling process limits. Thus, the experiment is divided on three stages (Fig. 2) which differ on the

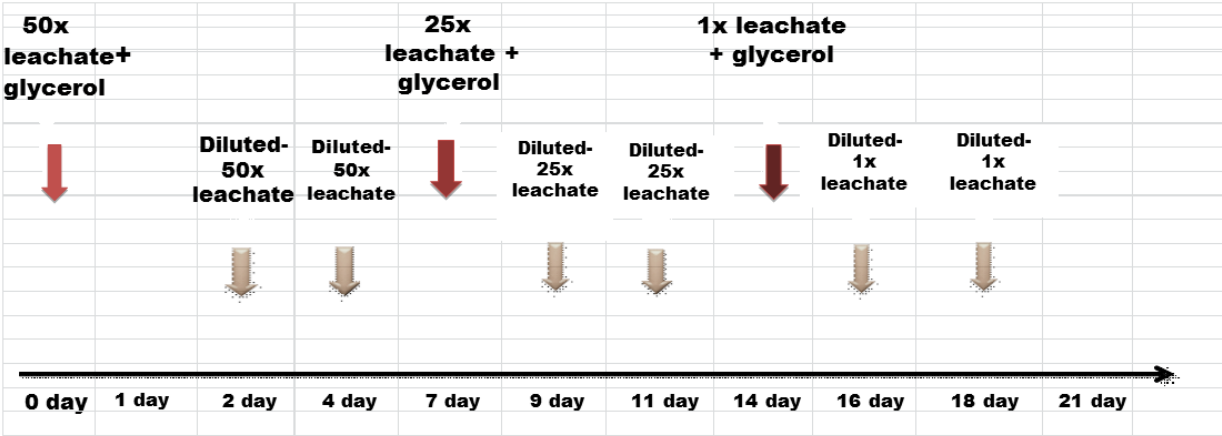

Fig. 2. Duration of process with addition of new leachate.

divided on three stages (Fig. 2) which differ on the incoming concentration of pollutants in leachate and it is expected to have a different effect on the biological system. It is important to note that in each stage of leachate addition the followed actions were accomplished in SBR: addition of influent and filling the reactor, aeration and biodegradation processes, stopping the aeration and settling the sludge, decanting the supernatant and filling the bioreactor with new leachate with the appropriate dilution for the next stage.

The first stage of the experiment occurs from the zero to the 7^{th} day inclusive. At the beginning of the experiment to the activated sludge is added 50 times diluted leachate and a co-substrate glycerol. The first samples for analysis of technological, hydrochemical and microbiological parameters have been taken at the beginning of the experiment when the leachate has been added to the activated sludge. During the second and the fourth day the leachate has been changed with a fifty times diluted leachate but without the addition of a co-substrate glycerol.

The second stage of the experiment occurs from the seventh to the fourteenth day inclusive. On the 7^{th} day has been added 25 times diluted leachate and a co-substrate glycerol. The samples from the activated sludge on the 7^{th} day have been taken before the addition of the leachate and the glycerol as the analyzed parameters have been: technological, hydrochemical and microbiological. On the ninth and the eleventh day the leachate has been changed with a new one with a dilution of twenty five times without an addition of glycerol.

The third stage of the experiment is from the fourteenth to the twenty first day inclusive. On the fourteenth day have been added an undiluted leachate and a co-substrate glycerol. Samples from the experiment have been taken at the same day before the addition of the leachate and the glycerol as analyzed have been: technological, hydrochemical and microbiological parameters. During the sixteenth and the eighteenth day the leachate has been changed with a new one but undiluted and without an addition of glycerol. Samples from these days have been taken for technological and hydrochemical parameters.

Analyzed parameters and methods

During the adaptation have been analyzed hydrochemical, technological and microbiological parameters. From the technological parameters have been analyzed dry matter and sludge volume index (SVI). The last one is important because it gives information about the presence or the absence of structural deformation of the AS. Sludge volume index (SVI) was analyzed according to BDS EN 14702-1:2006 [34]. SVI presents the volume in mL, which occupies 1 g of sludge after 30 minutes of precipitation. Mixed liquor suspended solids for calculation of SVI was determined by a standardized method [35].

From the hydrochemical parameters has been analyzed the chemical oxygen demand (COD) that indicates about the organic content in the leachate and its assimilation during the process. COD was determined by method with potassium dichromate and sample heating in presence of H_2SO_4 [35].

For a determination of the effectiveness (Eff) of the organic matter decrease (measured by COD) has been used the following formula (Eqn. 1):

Eqn. 1) $$Eff = \frac{Ct_1 - Ct_2}{Ct_1} \cdot 100, \%$$

where Ct_1 is the value of the chemical oxygen demand in the influent and Ct_2 is the value of the chemical oxygen demand in the effluent.

From the microbiological parameters has been analyzed the quantity of the bacteria from the genera *Pseudomonas* and *Acinetobacter*. They are important because they together eliminate carbon, nitrogen and phosphorus and degrade a wide circle of xenobiotics including such with an aromatic structure. The quantity of g. *Pseudomonas* and g. *Acinetobacter* were analyzed by classical cultivation method for determining the number of *Pseudomonas sp*. on Glutamate Starch Pseudomonas Agar and *Acinetobacter sp*. on Sellers Differential Agar. The bacterial quantity was presented as CFU/g and mixed liquor suspended solids was determined by a standardized method [35]. All of the analyses have been repeated at least three times.

RESULTS AND DISCUSSION

Organic biodegradation during the experiment

One of the key hydrochemical parameters for assessment of the water treatment processes regarding the organics are the chemical oxygen demand /COD/ and the biochemical oxygen demand /BOD_5/. Indirectly, by the decrease of these complex parameters, could be obtained information about the level of the organic pollutant's biodegradation. The data for COD during the experiment is presented on Fig. 3. It is registered that the COD values at the beginning of the experiment are high (2154.49 mgO_2/L) but at the end of the first stage (to the 7^{th} day) the organic matter concentration decreases to 108.91 mgO_2/L.

At the end of the first week of the experiment with the 50 times diluted leachate and the added glycerol the activated sludge starts to adapt to the xenobiotics in the leachate. During the second stage also there is an organic biodegradation as the COD is on the average of 439.32 mgO_2/L. On the graph is registered a sharply increase of the COD values in the last days to 3403.305 mgO_2/L that indcates for a hard adaptation to the pollutants in undiluted leachate. The dynamic of the COD values at the end of the experiments indirectly indicates for an inhibition of the biodegrading activity of the AS in the last days of simulated process. As a whole, we could conclude that during the first and the second stage it is registered a high decrease of the organic matter measured as COD but during the third stage of the experiment the adapting opportunities of the sludge are exceeded and the COD rests high.

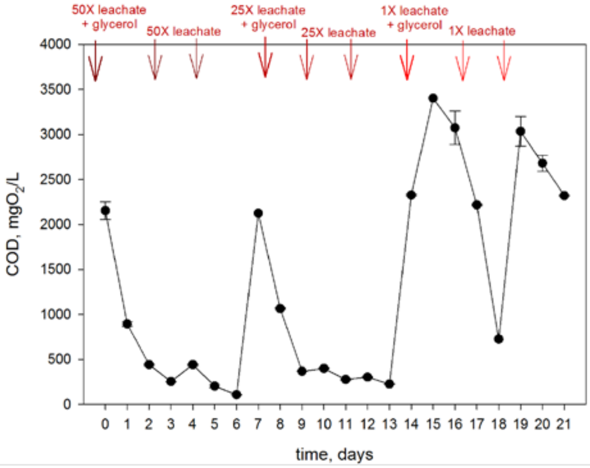

Fig. 3. Dynamics of organic matter concentration, measured as chemical oxygen demand (COD) during the experiment.

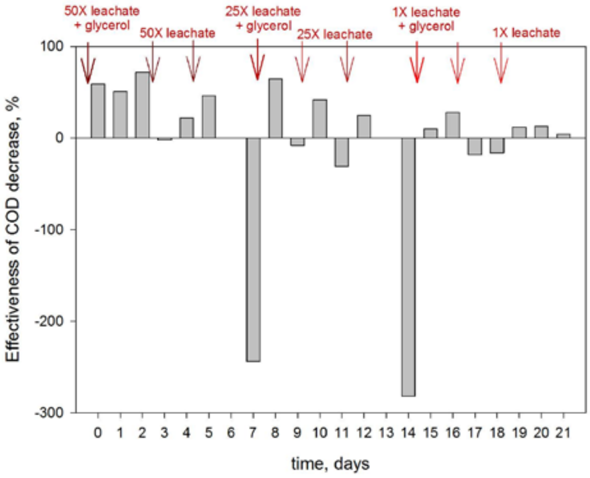

Fig. 4. Effectiveness of organic matter (measured as COD) removal during the process.

During the first stage of the experiment the effectiveness of the organic decrease measured as COD is highest (Fig. 4). The average value of the effectiveness for the first stage is 41% as the most probable reason is the low concentration of the pollutants in the 50X diluted leachate. In the second and the third stage the average values of the effectiveness are – 25% and – 36% namely more and more negative due to the leachate concentrating in these periods and the higher level of AS inhibition. In the 25 X dilution the adaptive potential of the activated sludge is sufficiently developed to be kept the treatment process in this concentration of the pollutants in the leachate. At the same time, the added quantity of glycerol is acceptable for the realization of the treatment of a leachate containing a complex of toxic pollutants. In future experiments, it could be studied the state of the adaptive potential of the activated sludge in smaller steps of dilution regarding an additional approach to the critical concentration for the complex of toxic pollutants. It should be taken under consideration the fact that this complex is not constant in spite of the large level of average in the accumulation tanks in real conditions. This inconstant complex and concentrations are a sufficient argument to conduct a following treatment process in 25X without the risk to approach tightly to the values of the fluctuating critical concentration. In this dilution it is reached the necessary balance between the processes of intoxication/detoxification in the activated sludge and the relatively sustainable realization of the treatment process. This statement is confirmed by the below exhibited and commented parameters of the activated sludge.

Activated sludge assessment during the process

The activated sludge assessment during the adaptation has been made through an analysis of the changes in its structure as analyzed have been the sludge volume index, the macrostructure through a light microscope and the quantity of key taxonomic groups of microorganisms that participate in the xenobiotic biodegradation.

The indicator sludge volume index (SVI) is used for the determination of the settling capability of the sludge. The index, together with the macrostructure of the sludge, are convenient indicators for the assessment of the treating process and for the presence of a deformation in the structure of the activated sludge as well as for the eventual reasons. The high SVI (over 120-150 mL/g) is an indicator for a sludge bulking that could be filamentous or non-filamentous. The low SVI (under 70 mL/g) indicates for another sludge deformation in which predominate the so called pin-point (small) flocs. In this case the sludge is starving. The normally functioning activated sludge with a good settling capability is with an index between 100-120 mL/g [36]. The data about it is presented on Fig. 5. At the starting of the experiment it is registered that the activated sludge from WWTP "Sadinata", part of Municipal enterprise for waste treatment of Sofia City, is characterized with pin-point flocs as the sludge volume index has the value of 29.50 mL/g. In the first days the activated sludge starts to improve its settling capability from 29.50 mL/g on the 1[st] day to 67 mL/g on the 7[th] day. In the next two stages of the experiment when the pollutants concentration increases (from 25 times diluted to undiluted leachate) SVI bit by bit decreases its values. This data is an indicator for a deformation of the activated sludge and for a decrease of the biodegrading activity. The reason for this result is in the concentrated pollutants in the leachate and in the low concentration of the biodegradable organics.

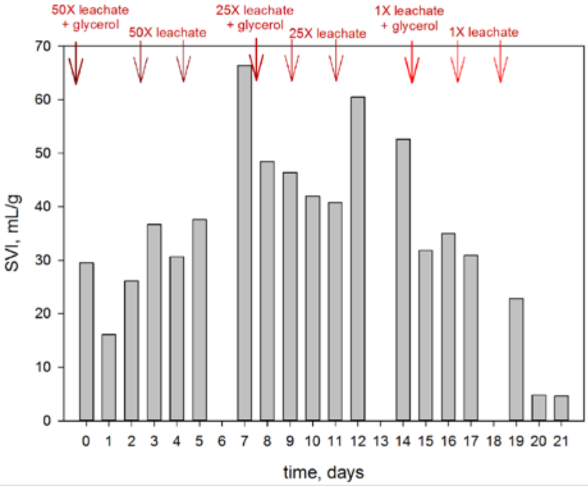

Fig. 5. Dynamics of sludge volume index (SVI) during the process.

By the obtained results we could conclude that the activated sludge is with a deformed structure by the type of the pin-point flocs (SVI under 70 mL/g). The deformation could be caused by the presence of toxic pollutants and/or hardly degrading compounds in the leachate. In the experiments conducted by the team of Center of competence "Clean technologies for sustainable environment – water, waste, energy for circular economy" the research group of prof. Djingova has registered the presence of aromatic amines, mono- and polyvalent alcohols, phenols, aldehydes and ketones.

The results obtained by the microscopic analysis of the samples from the 7[th], 14[th] and 21[st] day (Fig. 6) completely approve the hypotheses expressed for the parameter SVI. On Fig. 6a during the 7[th] day of the conducted experiment the microscopic picture of the activated sludge shows formed pin-point flocs but also it could be seen a presence of a little part of filamentous microorganisms that indicates an attempt for restructuring to a normally functioning activated sludge. The following microscopic images presented on Fig. 6b and Fig. 6c from the 14[th] and 21[st] day show the presence of pin-point flocs with accumulated in them pollutants. In these two phases of the experiment

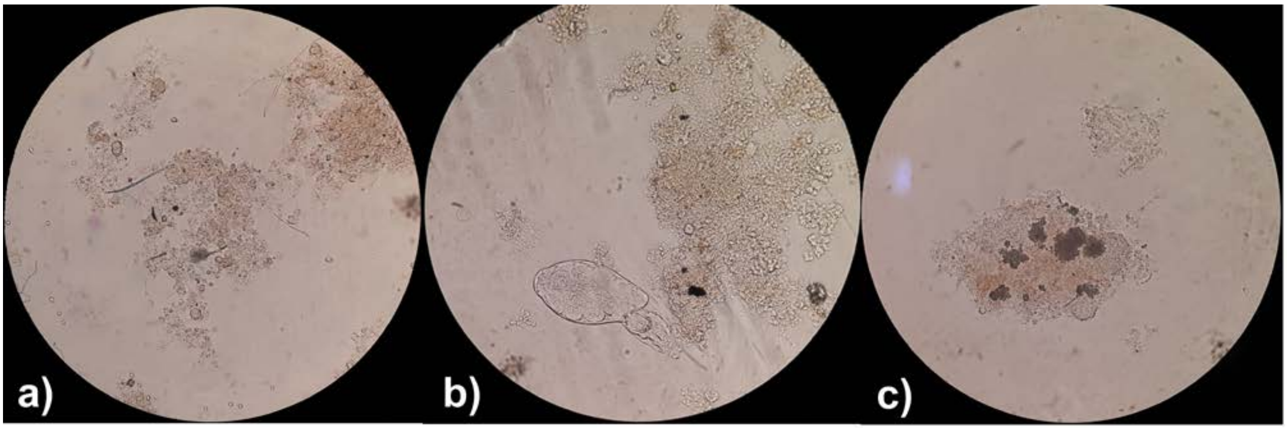

Fig. 6. Pictures of activated sludge on: a/ 7th day; b/ 14th day; c/ 21st day of experiment (Light Microscope – 100X).

the flocs represent a deformation of the activated sludge that is an indicator for a starving sludge.

The quantity of the bacteria from g. *Pseudomonas* and from g. *Acinetobacter* plays the role of an indicator for the flexibility of the biological system and its capability to adapt to degrade xenobiotics. These bacteria possess opportunities for alternative ways for energy provision and have more opportunities for a resistance development and unlocking of a xenobiotic degrading potential. The data for the bacteria from the g. *Pseudomonas* and from g. *Acinetobacter* is presented on Fig. 7.

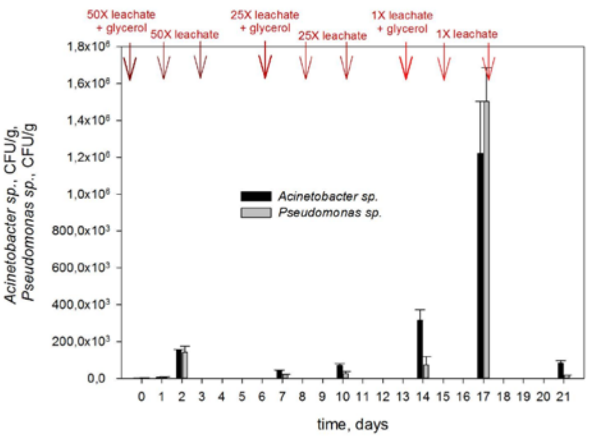

Fig. 7. Dynamics of quantity of *Pseudomonas sp.* and *Acinetobacter sp.*

The changes in the quantity of the bacteria from g. *Pseudomonas* and from g. *Acinetobacter* follow the same tendency in the simulated experiment (Fig. 7). In the first stage, the representatives of the both genera are in low quantities. After the transition in the second stage it is registered a quantitative increase to the 14^{th} day. In the third stage, the microorganisms of the both genera sharply increase their quantities 4 times for g. *Acinetobacter* and 20 times for g. *Pseudomonas*. This increase is a response to the addition of the highly concentrated leachate. According the previous results the first response of shock increase of toxic xenobiotic is the increase of the amount of homogenic bacteria of the genera from g. *Pseudomonas* and from g. *Acinetobacter*. The possible reason for that is the disruption and deformation of the flocs as well as the decrease the amount and inhibition of micro- and meta-fauna [9, 10]. These results as well as results for SVI and biodegradation activity of AS once again confirm that the adaptation process at these conditions riches the critical concentration for the complex toxic pollutants in the leachate. After reaching the critical concentration the bacteria from g. *Pseudomonas* and from g. *Acinetobacter* decrease their quantities (from $1,5.10^6$ CFU/g and $1,2.10^6$ CFU/g to $1,7.10^4$ CFU/g and $8,3.10^4$ CFU/g).

In spite of the vast potential for xenobiotics biodegradation of the microorganisms of genera *Pseudomonas* and *Acinetobacter*, in the simulated experiment the shocking addition of undiluted leachate causes a negative impact on the xenobiotic degrading bacteria and on the biodegradation process. The results give the base to propose that 25X dilution of the leachate is close to the critical concentration for the development of adaptive potential of AS in the specific experimental conditions. Our proposal is that the real wastewater treatment process to be accomplished at dilution of leachate 25 times with water, containing easily degraded trivial substrates.

If the strategy of the technologists is to increase the concentration of leachate it is necessary to make that very slowly and carefully. This is risky technological process. So, recommendation is to test other mechanisms for the improving the wastewater treatment process, for example to rotate aerobic and anoxic conditions. This rotation will stimulate purposely detoxification, denitrification and annamox process. Less risky, cost effective solution, will be replacement of glycerol with other cheaper and easily degradable substrate for acceleration of degradation of xenobiotic pollutants in leachate with high effectiveness. All these conclusions will be tested and verified in the next our research.

CONCLUSION

The obtained results clearly indicate that from the three stages of the conducted experiment the most favorable conditions for an adaptation of the activated sludge towards the pollutants in leachate are in the first stage. The improving of the settling capability and the macrostructure of the AS, as well as the higher biodegrading capability registered through the indicator effectiveness of organic matter decrease are the precondition for the adaptation of the activated sludge to a 50X diluted leachate. With the increase of the concentration in the second stage some of the parameters such as a presence of pin-point flocs with an absence of filamentous microorganisms and a negative effectiveness of organic matter decrease indicate a deterioration namely the functioning of the adaptive mechanisms of the activated sludge becomes difficult. In the third stage, by the results of the analyzed parameters, it is registered that the metabolic processes of the microorganisms of the activated sludge are inhibited by the high concentration of xenobiotics in the added undiluted leachate. The results confirm that the leachate diluted 25X contains toxic xenobiotics, which concentration is close to the critical one for the development of adaptive potential of AS in the concrete experimental conditions. This dilution of the leachate and the ratio COD:BOD_5 are appropriate for accomplishment of wastewater treatment process.

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REFERENCES

1. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Waste_statistics#Total_waste_generation
2. T. H. Christensen, Solid Waste Technology and Management, Blackwell Publishing Ltd., UK, 2011.
3. P. Kjeldsen, M. A. Barlaz, A. P. Rooker, A. Baun, A. Ledin, T. H. Christensen, *Crit. Rev. Environ. Sci. Technol.*, **32**, 297 (2002).
4. R. Stegman, U. K. Heyer, R. Cossu, in: *Proceedings Sardinia*, Vol. 10 (Proc. Sardinia 2005, 10th Int. Waste Manage. Landfill Symp.), 2005.
5. A. A. Abbas, G. Jingsong, L. Z. Ping, P. Y. Ya, W. S. Al-Rekabi, *J. Appl. Sci. Res.*, **5**, 534 (2009).
6. J. Wiszniowski, D. Robert, J. Surmacz-Gorska, K. Miksch, J. V. Weber, *Environ. Chem. Lett.*, **4**, 51 (2006).
7. Y. Ren, F. Ferraz, M. Lashkarizadeh, Q. Yuan, *J. Water Process. Eng.*, **17**, 161 (2017).
8. I. Schneider, M. Beluhova, Y. Topalova, *Ecol. Eng. Environ. Protect.*, **4**, 50 (2001).
9. I. Schneider, M. Ducheva, I. Yotinov, Y. Todorova, E. Daskalova, Y. Topalova, V. Stefanova, *Ecol. Eng. Environ. Protect.*, **7**, 27 (2016).
10. Y. Topalova, Y. Todorova, I. Schneider, I. Yotinov, V. Stefanova, *Water Sci. Technol.*, **78**, 588 (2018).
11. A. B. Yahmed, N. Saidi, I. Trabelsi, F. Murano, T. Dhaifallah, L. Bousselmi, A. Ghrabi, *Desalination*, **246**, 378 (2009).
12. B. Xie, S. Xiong, S. Liang, C. Hu, X. Zhang, J. Lu, *Bioresource technol.*, **103**, 71 (2012).
13. I. Yotinov, M. Belouhova, A. Kavazov, I. Hristova, N. Dinova, I. Schneider, E. Daskalova, S. Lincheva, Y. Topalova, in: Youth Scientific Conference Kliments Days Abstracts 2019, p.67.
14. K. Wang, L. Li, F. Tan, D. Wu, *Archaea*, **2018**, 10 (2018).
15. A. M. Costa, R. G. D. S. M. Alfaia, J. C. Campos, *J. Environ. Manage.*, **232**, 110 (2019).
16. R. Dimkov, Y. Topalova, I. Schneider, Environmental biotechnology, Publish Sci-Set Eco, Sofia, 2017.
17. S. Kheradmand, A. Karimi-Jashni, M. Sartaj, *Waste Manage*, **30**, 1025 (2010).
18. E. M. de Albuquerque, E. Pozzi, I. K. Sakamoto, P. Jurandyr, *J. Water Process. Eng.*, **23**, 119 (2018).
19. S. F. Corsino, M. Capodici, D. Di Trapani, M. Torregrossa, G. Viviani, *New Biotechnol.*, **55**, 91 (2020).
20. A. I. Zouboulis, M. X. Loukidou, K. Christodoulou, *Chemosphere*, **44**, 1103 (2001).
21. F. Spina, V. Tigini, A. Romagnolo, G. C. Varese, *Life*, **8**, 27 (2018).
22. S. Renou, J. G. Givaudan, S. Poulain, F. Dirassouyan, P. Moulin, *J. Hazard. Mater.*, **150**, 468 (2008).

23. Y. Liu, Z. Chen, L. Yang, Z. Liao, T. T. Sun, J. Wang, H. Wang, *Bulg. Chem. Commun.*, **47**, 193 (2015).
24. D. Bove, S. Merello, D. Frumento, S. A. Arni, B. Aliakbarian, A. Converti, *Chem. Eng. Technol.*, **38**, 2115 (2015).
25. P. Ghosh, I. S. Thakur, A. Kaushik, *Ecotox. Environ. Safe.*, **141**, 259 (2017).
26. H. Luo, Y. Zeng, Y. Cheng, D. He, X. Pan, *Sci.Total Environ.*, **703**, 135468 (2020).
27. M. Capodici, D. Di Trapani, G. Viviani, *Water Sci. Technol.*, **69**, 1267 (2014).
28. M. X. Loukidou, A. I. Zouboulis, *Environ. Pollut.*, **111**, 273 (2001).
29. M. S. Bilgili, A. Demir, B. Özkaya, *Environ. Manage.*, **38**, 189 (2006).
30. I. Ćosić, M. Vuković, Z. Gomzi, F. Briški, *Chem. Pap.*, **67**, 1138 (2013).
31. J. J. González-Cortés, F. Almenglo, M. Ramírez, D. Cantero, *J. Environ. Manage.*, **281**, 111902 (2021).
32. Z. Belhachmi, Z. Mghazli, S. Ouchtout, *Math. Comput. Simulat.*, **185**, 174 (2021).
33. Y. Topalova, Biological Control and Management of Water Treatment, Publish Sci-Set Eco, Sofia, 2009.
34. BDS EN 14702-1, (Characterization of sludges – settling properties. Part 1: Determination of settleability (Determination of the proportion of sludge volume and sludge volume index)), European Committee for Standardization, 2006, 16 p.
35. A. D. Eaton, L. S. Clesceri, E. W. Rice, A. E. Greenberg, Standard Methods for the Examination of Water and Wastewater, vol. 21, APHA-AWWA-WEF, Washington, D.C., 2005
36. D. Jenkins, M. G. Richard, G. Daigger, Manual on the Causes and Control of Activated Sludge Bulking, Foaming and Other Solids Separations Problems, Lewis Publishing, Boca Ration, FL, 2003.