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ACTIVITY OF NITRIFYING BIOFILM IN THE PROCESS OF WATER TREATMENT IN DIATOMITE BED

The study deals with the efficiency of removing ammonium nitrogen from water in biofiltration process. For the research purposes diatomite was used as packing of biological reactor. As a biofilter filler, diatomite was simultaneously the sorbent, the culture medium and the carrier of microorganisms. The biological activity of the bed was assessed taking account of the effectiveness of removing ammonia nitrogen in the process of nitrification and the time of biofilm formation. Diatomite stimulates the growth of biomass and the process of effective removal of ammonia nitrogen, reaching even 100%. SEM image proved that the surfaces of diatomite bed grains were overgrown with microorganisms. The biofilm structure was loose and porous, being characteristic of aerobic biofilm. The biofilm covered the entire grain surface.

1. INTRODUCTION

The removal of nitrogen compounds from water to be used for drinking is a serious problem in water treatment technology. Because of the costs of removing nitrogen-containing contaminations, biological methods of achieving this goal are being proposed more and more often. Technological systems based on the process of nitrification have been developed and implemented wherever there is the necessity to remove ammonium ions without removing all nitrogen compounds. The biofiltration is numbered among highly-effective processes, but it is also a process influenced by many factors [1], i.e., bed height, hydraulic loading rates and the concentration of total ammonia nitrogen [2], concentration of oxygen [3], [4], [5], concentration of free ammonia [6], concentration of nitrite [7] and temperature [8], [9]. A long time needed for biofilm formation and that for reaching suitable concentration of biomass are the problems that still remain to be solved. The effectiveness of the bioprocess and the time for biofilter to reach full capacity are determined by the properties of filler or packing material [10], [11]. The important parameters are not

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only its granulation or specific surface, but also its capability to generate durable combinations of microorganisms and the carrier or the bonds between them [12]. Quite important is the character of surface resulting from the properties of the basis and structures of pores. The character of the external structure of grain surface affects its overgrowing with microorganisms. Multiplication of microorganisms takes place both in intergranular spaces and in macropores, the sizes of which enable microorganism cells to penetrate into the so-called niches that protect the cells against disadvantageous shearing effect of water flow [13], [14]. A packing material of biofilter may play a role of not only the substrate for biofilm formation, but also that of absorbent, ion-exchanger, nutrient and a buffer medium for biochemical reactions [9], [15]. Making the selection of biofilter packing we take account of both its effectiveness and price. Hence it should be cheap, efficient and it should stimulate biomass development.

This paper deals with the possible application of diatomites as packing material for nitrification filters. Based on the effectiveness of removing ammonia nitrogen in the process of nitrification and the time of biofilm formation, the biological activity of the bed has been estimated.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL SET UP

The tests were performed on model solution prepared on the basis of tap water without chlorine. Water to be fed onto bed was enriched with ammonium nitrogen in the form of NH_4Cl solution and a bio-preparation containing nitrifying bacteria (table 1). The biopreparation was added to the water feeding biofilters for 10 days.

Table 1

Some parameters of synthetic influent

Parameter	Unit	Min	Max
Ammonia nitrogen	g N/m^3	1.15	2.3
Nitrite nitrogen	g N/m^3	0.0015	0.74
Nitrate nitrogen	g N/m^3	0.6	2.76
Dissolved oxygen	g O_2/m^3	8.4	12
pH	–	7.05	8.10
Colour	g Pt/m^3	0	9
Turbidity	NTU	0	5
Temperature	°C	16	18

The parameters of the biofilter (figure 1) were as follows:

- bed height: 1.2 m,
- bed diameter: 35 mm,
- approximate filtration rate: 3 m/h,
- upward flow (there was no bed flushing).

Diatomite of the granulation ranging from 1.2 to 2.0 mm from Jawornik Ruski-Borownica strip pit located in the northern parts of the Leszczawka deposit was used for tests. Physical and chemical properties of diatomite are specified in table 2.

Table 2

Physical and chemical properties of diatomites

Parameter	Unit	Value
Specific surface	m^2/g	20
Granulation	mm	1.2–2.0
Specific weight	g/cm^3	2.10–2.25
Loss of roasting (900 °C)	%	7–10
Porosity	%	25–35
Absorbability	%	20–30
SiO_3	%	68–73
Al_2O_3	%	9–12
Fe_2O_3	%	4–6
CaO	%	2–3
P_2O_5	%	0.2

Bed performance was estimated on the basis of chemical and physical parameters, i.e., ammonia nitrogen, nitrite nitrogen(III), nitrate nitrogen(V) and oxygen content, pH, colour, turbidity, as well as on the basis of such bacteriological indicators as a total number of psychrophilic and mesophilic bacteria. Additionally, the biofilm was tested for titre of nitrification bacteria and enzymatic activity of microorganisms of the biofilm (TTC dehydrogenase test).

2.2. METHODS OF PHYSICOCHEMICAL ANALYSIS OF WATER

Quantitative determination of physicochemical parameters of water was based on standard testing procedures:

- ammonia nitrogen content – colorimetric method with Nessler's reagent, PN-94/C-04576-04,
- nitrate nitrogen(V) – colorimetric method with sodium salicylate, PN-82/C-04576-08,
- nitrite nitrogen(III) – standard methods for the examination of water and waste-water [16] by colorimetric method based on the reaction of nitrite nitrogen(III) with

4-diazobenzenesulphonic acid, which produces pink coloration of intensity directly proportional to nitrate concentration in water,

- pH – electrometric method according to standard methods for the examination of water and wastewater; testing pH, acidity and alkalinity, PN-90/C-04540.01-1489,
- colour – standard methods for the examination of water and wastewater, PN-74/C-04558,
- turbidity – standard methods for the examination of water and wastewater, PN-79/C-04883-03,
- oxygen – electrochemical method with the use of oxygen probe Oxi 330/SET manufactured by German WTW Company.

2.3. METHODS OF MICROBIOLOGICAL ANALYSIS

Quantitative determination of bacteriological parameters of water, i.e., total number of both psychrophilic and mesophilic bacteria, was based on standard testing procedure (PN-EN ISO 6222:2002).

The biofilm was tested for:

- titre of nitrifying bacteria [17], [18],
- enzymatic dehydrogenase activities (TTC test) of microorganisms of biological film [19].

Autotrophic nitrifying bacteria that populate the bed were determined by the method of their culturing on a liquid media. Bed packing material sampled (1g) from various bed/deposit heights was transferred to the Ringer liquid, then shaken and centrifuged. Dilutions in the range from 10^{-1} to 10^{-14} were prepared from the suspensions. Then 1 cm^3 of sample from each dilution was cultivated on the Winogradski medium and incubated at of 28 °C for 14 days. After fourteen days of incubation nitrate nitrogen was detected in the culture.

Detecting of nitrates(III). Nitrates on the Winogradski medium were detected using the Griess reagent. 0.5 cm^3 of the Griess reagent was added to test glasses with at least five-day culture. After several minutes a pink tinge appeared which testified to a positive result. The last sample of pink tinge was considered to be the titre of nitrifying bacteria of the first phase.

Detecting of nitrates(V). Nitrates on the Winogradski medium were detected using brucine and diphenylamine. From the 1st day of the research till the 37th day of filter operation the results were read with brucine, and afterwards the diphenylamine was used, because it was considered to be more reliable.

Dehydrogenase activity. Dehydrogenase activity of biofilm was determined spectrophotometrically, with TTC (2,3,5-triphenylotetrazolium chloride) reduced to TF (triformazan). The activity of a packing material from the upper and lower parts of the bed was

also measured. 15 g of the bed were sampled into centrifuge test tubes and centrifuged with 5 cm^3 of the Ringer liquid and 5 cm^3 of Tris-HCl buffer. After centrifuging, 1 cm^3 of sodium sulfate and 1 cm^3 of TTC solution were added to the test tubes. Samples were incubated in darkness, in steam bath, at the temperature of 37 °C for 30 minutes.

Extinction coefficient of the solution from the test tubes was measured spectrophotometrically at the wavelength $\lambda = 480$ nm. The amount of formazan formed in the samples tested was determined based on the analytical curve. Dehydrogenase activity was calculated taking account of the amount of formazan formed per 1 g of the packing.

The samples for testing were taken at two different heights of the filter: $H1 = 0.65$ m (hereafter lower filter section) and $H2 = 1.15$ m (hereafter upper filter section). Samples were also subjected to microscopic examination.

3. RESULTS

The diatomite bed enriched with the biopreparation was quickly ready to operate as biosorption bed. As early as in the first days, the nitrification could be observed. Its effectiveness ranged from 20 to 30% and increased gradually to reach 100% on the 20th day (figure 1). After 20 days of bed maturing the concentration of ammonia nitrogen in filtrate was within 0.00–0.15 mg N/dm^3 and was lower than its maximum acceptable content in water to be used for consumption (0.5 mg NH_3).

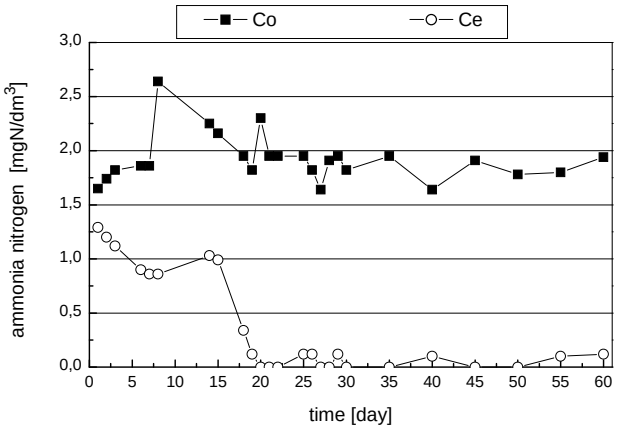


Fig. 1. Ammonia nitrogen changes in raw water (Co) and the water treated (Ce) in diatomite bed

The concentration of nitrite nitrogen(III) increased in proportion to the changing content of ammonia nitrogen and reached the maximum (0.72 mg N/dm^3) on the 18th day. Then it decreased gradually to 0.24–0.36 mg N/dm^3 (figure 2). By the end of the

experiment, the content of 0.5 mg NO_2/dm^3 exceeded the maximum acceptable concentration of 0.15 mg $N\text{-}NO_2/dm^3$ given in the recent Order of the Minister of Health from March the 29th 2007 for potable water (Official Gazette Dz.U. No. 61, item 417) [20]. The concentration of nitrate nitrogen(V) in the treated water was higher than that in the raw water, except for first few days of bed operation (figure 3).

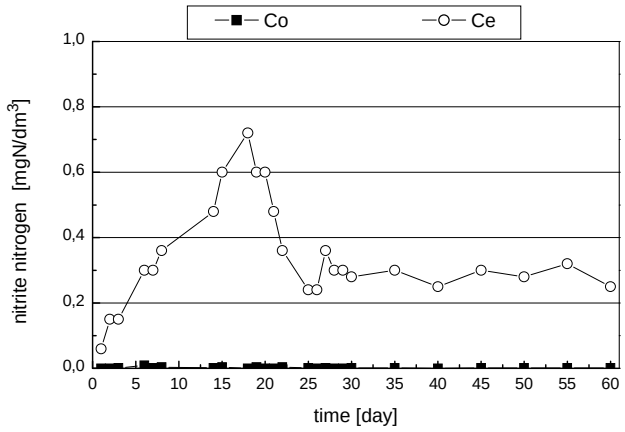


Fig. 2. Nitrite nitrogen changes in raw water (Co) and the water treated (Ce) in diatomite bed

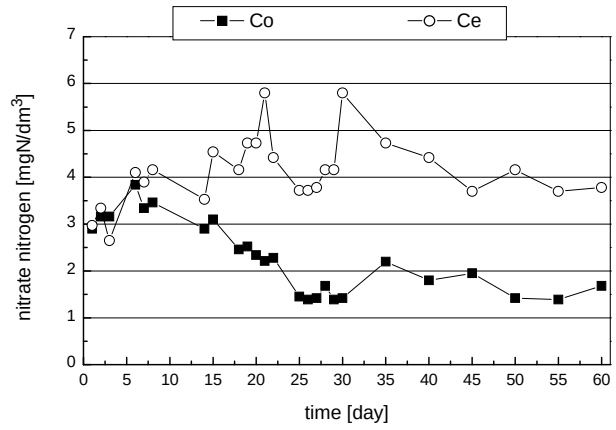


Fig. 3. Nitrate nitrogen changes in raw water (Co) and the water treated (Ce) in diatomite bed

During biofiltration of ammonia nitrogen-containing water, only some per cent of this compund are transformed into nitrate nitrogen(III) and nitrate nitrogen(V). The rest is oxidized and accumulated by nitrifying bacteria and heterotrophic bacteria that

form a biofilm on the grains of filtration material. Heterotrophic bacteria assimilate some portion of ammonia nitrogen while using hydrogen dioxide present in the filtered water. Removal of ammonia nitrogen is associated with the processes taking place in the biofilm, as the number of nitrifying and heterotrophic bacteria in the biofilm depend on its thickness [21].

The content of oxygen dissolved in raw water oscillated between 6.2 and 7.7 mg O_2/dm^3. The content of oxygen in the treated water was lower compared to that of raw water. In the water subjected to biofiltration, the concentration of oxygen ranged from 5.13 to 6.38 mg O_2/dm^3 (figure 4).

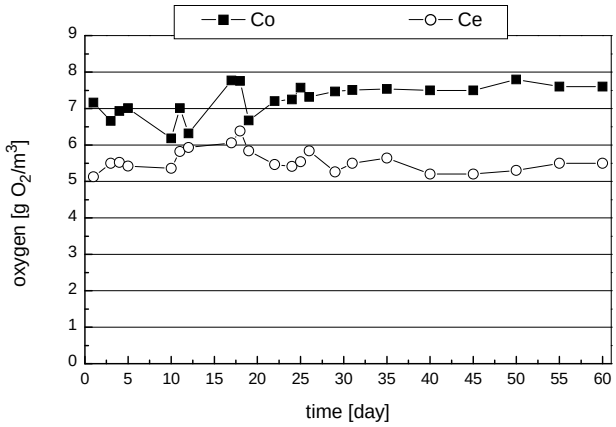


Fig. 4. Oxygen changes in raw water (Co) and the water treated (Ce) in diatomite bed

The analysis of the quantity of the ammonium nitrogen removed and the oxygen content indicates that the actual quantity of oxygen indispensable for oxidizing one mg of ammonium nitrogen was not stoichiometric. As much as 3.43 g of oxygen is needed to oxidize one g of ammonium nitrogen to nitrite nitrogen(III) and 1.14 g of oxygen to oxidize one g of ammonium nitrogen to nitrate nitrogen(V). Thus, a total stoichiometric consumption of oxygen in nitrification reactions is 4.57 g of O_2 per one g of ammonium nitrogen. The actual consumption of oxygen ranged from 0.39 to 2.5 mg O_2/dm^3, being lower than theoretical consumption, which should be from 2.4 to 8.8 mg O_2/dm^3. The quantity of generated nitrate nitrogen(III) and (V) does not correspond to that of ammonium nitrogen removed during filtration, the difference being the ammonium nitrogen assimilated by nitrifying bacteria.

After filtration, pH of water ranged between 7.11 and 7.77 (figure 5). By the 5^{th} day, pH of treated water was higher than that of raw water. In the period from the 6^{th} to the 30^{th} day, pH was comparable with that of raw water, and after the 30^{th} day, pH of treated water was lower, which proved that nitrification process took place.

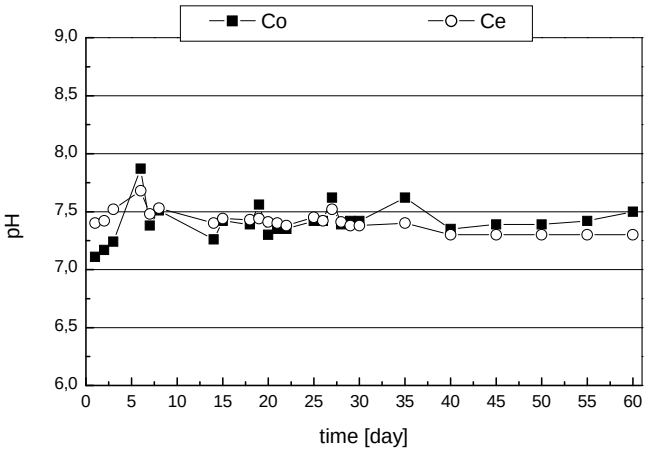


Fig. 5. pH changes in raw water (Co)
and the water treated (Ce) in diatomite bed

The turbidity of treated water was higher, and the colour deeper compared to raw water (figures 6, 7).

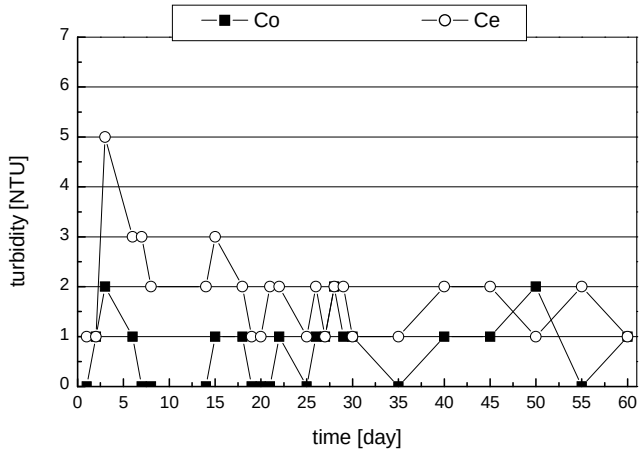


Fig. 6. Turbidity changes in raw water (Co)
and the water treated (Ce) in diatomite bed

Water treated in diatomite bed met the requirements for potable water in respect of the concentration of nitrogen ammonium and nitrate nitrogen(V), pH and colour. Turbidity and nitrite nitrogen(III) concentration exceeded the standard values.

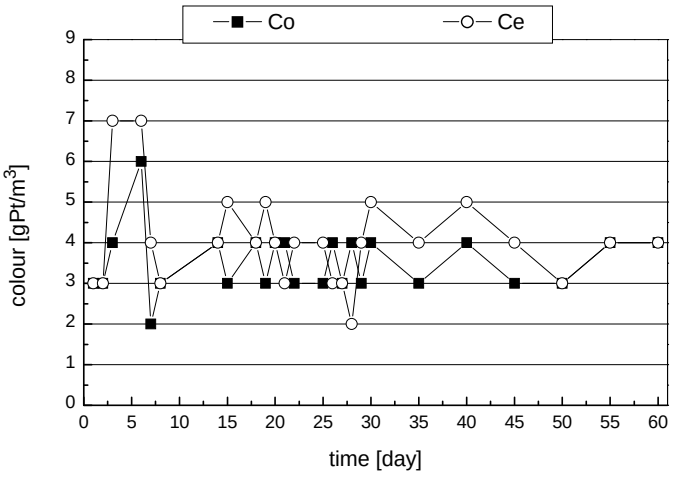


Fig. 7. Colour changes in raw water (Co)
and the water treated (Ce) in diatomite bed

The number of psychrophilic bacteria in raw and treated water were determined after the 7th day of filter operation. By the 15th day of filter operation, the bacteria in water occurred very abundantly, in the range of several tens to several hundred thousand in 1 cm^3 of water. Raw water was richer in bacteria than treated water, which proved that bacteria were deposited in filter packing (figure 8).

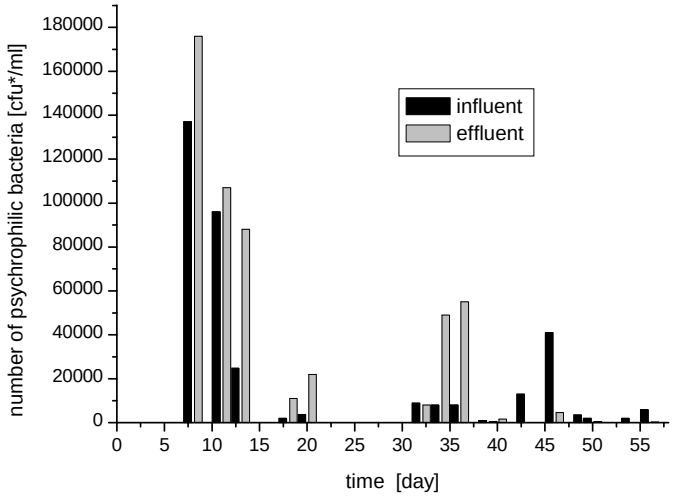


Fig. 8. Total psychrophilic bacteria changes in influent and effluent

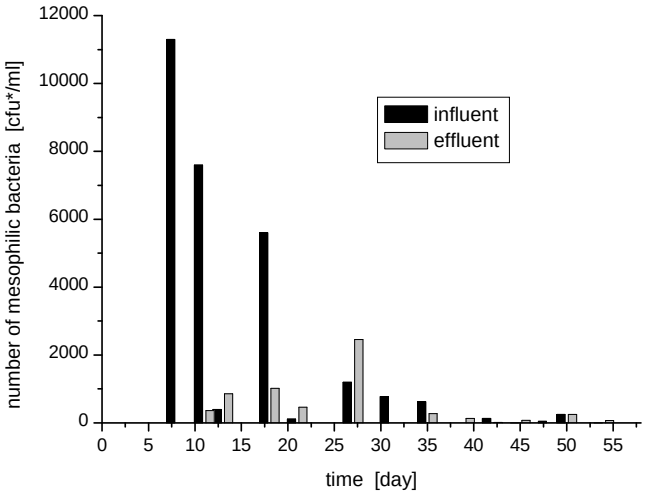
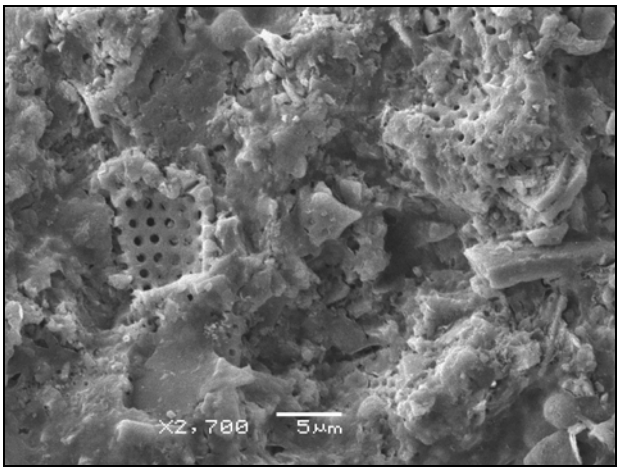
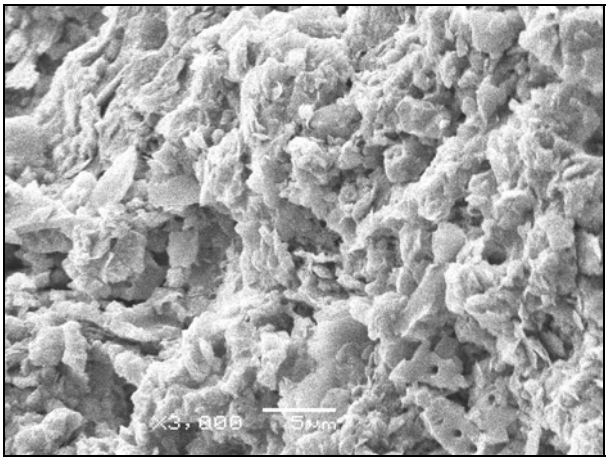


Fig. 9. Total mesophilic bacteria changes in influent and effluent

By the 22nd day, considerable oscillations of the number of psychrophilic bacteria were observed in water subjected to the treatment process. They resulted from the addition of a biopreparation to the treated water. High number of bacteria in the treated water could be due to too high filtration rates and/or due to poor bed operation. Microscopic examinations showed that the biofilm on the bed/filling was observed in its lower part only. A definitely lower number of bacteria and slight variations of the quantity of these bacteria in the treated water were observed as late as after the 34th day of filter operation. The number of mesophilic bacteria were similar (figure 9).



Phot. 1. Biofilm on diatomite grain on the 5th day of bed operation



Phot. 2. Biofilm on diatomite grain on the 60th day of bed operation

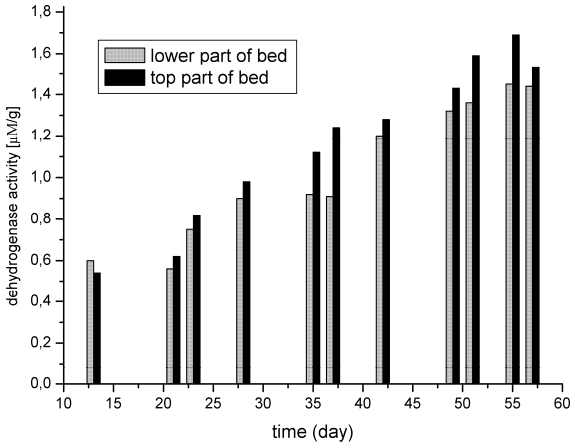


Fig. 10. The changes of microorganism activity in the lower and top parts of bed

The activity of microrganisms in biofilter packing was tested from the 13th day of the experiment (figure 9) and a gradual increase in their enzymatic activity was observed. The dehydrogenase activity was higher in the samples taken from upper parts of the bed, which could most likely result from a higher number of heterotrophic microorganisms in the biofilm in these parts of the filter. Such a result testified to a stable operation of nitrification filter. Heterotrophic organisms were observed to occur more abundantly in the lower parts of the packing, while autotrophic organisms were found in its upper parts [15]. As the filtration in the experiment was conducted upward, the quantitative proportions of microorganisms were reversed.

The tests of the titre of nitrifying bacteria began from the 13th day. On the 13th day of bed operation, no biofilm was observed in the filter packing. This was confirmed by the tests of the titre of nitrifying bacteria and microscopic examinations. On the 21st day, the titre of nitrifying bacteria in the sample taken from the lower parts of the bed reached 10^{-1}, and in the sample from the top parts of the bed it was 10^{-2}. On the 23rd and 28th day, the titre of nitrifying bacteria was 10^{-5} and 10^{-8}, respectively, indicating still a small number of these bacteria in the biofilm. From the 35th day of the filter operation, the values of the titre of nitrifying bacteria suggesting the presence of well developed biofilm were recorded. No greater differences were observed in the number of autotrophic nitrifying bacteria in the samples from the upper and lower parts of the filter.

4. DISCUSSION OF RESULTS

Many methods have been developed for testing biosorptive beds. Most take into account the manner of sampling, biomass extraction and the assessment of its activity or intensity of its development [12]. As the parameters, i.e., biomass concentration and metabolic activity of bacteria, correlate with the technological effect obtained, the assessment of nitrifying bed performance was based on that effect.

Due to its properties, diatomite stimulates the growth of biomass. A 100% effectiveness in the removal of ammonia nitrogen was achieved after 20 days. Simultaneously, the number of psychrofilic and mesophilic bacteria in water after biofiltration were small. The titre of nitrifying bacteria in biofilm was very low, which testified to a great number of these bacteria.

ŁOMOTOWSKI and HALINIAK [13], who studied the removal of ammonia nitrogen from underground water in biofilters, found that the number of nitrifying bacteria grow with an increase in the bed depth and, irrespectively of the bed/packing height and type (sand, active carbon). *Nitrobacter* bacteria were more abundant compared with *Nitrosomonas*. At a sand filter 2.5 m high the bacteria growth was observed to the depth of 1.5 m. At a greater depth their number decreased [13]. Both in sand filter and carbon filter the number of nitrifying bacteria ranged from ca 10^5 to 10^7 cells per gram of a dry matter of the bed.

In the case of fluidal filters, a large number of *Nitrosomonas* bacteria in water after filtration were observed in the initial phase. The longer the cycle of bed operation, the lower the number of nitrifying bacteria in water [13].

Both the number and the activity of microorganisms overgrowing the bed are of a great importance if the results of water conditioning are taken into account. According to WANG [22] the metabolic activity of the biomass rather than the bacteria number determines the effectiveness of biodegradation.

The biofilm is composed of active microorganisms and inactive substances. The former are responsible for the removal of substrate from the areas of the film–liquid

interface. The latter do not participate in biological oxidation but they affect the film thickness. Accumulation of inactive substances in the biofilm contributes to the limiting of an average microbiological activity [23]. Due to the lack of oxygen, the reactions that involve heterotrophic bacteria take place in grain pores and on their surfaces, while the biochemical activity of nitrifying bacteria becomes hampered.

In order to maintain the maximum activity of microorganisms, a suitable thickness of the biofilm overgrowing bed is necessary. The thickness recommended for aerobic biofilm ranges from 15 to 25 μm, whereas calculations indicated that the thickness of an active biofilm as small as 7 μm allows the reaction of ammonium nitrogen oxidation to take place. The remaining part of biofilm are the so-called inactive supporting substances [12]. For this reason the counter-gravitational flow permits the control of the biofilm thickness and a constant microbiological activity of the biofilm.

Gravitational flow allowed a dead biomass to be accumulated in the lower parts of the biofilter, which could be responsible for lower oxygen content in the water subjected to purification process. The counter-gravitational flow removed dead cells of microorganisms from the biofilter and simultaneously enabled natural regulation of biofilm thickness, thus favouring a lower oxygen demand.

It should be stressed that the biofiltration is a complex process, since biological nitrification is accompanied by sorption and assimilation phenomena. Therefore, it can be assumed that higher concentration of biomass and greater biofilm thickness may lead to higher oxygen consumption connected with mineralization of dead organic matter deposited in the biofilter.

The changes in the colour and turbidity of treated water can explain the relationships between microorganisms in the bed. High turbidity of treated water testifies to higher number of microorganisms in filtrate (if turbidity is not caused by mechanical abrasion of a filling material in the bed). Small changes in turbidity and colour prove that the properties of the biofilm formed are favourable and that the microorganisms are permanently attached to the carrier. The cause of high turbidity and colour can be the upward flow.

The counter-gravitational flow is more favourable in the nitrification process, because:

- oxygen consumption is lower, thus creating more favourable conditions for nitrification process,
- it permits the control of biofilm thickness and maintains a constant microbiological activity of the biofilm as well as its uniform distribution on the entire biofilter cross-section.

The colour and turbidity variations of treated water may provide an answer to the question, which packing material is better overgrown with microorganisms and stimulates biofilm formation. High turbidity of the treated water may testify to a high number of microorganisms. Turbidity was observed to rise to 2 NTU for aerobic biofilm, and even to 22 NTU for anaerobic biofilm [24]. The maximum turbidity during the

experiment did not exceed 2 NTU. It is the evidence that biofilm is firmly attached to the granular packing material and mutual interactions between microorganisms are observed. Microscopic examination confirmed spongy and corrugated structure of biofilm, covering tightly the packing grains (photo 1). Numerous capillaries and gaps in biofilm structure enabled deep penetration of oxygen and nutrients and, consequently, highly effective removal of ammonia nitrogen.

The research on the biofilm formation on various packing materials have revealed that surface features, resulting from bed characteristics and the structure of pores, are of crucial importance [18]. The character of the external surface of grains affects their overgrowing with microorganisms.

Microorganisms grow both in the spaces between grains and in macropores whose sizes enable microorganism cells to penetrate them and to create niches being protected against disadvantageous effects of water-flow forces [25], [26]. Some spaces in the same packing materials may be more slowly overgrown with microorganisms because of exceptionally smooth surfaces of capillaries and a poorer availability of substrates inside of not-through-flow macropores.

Moreover, one of the suggestions is that the growth of biomass in the bed is stimulated by the number of protected niches [25], another claims that it is the character of the external surface of grains that is important in the colonization [14], [26]. The biofilm structure may be described by the surface and volumetric parameters. The surface parameters characterise the biofilm structure (density and porosity), while volumetric parameters – the quantity and morphology of biomass. It was confirmed that aerobic biofilm has a corrugated structure, and the structure of anaerobic biofilm is rather compact with less EPS (matrix) and is formed faster than aerobic biofilm. The image of diatomite bed in scanning microscope confirmed that microorganisms overgrew the grain surfaces. This biofilm had a loose and porous structure characteristic of aerobic biofilm and covered the entire grain surface.

The diatomite packing was efficient in ammonia nitrogen removal. High effectiveness of nitrification in diatomite bed can be explained by its properties stimulating reproduction of nitrifying bacteria. Presumably diatomite, made up of carbonates and phosphates, becomes a nutrient for them, thus accelerates considerably the biomass growth.

5. CONCLUSIONS

1. Diatomite properties stimulate the growth of nitrifrying biofilms, hence the efficiency of ammonia nitrogen removal as high as 100%.

2. The chemical composition of biofilter packing that stimulates the growth of microorganisms has a decisive effect on the time of biofilm growth.

3. Diatomite may be the buffer medium and nutrient for microorganisms, which al-

lows them to overgrow rapidly the surface of diatomite.

4. A constant number of psychrophilic and mesophilic bacteria in treated water may be the indicator of a stable biofilter bed operation.

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AKTYWNOŚĆ BIOFILMU NITRYFIKACYJNEGO W PROCESIE UZDATNIANIA WODY NA ZŁOŻU DIATOMITOWYM

Zbadano efektywność usuwania azotu amonowego podczas biofiltracji. Jako wypełnienie reaktora biologicznego zastosowano diatomit, który pełnił rolę sorbenta, pożywki i nośnika mikroorganizmów. Oszacowano biologiczną aktywność złoża w powiązaniu zarówno z efektywnością usuwania azotu amonowego w procesie nitryfikacji, jak i tworzeniem się biofilmu. Diatomit stymuluje rozwój biomasy, dlatego usuwanie azotu amonowego z wody przebiegało z dużą efektywnością, osiągającą wartość 100%. Obraz z mikroskopu skaningowego potwierdził, że mikroorganizmy porosły powierzchnię ziaren. Biofilm miał luźną i porowatą strukturę charakterystyczną dla biofilmu aerobowego i pokrywał całą powierzchnię ziarna.