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EFFECT OF IRON(III) ON EFFECTIVENESS OF AMMONIUM NITROGEN REMOVAL IN PROCESS OF NITRIFICATION IN CHALCEDONITE BEDS

WPŁYW ŻELAZA(III) NA EFEKTYWNOŚĆ USUWANIA AZOTU AMONOWEGO W PROCESIE NITRYFIKACJI W ZŁOŻACH CHALCEDONITOWYCH

W artykule przedstawiono ocenę efektywności usuwania azotu amonowego z wody w procesie nitryfikacji w złożach chalcedonitowych. Badania przeprowadzono metodą biofiltracji, z przepływem odwrotnym do grawitacyjnego, z prędkością: 1,5 m/h ; 3,5 m/h ; 6 m/h. Zastosowano dwa modele biofiltrów, których wypełnienie stanowił modyfikowany termicznie chalcedonit (odmiana czerwono-brunatno-żółta). Na jeden z nich kierowano roztwór modelowy zawierający 2 mg $N\text{-}NH_4^+/dm^3$ i 1 mg $Fe(III)/dm^3$, na drugi: roztwór modelowy zawierający tylko azot amonowy o takim samym stężeniu. Celem przyspieszenia czasu dojrzewania złóż roztwory modelowe wzbogacano dodatkowo w biopreparat zawierający bakterie nitryfikacyjne I i II fazy. Na podstawie wyników badań fizykochemicznych i bakteriologicznych dokonano oceny wpływu żelaza(III) na czas wykształcenia biofilmu i zużycie tlenu w procesie biofiltracji. Stwierdzono, że czas formowania biofilmu (dojrzewanie złoża) zależy od składu chemicznego wody kierowanej na biofiltry. Obecność żelaza(III) w wodzie uzdatnianej w procesie nitryfikacji wpływa na wzrost skuteczności usuwania azotu amonowego i skrócenie czasu wykształcenia biofilmu. W czasie trwania eksperymentu chalcedonit usuwał żelazo w prawie 100%, a stężenie azotu amonowego osiągnęło poziom poniżej wartości normatywnej. Obecność żelaza miała również pozytywny wpływ na czas rozpoczęcia II fazy procesu nitryfikacji, jednak zużycie tlenu było większe niż na złożu gdzie uzdatniano wodę zawierającą tylko azot amonowy. Badania mikroskopowe błony biologicznej oraz wyniki badań bakteriologicznych wody uzdatnionej potwierdzały większą aktywność biofilmu na złożu, na które kierowano wodę z dodatkiem żelaza. Natomiast obecność żelaza w wodzie kierowanej na biofiltr hamowała rozwój heterotrofów w błonie biologicznej, co wpływało na lepszą jakość bakteriologiczną wody uzdatnionej. Stwierdzono, że o końcowym efekcie procesu uzdatniania decyduje nie tylko prędkość filtracji, ale także powierzchnia aktywnego biofilmu.

1. Introduction

The research studies conducted hitherto suggest that chalcedonite is a heterogeneous mineral, i.e. mineral with a very wide range of possible applications [1,2,3,4]. One of them, deserving special attention, is its usability as sorbent in water purification technology. The effectiveness of its performance is confirmed in case of removing compounds of iron, manganese, plankton, coloring, turbidity and COD_{Mn} [1,3,5,6]. Additional advantage of chalcedonies is their extended filtering cycle, as well as shorter periods of bed ripening in the process of removing iron and manganese, compared to traditional quartz beds [1]. As ammonia nitrogen is a very frequent impurity of underground waters, next to iron and manganese, attempt was made at applying a chalcedonite bed as filling mass for the nitrification filter, and at assessment of iron(III) presence on the biological film formation time, as well as on nitrification process effectiveness.

2. Study Methods

Two chalcedonite-filled biological reactors with bottom-up flow were applied in the study. Biofilters were filled with thermally modified chalcedonite (red-brown-yellow variety).

One of them (marked with symbol F_{NH4-Fe}) was supplied with dechlorinated tap water, 'enriched' with:

- ammonia nitrogen in form of NH_4Cl (approx. 2,0 mg $N\text{-}NH_4^+/dm^3$),
- iron in form of $FeCl_3$ (approx. 1,0 mg Fe/dm^3),
- bio-preparation prepared on the basis of author's own culture.

The other one (marked with symbol F_{NH4}) was supplied with water with the same content of ammonia nitrogen and the bio-preparation, but no iron(III) compounds.

The parameters of both biofilters were as follows:

- bed height, h = 1,2 m,
- bed granulation, ∅ 1,0-2,0 mm,
- bed diameter, 32 mm,
- filtration rate, approx. 1,5 m/h; 3,5 m/h; 6,0 m/h, with respective retention times: 0,36h (21,6 min); 0,18 h (10,8 min); 0,11 h (6,6 min).

The parameters of raw water directed onto bed were within the following range of values:

- ammonia nitrogen ($N\text{-}NH_4^+$): 1,75-2,45 mg N/dm^3
- nitrate nitrogen ($N\text{-}NO_3^-$): 1,4-3,7 mg N/dm^3
- nitrite nitrogen ($N\text{-}NO_2^-$): 0,001-0,210 mg N/dm^3
- temperature: 14,7-20,1°C
- DO: 4,8-10,8 O_2/dm^3
- COD_{Mn} 1,4-4,2 mg O_2/dm^3
- iron: 0,98-1,25 mg Fe/dm^3.

Water samples for physicochemical determinations were taken once a day at the same time. The following chemical parameters of water were inspected: ammonia nitrogen ($N\text{-}NH_4^+$), nitrate nitrogen ($N\text{-}NO_3^-$), nitrite nitrogen ($N\text{-}NO_2^-$), DO, iron, as well as bacteriological indicators: total number of psychrophilic and mesophilic bacteria.

Additionally, biological film of the bed was examined in these respects:

- titre of nitrifying bacteria,
- enzymatic dehydrogenase activities (TTC-test) of biological film microorganisms,
- presence of iron bacteria.

Microscopic observations of bed were also conducted during filter operation, with either fluorescent microscope or electron scanning microscope.

3. Analysis of the nitrification process and assessment of effectiveness ammonia nitrogen removal

Ammonia nitrogen removal in both chalcedonite beds started on the 6th day of the experiment (refer to Fig. 1 and Fig. 2). However, ammonia nitrogen removal was observed to be more effective in the bed fed with water containing iron(III). The acceptable content of ammonia nitrogen in purified water was reached on the 30th day in case of $F_{NH4\text{-}Fe}$ bed, and on the 35th day in case of F_{NH4} bed.

Changes in nitrite nitrogen in conditioned water were comparable for both beds. They were within the range from 0,01 to 0,83 mg $N\text{-}NO_2^-/dm^3$ in the initial phase of bed operation. A sudden growth in nitrite content occurred from the 30th day and then it oscillated in the range of 1,00-1,66 mg $N\text{-}NO_2^-/dm^3$ (Fig. 3, Fig. 4).

This growth was the evidence of the course of the 1st stage nitrification process. However, the nitrite concentration exceeded the acceptable standard values for potable water more than 10 times (0,5 mg NO_2^-/dm^3; 0,1 mg $N\text{-}NO_2^-/dm^3$). In both cases there was no reduction of nitrite content that would lead to a nitrate concentration rise characteristic for the 2nd phase of the nitrification process (Figs. 3 and 4). Differences between initial concentration and final concentration were 0,0-0,46 mg $N\text{-}NO_3^-/dm^3$, for $F_{NH4\text{-}Fe}$ bed and 0,0-0,12 $N\text{-}NO_3^-/dm^3$ for F_{NH4} bed (Fig. 5., Fig. 6.).

A lack of any significant rise in nitrate ion concentration was the evidence of nitrification not occurring in the 2nd phase. Extending of retention time through filtration rate lowering to 1,5 m/h, did not affect the quantity of removed ammonia nitrogen in any significant way, it has also not caused any reduction in nitrite concentration (ref. to Figs. 7,8,9,10). Raising of filtration rate to 6 m/h has not caused any significant changes in chemical parameters of water subjected to biofiltration process. Analysis of tests results indicates that not just the filtration rate and the respective retention time determine the final result of nitrification; the surface area of active biofilm is also of major importance. Most probably, in order to achieve acceptable level of nitrite nitrogen, the bed quantity should be increased through application of 2nd stage of filtration, or through applying larger-diameter biofilters.

Insufficient quantity of oxygen may have caused nitrification process inhibition, but in this case the oxygen concentration in water after biofiltration process has not become reduced below the level of 2 mg O_2/dm^3. The oxygen concentration in water after biofiltration process had diminished from its level in water subjected to conditioning and it reached its minimum at 2,3 mg O_2/dm^3 on the 43rd day in the $F_{NH4\text{-}Fe}$ bed, and at 3,2 mg O_2/dm^3 the 41st day in the F_{NH4} bed. However, the cause of oxygen concentration reduction over the first days of the experiment, when the nitrification process has not yet begun, still needs clarification.

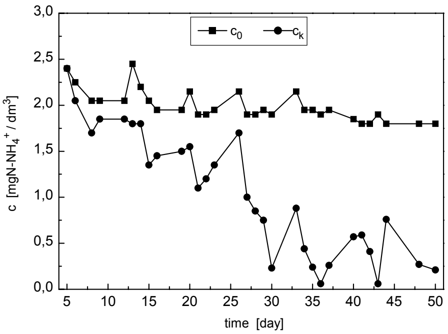

Fig.1. The changes of ammonia nitrogen in the F_{NH4-Fe} bed

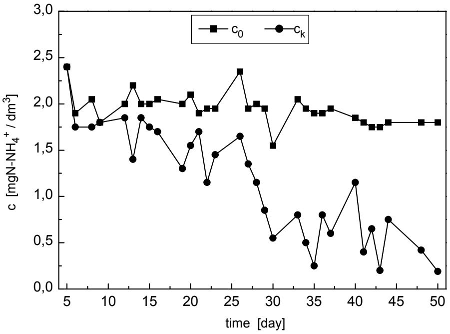

Fig 2. The changes of ammonia nitrogen in the F_{NH4} bed

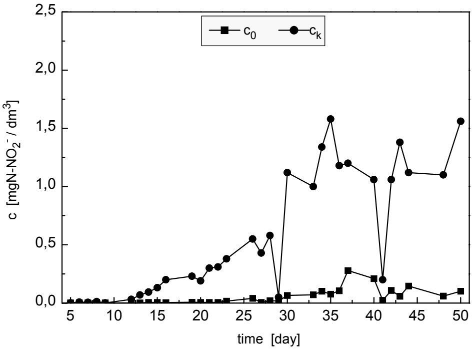

Fig 3. The changes of nitrite nitrogen in the F_{NH4-Fe} bed

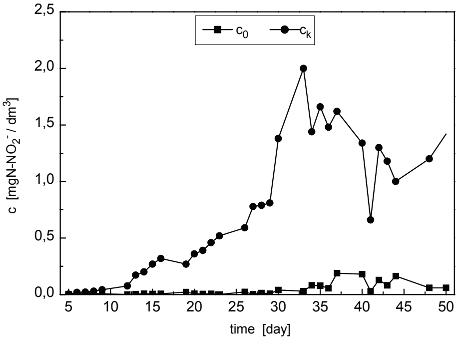

Fig.4. The changes of nitrite nitrogen in the F_{NH4} bed

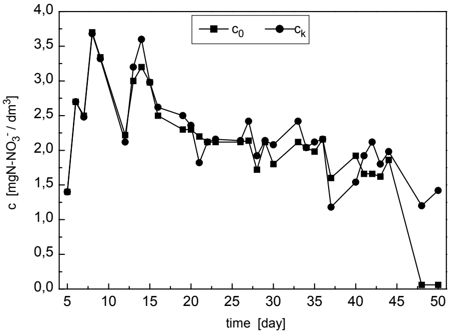

Fig 5. The changes of nitrate nitrogen in the F_{NH4-Fe} bed

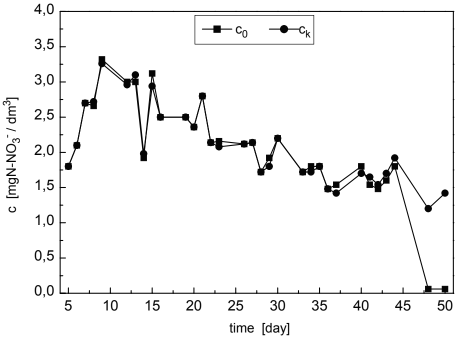

Fig 6. The changes of nitrate nitrogen in the F_{NH4} bed

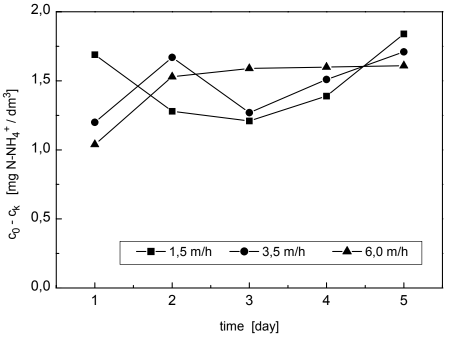

Fig 7. The changes of ammonia nitrogen in the F_{NH4-Fe} bed

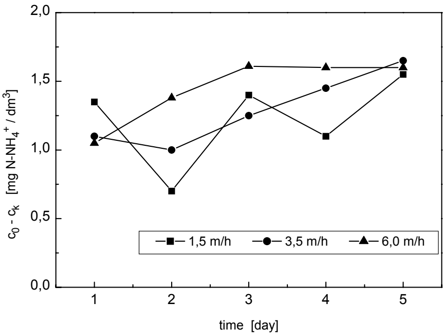

Fig 8. The changes of ammonia nitrogen in the F_{NH4} bed

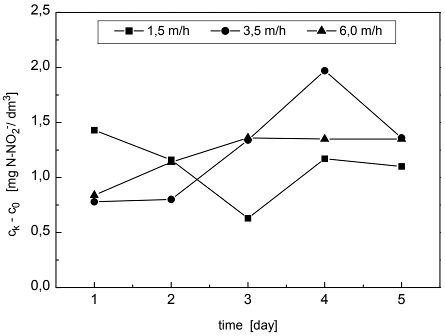

Fig 9.The changes of nitrite nitrogen in the F_{NH4-Fe} bed.

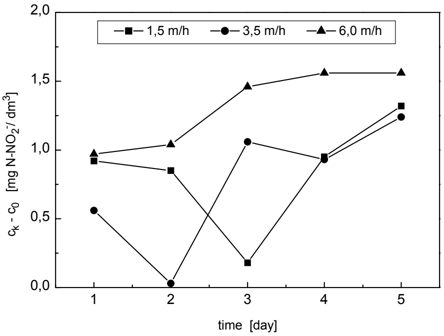

Fig.10. The changes of nitrite nitrogen in the F_{NH4} bed.

Oxygen consumption on the F_{NH4-Fe} bed ranged between 1,00 and 6,00 mgO_2/dm^3, whereas the F_{NH4} bed exhibited smaller loss in oxygen content – from 1,00 to 3,60 mg O_2/dm^3. Oxygen consumption was higher on that bed where both ammonia nitrogen and iron were removed, than it was on the bed removing ammonia nitrogen only (Fig. 11). The reason may be the oxygen consumption by developing mixotrophic iron-oxidizing bacteria, as well as other heterotrophic bacteria. The numbers of iron-oxidizing bacteria in biological film was very low (2 300 cfu /1 g of bed dry matter). Higher values of dehydrogenase activity on the F_{NH4-Fe} bed may be the evidence of greater numbers of heterotrophic bacteria in the bed. In comparison, the activities of enzymes of respiratory microorganisms of biological film on filter grains till the 10th day of bed operation were similar (Fig. 12). Then, dehydrogenase activity was invariably higher in the F_{NH4-Fe} bed.

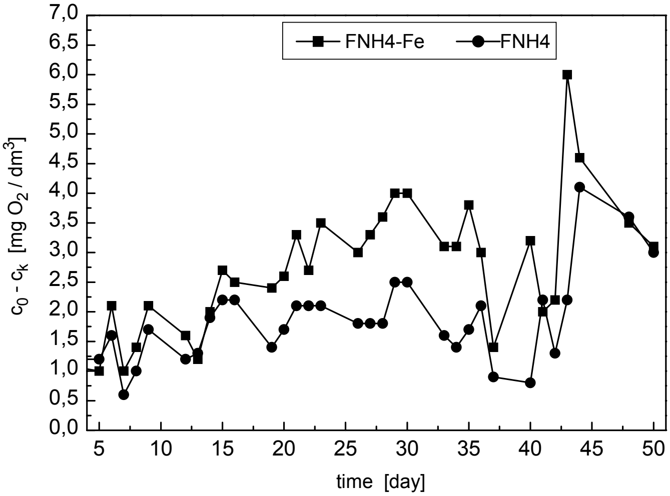

Fig 11. Comparison of oxygen changes in treated process in $F_{NH4\text{-}Fe}$ and F_{NH4} bed

In the initial phase of bed operation the real oxygen consumption, ranging within approx. 1,00-4,00 mg O_2/dm^3 on the $F_{NH4\text{-}Fe}$ bed and 1,00-2,00 mg O_2/dm^3 on the F_{NH4} bed, was higher than its theoretical demand in relation to the quantity of removed ammonia nitrogen. Later on these proportions became reversed, as indicated especially by filtration rate reduced to 1,5 m/h. Theoretically the oxygen demand varied within 4,0 and 6,0 mg O_2/dm^3, whereas its actual consumption was within approx. 1,00-4,00 O_2/dm^3 As much as 3,43 g of oxygen is needed to convert 1 g of ammonia nitrogen into nitrites and 1,14 g of oxygen - to convert 1 g of nitrites into nitrates, thus, the summary stoichiometric oxygen consumption in nitrification reactions equals to 4,57 g O_2 per 1g of ammonia nitrogen. In experimental conditions, i.e. the process of single-stage biofitrtion, the oxygen content was theoretically sufficient for removing max. 2 g ammonia nitrogen. It would seem, therefore that the process should proceed without any disturbances, and all nitrites should be oxidized to nitrates.

The reduction oxygen concentration was not stoichiometric to the quantity of removed ammonia nitrogen, reaching 1,9-4,2 mg O_2 consumed per 1 g N_{Ni}, as observed for each 1 g of nitrogen undergoing nitrification, whereas the actual oxygen usage in biofiltration process was .1,1-3,0 mg O_2 per 1g N_C. The oxygen paradox phenomenon could be caused by ammonia nitrogen removal in some different way than the nitrification. The available literature mentions a high share of the assimilation process, as well as of possible occurrence of ANAMMOX process [7]. The cause of lower oxygen consumption for ammonia nitrogen transformation in nitrification process is more difficult to explain. It was shown that in the first stage of ammonia nitrogen oxidation to hydroxsylamine oxygen may come from water particle rather than from molecular oxygen [8]. If, additionally, the fact that oxygen is released during biosynthesis is taken into account, then the real oxygen consumption may be lower than it results from chemical equations.

The research on the numbers of psychrophilic and mesophilic bacteria in conditioned water indicates a significant bacteria washing-out at water flow rates of approx. 1,5 m/h; 3,5 m/h. At the flow rate of 6 m/h the observed washing out of bacteria from biofilter filling was most modest (Fig. 13). In all tested water samples the acceptable value in the number of bacteria psychrophilic and mesophilic bacteria, as determined in the Decree of the Ministry of Health dated 29th March 2007 (*Official Gazette* Dz.U. Nr 61, item 417) on quality of potable water for human consumption has been exceeded.

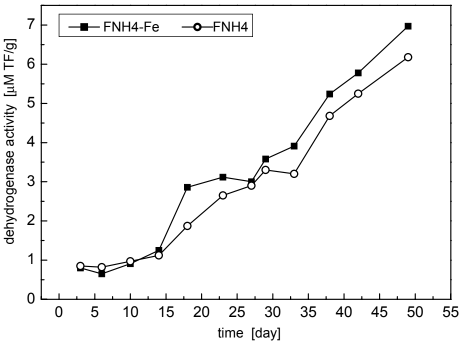

Fig 12. The activity of microorganisms in FNH4-Fe and FNH4 bed.

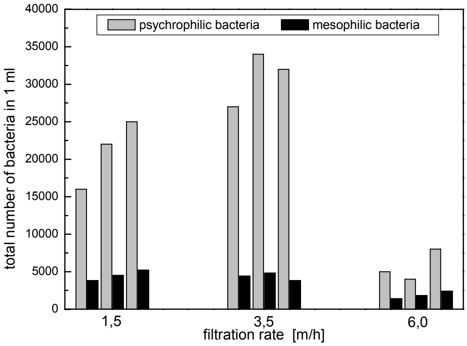

Fig 13. Total psychrophilic and mesophilic bacteria in effluent

4. Effect of iron presence on the time of biofilm formation and nitrification effectiveness

Chalcedonite bed reduced the content of Fe(III) to acceptable standard values (0,2 mg Fe/dm^3) up to 29 day of the $F_{NH4\text{-}Fe}$ bed operation (Fig. 14). Once the sorptive capacities were exhausted, a continuous rising of iron content in the filtrate was observed.

Up to the 12th day the bed operation was characterized by small differences in the quantity of removed ammonia nitrogen (Fig.15). Subsequent days of the bed operation brought increasing difference between the initial and final concentrations of ammonia nitrogen irons, whereas the observed drop in ammonia nitrogen content values on the $F_{NH4\text{-}Fe}$ bed was greater than that on the F_{NH4} bed.

Moreover, the time to 1st phase of nitrification starting was shorter and the growth in nitrite content was more in $F_{NH4\text{-}Fe}$ bed (Fig. 16). Water subjected to biofiltration on the F_{NH4} bed was characterized by small changes in nitrate nitrogen content, $-0,18 \div 0,12$ mg $N\text{-}NO_3^-/dm^3$, whereas in conditioned water on the $F_{NH4\text{-}Fe}$ bed the differences were larger and were in the range of $-0,42 \div 0,46$ mg $N\text{-}NO_3^-/dm^3$ (Fig.17).

On the basis of the test results, it may be said that iron presence in conditioned water may contribute to raising the degree of ammonia nitrogen removal from it. That is confirmed by the research conducted in wastewater treatment station in Nieuw-Lekkerland, Holland [9]. That research was based on the assumption that subsurface aeration leads to oxidation of iron(II) to iron(III), while generating mobile colloids, which contributed to an approx.10%-more-effective ammonia-nitrogen removal from water, compared to the operation of filters that conditioned water with ammonia nitrogen only. However, it was noticed that bed volume may become locally reduced because of iron precipitation. Therefore, a reverse-flow flushing with water is recommended in order to prevent it.

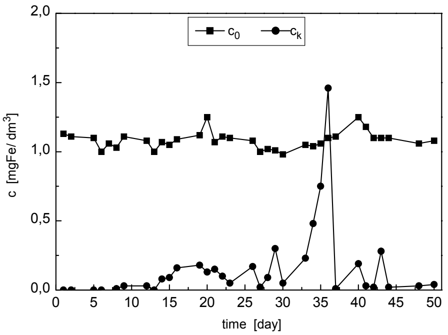

Fig 14. The changes of iron in F_{NH4-Fe} bed

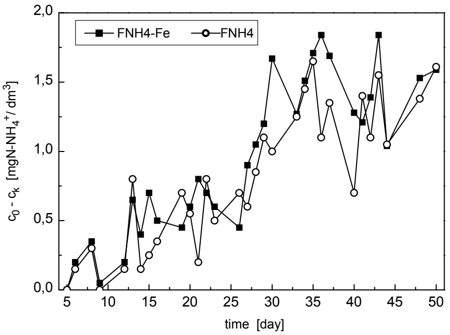

Fig 15. Comparison of ammonia nitrogen changes in F_{NH4-Fe} and F_{NH4} bed

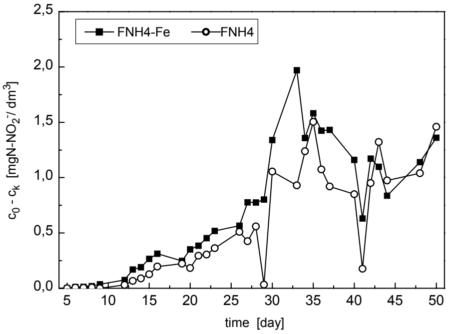

Fig 16. Comparison of nitrite nitrogen in F_{NH4-Fe} and F_{NH4} bed.

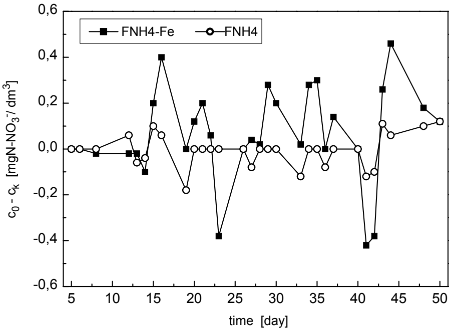

Fig 17. Comparison of nitrate nitrogen in F_{NH4-Fe} and F_{NH4} bed.

In the 30th day of bed operation, the nitrification bacteria numbers were determined in biological film formed on the bed grains. The numbers of nitrification bacteria on both beds were similar, the number of nitrification bacteria being 240 x 10^6 /1g of dry matter in both the upper and lower bed sections. It was the evidence of very high numbers of autotrophic nitrification bacteria. Independently of bed height and filling type the numbers *Nitrobacter* bacteria is higher than that of *Nitrosomonas* and it grows with bed depth to 1,5 m, further down the numbers of nitrification bacteria become smaller [10]. Small bed height was the probable cause of no differentiation in numbers of nitrification bacteria in studied filters.

The effect of iron presence on biofilm formation time and nitrification effectiveness was assessed on the basis of test results and photographs taken under JOEL JSM-5500 LV scanning microscope.

The study on the biofilm formation on various fillings have proven the significance of the character of their surface, resulting from substrate characteristics and pore structure [11]. The character of the structure of external grain surface affects the colonization by microorganisms. Multiplication of microorganisms takes place both in the intergranular spaces and in macro-pores that have dimensions enabling penetration of microorganism cells into them where they create spots sheltered from detrimental action of shearing forces from water flow [12].

The cause of inferior colonization of some fillings may be too smooth surfaces of their capillaries as well as lower accessibility of substrates inside non-through *(dead-end)* macro-pores. Besides, one of the theses says that biomass growth on bed is stimulated by the number of shelter spots [12], while the other states no relationship between biological activity and the type of bed. The third related these says that colonization character of external grain surface does matter for the colonization [13].

By comparing of microscopic pictures of raw chalcedonite grains with grains taken from filtration beds, one may observe a well developed mucous biological film surrounding bed grains, created by the activity of microorganism cells (photos 1, 2, 3). The other thing noticeable is that cell film sampled from the F_{NH4-Fe} bed indicated more differentiated (undulated) surface than that of the F_{NH4} bed. Besides, it was found that the film was thicker when sampled from lower layers of bed. The latter may be the evidence of higher numbers of heterotrophic bacteria, additionally confirmed by higher enzymatic activity of the biological film.

5. Conclusions

1. Chalcedonite bed may be used as filling of nitrification filter.
2. Iron presence in conditioned water affected the nitrification process by increasing its effectiveness, as well as contributed to shortening of biofilm formation time.
3. Both the filtration rate rise to 6 m/h and a twofold reduction of filtration rate to 1,5 m/h did not cause any significant changes in chemical parameters of water subjected to biofiltration process.
4. The final result of nitrification process is much affected by the surface of active biofilm, which is mainly determined by the characteristics of its substrate and the structure of pores.
5. The applied parameters of biofilters appear insufficient to achieve full nitrification process. No 2^{nd}-phase nitrification, leading to nitrite oxidation to nitrates, was observed.
6. Actual oxygen consumption was approx. 3 times lower than its theoretical value. That may indicate ammonia nitrogen removal by other ways than the nitrification.
7. Compared to bed where filtered water was without iron(III), higher oxygen consumption was observed on the bed, where iron was removed from water in addition to nitrification process.
8. The phenomenon of bacteria washing out of the biofilm occurred to least degree when higher filtration rates were applied.
9. Because of high numbers of psychrophilic and mesophilic bacteria in the filtrate, water disinfection process had to be conducted.
10. Dehydrogenase activity values indicate higher numbers of heterotrophic bacteria in the F_{NH4-Fe} bed.

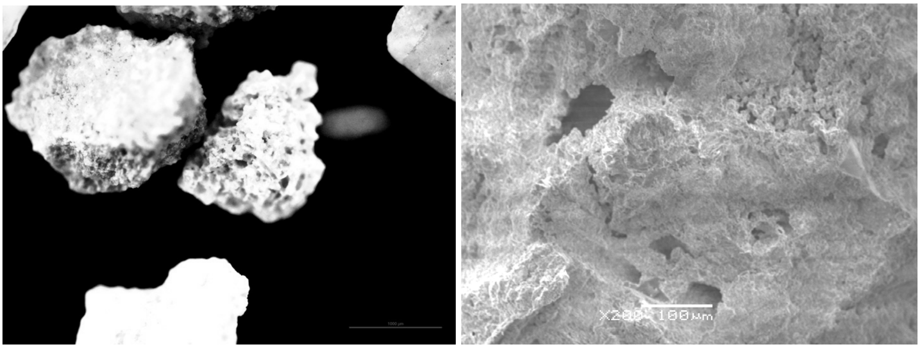

Photo 1. Natural raw chalcedonite grain

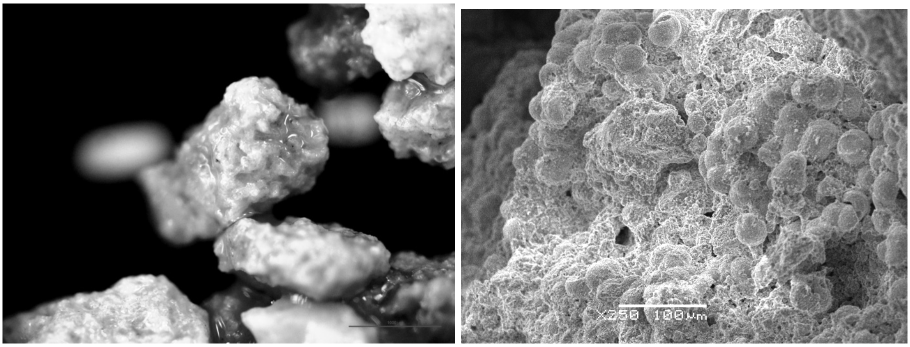

Photo 2. Biofilm on chalcedonite grain ($F_{NH4\text{-}Fe}$ bed)

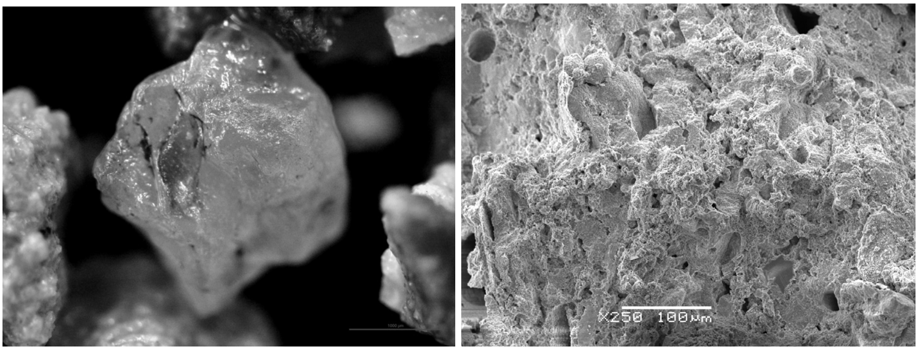

Photo 3. Biofilm on chalcedonite grain (F_{NH4} bed)

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