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# ISOLATION OF PURE CULTURES IRON- AND MANGANESE-OXIDIZING BACTERIA FROM RAPID FILTERS

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The aim of the research was the isolation from drinking water the pure cultures of iron- and manganese-oxidizing microorganisms with further assessment of their efficacy to remove these contaminants on rapid filters. To assess the effectiveness selected strains were grown on the solid nutrient medium; the suspension was prepared and was treated to zeolite loading. Ten pure cultures of iron- and manganese-oxidizing bacteria were isolated and identified as 6 genuses: *Siderocapsa, Leptothrix, Sphaerotillus, Galionella, Metallogenium, Hyphomicrobium*. Comparison the efficiency of genuses *Leptothrix, Sphaerotillus, Metallogenium* has shown that under conditions of these experiments *Leptothrix* more effectively removed iron and manganese at low concentrations in model solution.

***Key words***: iron- and manganese-oxidizing microorganisms, rapid filters, zeolite loading.

Iron compounds related to one of the common components in natural waters in Ukraine. Groundwater with iron is commonly found in almost all regions, sometimes the concentration of iron reaching more than 20–30 mg/dm$^3$. Iron removal from drinking water in low concentration does not cause difficulties, as long as it does not concern their high concentrations.

Well known that methods (both chemical and biological) for removal iron and manganese compounds differ in the degree of technological reliability, efficiency, ease of use, etc. [1–4]. However, the progressive development of biotechnological processes, and most importantly empower their implementation, does biotechnological methods as one of the most promising areas of water purification compounds of iron and manganese.

The basis of industrial biotechnology processes is accountability biological agent composition, so it is important to get a pure (in species belonging) culture. The manufacturability and efficiency of microorganisms are critical parameters for developing the technology of iron and manganese removal.

That is why the aim of our work was the identification of pure cultures of iron- and manganese-oxidizing microorganisms from drinking water with further assessment of their efficacy to remove these contaminants on rapid filters with zeolite filtration media. Rapid filters are widely used in the practice of water treatment. Usually, they are used for clarification of turbid and colored water after coagulation and settling, with reagent softening, iron removal and in other cases. It works by the principle of volumetric filtration when impurities remain in the pores of the filter throughout the entire volume of the charge as a consequence of the adherence of fine particles to the grains of the filter media.

## Materials and Methods

The cultures were isolated from water samples taken on the filters of the water treatment plant in Fastiv (Kyiv region, Ukraine). In raw water, the concentration of iron was 7.27 mg/dm$^3$.

The isolation of pure cultures was conducted by Drygalski method. Cultures were grown in two nutrient media No.1 and No.2 containing $(NH_4)_2SO_4$, $NaNO_3$, $K_2HPO_4$, $MgSO_4 \cdot 7H_2O$ — 0.5 g/dm$^3$ each of the reagents, citric acid — 10 g/dm$^3$, sucrose — 2 g/dm$^3$, pancreatic hydrolysate of casein — 1 g/dm$^3$,

$FeSO_4 \cdot 7H_2O$ — 5.9 g/dm$^3$ (for iron-oxidizing bacteria) and $MnSO_4 \cdot 5H_2O$ — 4.7 g/dm$^3$ (for manganese-oxidizing bacteria), agar-agar — 20 g/dm$^3$, distilled water — 1 dm$^3$, pH = 6.8 [5]. Capek medium with streptomycin was also used. The same media without iron and manganese were used for evaluating the effectiveness of strains.

Pure cultures were sub-planted for saving to the tubes on the media containing: $MnSO_4$ — 7 mg/dm$^3$, $(NH_4)_2SO_4$ — 1.5 g/dm$^3$, KCl — 0.05 g/dm$^3$, $K_2HPO_4$ — 0.05 g/dm$^3$, $Ca(NO_3)_2$ — 0.01 g/dm$^3$, glucose — 2 mg/dm$^3$, distilled water — 1 dm$^3$ [5].

To determine the species affiliation of isolated organisms their morphology, Gram staining, coloring iron and manganese oxides in capsules and covers were studied. The cells were observed under bright field at 1350 magnification (Leica ATC 2000); species affiliation was established by comparison with photos (Bergey's Manual of Systematic Bacteriology).

To determine the iron and manganese oxides in cellular structures cytochemical staining techniques were used: for $Fe^{3+}$ — potassium hexacyanoferrate (II); for $Mn^{4+}$ — benzidine solution. The presence of metal oxides, painted in blue color, found under a light microscope, as well as colonies that grew on the cups.

To assess the effectiveness selected strains were grown on solid nutrient medium; the suspension was prepared and was treated to zeolite loading. The inoculating process was taken place in non-flow mode.

After settling zeolite model solutions prepared on drinking water were passed through columns. Solution containing 1–2 mg/dm$^3$ Fe(II) and 0.2 mg/dm$^3$ Mn(II).

During flowing model solutions through columns biomass in 1 g per load, the total microbial count and residual concentration of iron and manganese: Fe(II) — with 2,2-bipyridyl; total manganese content — photo-colorimetric according to GOST 4974-72 (method B) were measured.

Columns with no processing zeolite seeds were controlled for quality of settling load with microorganisms (Output Fe(II) concentration — 1.3 mg/dm$^3$; output Mn (II) concentration — 0.1 mg/dm$^3$; Fe(II) removal efficiency — 65%; Mn(II) removal efficiency — 55%, TMN = 180 CCU/dm$^3$). The accuracy of the experiments was evaluated with common methods of processing of experimental data in chemical technology.

## Results and Discussion

*Stage 1. Bacterial Cultivation.* Growing of bacteria on Petri dishes with selective media No. 1 and No. 2 became noticeable for 4–5 day growth at 25 °C. Specific yellow-orange colonies were observed on a media for iron-oxidizing bacteria; the color of media had been changing from light green to ferruginous during cultivation. Specific brown colonies were observed on a media for manganese-oxidizing bacteria; the color of media had been changing from beige to brown during cultivation. The size and structure of colonies varied on both media.

In the application, Capek's medium with streptomycin development of iron- and manganese oxidizing bacteria was not observed. Accumulation of iron and manganese was identified only on the colonies' surface on a dish, which was insufficient for further work to identify the bacteria.

*Stage 2. Identification of Isolated Microorganisms.* The next step was the identification of isolated microorganisms with Gram's Method. Iron-oxidizing bacteria are Gram-negative, some strain have no cell wall, Gram-positive are not presented. Based on this principle and on morphology, were identified: spherical, ellipsoidal cells as *Siderocapsa*; cylindrical cells with a sheath as *Leptothrix*; rod-shaped cells as *Sphaerotillus*. Those cells, which are not stained by Gram and took the stalk cells, were assigned to genus *Galionella* (stalk cells a key feature of this genus).

The distinction between *Leptothrix* and *Sphaerotillus* was conducted with coloring iron oxides. It is known that *Leptothrix* accumulates iron oxides in a sheath, which painted in blue with potassium hexacyanoferrate (II); *Sphaerotillus* have a thin cover and hardly accumulate iron oxides, and thus not painted. This principle makes it possible to distinguish between these genuses of bacteria.

The same principle is for manganese-oxidizing bacteria, which grew on the selective medium No. 2. *Metallogenium* was identified by coloring with benzidine. This genus also has specific morphological features — colony in the form of "spider", allowing them fairly easy to detect, among other manganese-oxidizing bacteria. Research under the light microscope colonies on both media was allowed to identify *Hyphomicrobium*, a characteristic feature of which is the formation of the filament.

Ten pure cultures of iron- and manganese-oxidizing microorganisms have been isolated during experimental research. All strains were passaged to a liquid medium for storage.

*Stage 3. Evaluate the Effectiveness of Isolates.* On solid medium three cultures — *Siderocapsa*, *Galionella*, *Hyphomicrobium* – shown prevented growth: colonies were shallow. Although, the same cultures showed rise growth on the medium without iron, which can cause the ability of these bacteria to remove iron in association with other microorganisms.

Other strains — *Leptothrix, Sphaerotillus, Metallogenium, Siderocapsa, Galionella, Hyphomicrobium* — shown appreciable growth on solid medium: colonies were of yellow-orange color, the nutrient medium had changed color from light green to ferruginous, indicating that oxidation of ferrous iron to ferric. A key feature was that the colonies *Leptothrix, Sphaerotillus, Metallogenium* larger than others were and manifested their growth faster (5–6 days cultivation). Therefore, these cultures were used in further studies.

Table 1 shows the change of iron and manganese after passing model solution with a concentration of 2 mg/dm$^3$ Fe(II) and 0.2 mg/dm$^3$ Mn(II) through columns with microorganism *Leptothrix, Sphaerotillus, Metallogenium*.

Change of concentration during the whole filter period (8–48 h) was negligible: the effectiveness of iron removal on loadings with microorganisms lay within the 90–92%; for manganese — 80–90%. Therefore, we can assume that at low concentrations of elements, physical and chemical processes dominate biological. According to the data (Table 1) it can be assumed that iron and manganese at low concentrations were not removed by the biological way.

To identify which culture is the most effective against the removal of iron and manganese, the number of bacteria in 1 cm3 on columns was measured and the average efficiency of removing items each separately was estimated. The data are shown in Table 2.

Based on the data presented in Table 1 the diagrams of the efficiency of iron removal and manganese (Figure) by different genuses of microorganisms have been constructed.

According to bar charts on Figure, *Leptotrix* removes manganese effectively than *Sphaerotillus* and *Metallogenium*. Chemical oxidation prevailed over biological — TMN was low value (300 CCU/dm$^3$) and almost the same efficiency removal of iron and manganese were observed.

Ten pure cultures of iron- and manganese-oxidizing bacteria were isolated from water samples taken on the filters of the water treatment plant in Fastiv and identified as 6 genuses: *Siderocapsa, Leptothrix, Sphaerotillus, Galionella, Metallogenium, Hyphomicrobium.*

In the experiments with a model solution was shown that in a low concentration of these elements they remove physic-chemical way. The evidence is lack of lag-phase on the beginning of filtration, low data of TMN, practically the same efficiency of removing manganese and iron with three cultures.

While evaluating the effectiveness of isolates by removal of iron and manganese was shown that three cultures — *Siderocapsa*, *Galionella*, *Hyphomicrobium* were weaker and growth slowly than other *Sphaerotillus, Leptothrix, Metallogenium.*

Obtained results on the isolation of iron and manganese-oxidizing bacteria correlate with the work of other authors [6, 7]. In [6] authors

*Table 1.* **Changing the concentration of iron and manganese**

| Time, hours | Output concentration, mg/dm$^3$ | | Removal efficiency,% | |
|---|---|---|---|---|
| | Fe(II) | Mn(II) | Fe(II) | Mn(II) |
| Leptothrix | | | | |
| 8 | 0.20* | 0.03* | 90** | 85** |
| 16 | 0.16* | 0.04* | 92** | 80** |
| 24 | 0.16* | 0.03* | 92** | 85** |
| 36 | 0.15* | 0.02* | 93** | 90** |
| 48 | 0.18* | 0.02* | 91** | 90** |
| Sphaerotillus | | | | |
| 8 | 0.18* | 0.03* | 91** | 85** |
| 16 | 0.17* | 0.03* | 92** | 85** |
| 24 | 0.18* | 0.03* | 91** | 85** |
| 36 | 0.17* | 0.03* | 92** | 85** |
| 48 | 0.18* | 0.02* | 91** | 90** |
| Metallogenium | | | | |
| 8 | 0.18* | 0.03* | 91** | 85** |
| 16 | 0.18* | 0.04* | 91** | 80** |
| 24 | 0.18* | 0.03* | 91** | 85** |
| 36 | 0.20* | 0.04* | 90** | 80** |
| 48 | 0.18* | 0.04* | 91** | 80** |

*Note: here and in Figure* * — $P < 0.05$, in comparison with control: output Fe(II) concentration — 1.3 mg/dm$^3$; output Mn (II) concentration — 0.1 mg/dm$^3$;

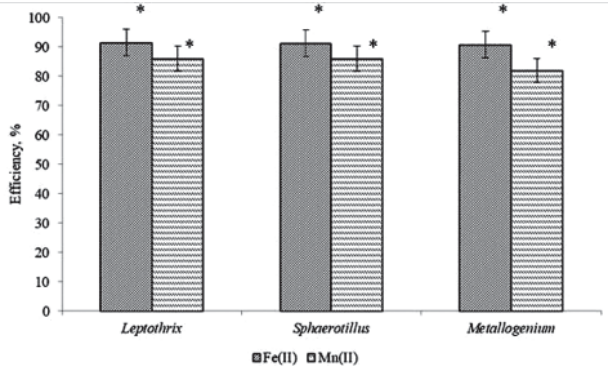
*here and in Table 2* ** — $P < 0.05$, in comparison with control: Fe(II) removal efficiency — 65%; Mn(II) removal efficiency — 55%.

*Table 2.* **Evaluation of removal of iron and manganese with microorganisms**

| Genus | TMN, CCU/dm$^3$ | The efficiency of removing Fe(II),% | The efficiency of removing Mn(II),% |
|---|---|---|---|
| *Leptothrix* | 300* | 91.5** | 86.0** |
| *Sphaerotillus* | 300* | 91.2** | 86.0** |
| *Metallogenium* | 250* | 90.8** | 82.0** |

*Note:* * — $P < 0.05$, in comparison with control: TMN = 180 CCU/dm$^3$;

** — $P < 0.05$, in comparison with control: Fe(II) removal efficiency — 65%; Mn(II) removal efficiency — 55%.




**Average efficiency of iron and manganese removing**

*Note:* * — $P < 0.05$, in comparison with control: Fe(II) removal efficiency — 65%; Mn(II) removal efficiency — 55%.

isolated iron- and manganese-oxidizing bacteria from the bottom sediments of Lake Baikal and carried them to six genera: *Metallogenim, Lepthotrix, Siderocapsa, Naumaniella, Bacillus* and *Pseudomonas*. Also it was found that cultured ferric bacteria possess high oxidative activity. In [7] it was established that it is possible to inhibit the development of some and the intensive growth of other cultures of *Galionella, Lepthotrix, Metallogenim* depending on the physicochemical composition of water with a relatively constant effect of treatment.

So, comparison the efficiency of genuses *Leptothrix, Sphaerotillus, Metallogenium* has shown that under conditions of these experiments *Leptothrix* effectively removed iron and manganese at low concentrations in solution. In further research is planned to evaluate the effectiveness of iron and manganese removal by mixed cultures and the ability to use biotechnology techniques to improve isolated strains.

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## ВИДІЛЕННЯ ЧИСТИХ КУЛЬТУР ЗАЛІЗО- ТА МАРГАНЕЦЬОКИСНЮВАЛЬНИХ БАКТЕРІЙ ІЗ ШВИДКИХ ФІЛЬТРІВ

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Метою дослідження було виділення з питної води чистих культур мікроорганізмів, здатних окиснювати залізо та марганець, з подальшим оцінюванням їхньої ефективності з метою видалення цих речовин на швидких фільтрах. Для оцінювання ефективності вибрані штами вирощували на твердому живильному середовищі, готували суспензію та обробляли нею цеолітове завантаження. Виділено 10 чистих культур мікроорганізмів, здатних окиснювати залізо та марганець, які віднесено до 6 родів: *Siderocapsa, Leptothrix, Sphaerotillus, Galionella, Metallogenium, Hyphomicrobium*. Порівняння ефективності родів *Leptothrix, Sphaerotillus, Metallogenium* показало, що в умовах цих експериментів бактерії роду *Leptothrix* ефективніше видаляють залізо і марганець за низьких концентрацій у модельному розчині.

***Ключові слова:*** мікроорганізми, здатні окиснювати залізо та марганець, швидкі фільтри, цеолітове завантаження.

## ВЫДЕЛЕНИЕ ЧИСТЫХ КУЛЬТУР ЖЕЛЕЗО- И МАРГАНЕЦОКИСЛЯЮЩИХ БАКТЕРИЙ ИЗ СКОРЫХ ФИЛЬТРОВ

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Целью исследования было выделение из питьевой воды чистых культур микроорганизмов, способных окислять железо и марганец, с последующей оценкой их эффективности для удаления этих веществ на скорых фильтрах. Для оценки эффективности выбранные штаммы выращивали на твердой питательной среде, подготавливали суспензию и обрабатывали ею цеолитовую загрузку. Выделено 10 чистых культур микроорганизмов, способных окислять железо и марганец, которые отнесены к 6 родам: *Siderocapsa, Leptothrix, Sphaerotillus, Galionella, Metallogenium, Hyphomicrobium*. Сравнение эффективности родов *Leptothrix, Sphaerotillus, Metallogenium* показало, что в условиях данных экспериментов бактерии рода *Leptothrix* более эффективно удаляют железо и марганец при низких концентрациях в модельном растворе.

***Ключевые слова:*** микроорганизмы, окисляющие железо и марганец, скорые фильтры, цеолитовая загрузка.