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USAGE OF *Lemna minor* DUCKWEED FOR MALT PLANT WASTEWATER TREATMENT FROM FERRUM COMPOUNDS

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The aim of the work was to determine the rational parameters of the malt plant wastewater treatment from ferric compounds in a flow-through experimental bioreactor with the use of duckweed. The main tasks of the study were as follows: to determine the effect of the initial concentration of the ferrum, the amount of biomass introduced and the duration of the purification process to reduce the content of ferric compounds in the wastewater.

The studies were carried out on existing sewage treatment plants. They used a semi-production unit incorporated into the technology before the decontamination step.

The results show that the logarithmic nature of the biological process was detected in the concentration range from 0.2 to 1.3 mg/dm^3. Under these conditions, the rational values of the of the purification process duration is found to be 3-8 h with the *Lemna minor* biomass value not exceeding 12 g/dm^3.

It has been first demonstrated that the effect of sewage treatment from ferric ions in the bioreactor with *Lemna minor* was up to 40% and depended on the initial concentration of the ferrum compounds in water at the existing wastewater treatment plants at a semi-production unit for biological treatment of wastewater from ferric compounds.

Key words: wastewater, biological treatment, iron, duckweed, fibrous carrier, malt plant.

The activities of food industry, in particular malt production, include technological processes of washing and processing of raw materials, which result in wastewater containing suspended solids, dissolved organic, nitrogen and phosphorus compound, ions of heavy metals, salts, etc. above maximum admissible concentrations and therefore need to be properly threaten before being discharged into the natural water body.

The technological process of malt production includes the following steps: reception of barley grain, its cleaning, sorting and soaking, germination of barley grains, drying and reflection of sprouts, storage and release of malt. As a result, wastewater with a high content of organic matter, nitrogen, phosphorus, etc. is formed. Also, wastewater contains ferric compounds (III) bound to organic substances in chelate complexes and covalent bonds in water-soluble compounds [1]. An additional source of ferric input to the water is the addition of $FeCl_3$ ferrous coagulant in the wastewater treatment process to increase the efficiency of precipitation of activated sludge (due to its swelling) and the purification of wastewater from phosphates.

A number of scientists studied the heavy metal ions removal from the water of various origin by duckweed *Lemna minor* [2–7]. At the wastewater treatment, a high degree of ion ferrous removal was obtained in 4–5 days, which was 80 ± 5% [4]. To date, no information has been found on the effect of duckweed planting density on the kinetics of the process and on the degree of removal of iron ions from water [8, 9]. *L. minor* has a high potential for removal a wide range of pollutants such as phosphorus, nitrogen, fluorine, copper, manganese, arsenic, cadmium, chromium, nickel, and ferrous from wastewater [2, 4, 10–13]. It is known that *L. minor* grows well and is stable in an environment with organic contamination and doubles its mass for

5–6 days [14–16]. Duckweed can be fermented with sludge in methane tank for biogas production [17, 18].

The iron in wastewater may be presented in the form of Fe (II), Fe (III), and ferrous organic compounds [19]. Fe (II) accumulates in the form of an oxidized film Fe (III) on the roots of plants due to oxygen release from the roots [2]. The formation of such a film promotes the adsorption of phosphorus compounds from water [20]. The mechanism of Fe (III) uptake into the duckweeds cell occurs due to the preliminary reduction of Fe (II) by ferric-chelate reductase at the surface of the cell membrane and subsequent transfer of Fe (II) into the cell by transport protein [21, 22]. A chelating agent is required for the process [23, 24]. In the presence of heavy metals ions in the environment of higher plants, phytochelatins are produced in the form of peptide tails constructed from γ-glutamylcysteine on the cell wall [25]. The mechanism is well studied in the model organism *Arabidopsis thaliana* and is characteristic of most plants, except herbs [26, 27]. The partial absorption of Fe (III) ions by the cell wall is possible, however, according to the results of studies [28], the iron ions is subsequently transported to the plant by the above mechanism. Further, Fe (II) accumulates in cells of the leaf`s mesophyll and serves as a catalyst in the synthesis of chlorophyll at the stage of formation of aminolevulinic acid and the synthesis of protoporphyrin. Iron is required in the synthesis of cytochromes [29].

The aim of the work was to determine the rational parameters of the malt plant wastewater treatment from ferric compounds in a flow-through experimental bioreactor with the use of duckweed. The main tasks of the study were to determine the effect of the initial concentration of the ferum, the amount of biomass introduced and the duration of the purification process to reduce the content of ferric compounds in the wastewater.

To achieve the aim, the following objectives were investigated:

1. To conduct a series of studies in a semi-production experimental bioreactor for a long time with the different technological parameters (biomass of duckweed and biofilm, wastewater flow) in the presence of fluctuations in wastewater composition at the inlet.

2. To analyze the experimental data obtained and set rational process parameters.

Materials and Methods

Lemna minor was picked up from a cultivator and grown in non-sterile conditions with the following composition of water: pH — 7.0; dissolved oxygen — 3.4 mg/l; NH_4^+ — 1.84 mg/l; NO_2^- — 0.74 mg/l; NO_3^- — 28.74 mg/l; PO_4^{3-} — 3.84 mg/l.

The studies were carried out on existing wastewater treatment facility. The chemical composition of wastewater is shown in Table. The wastewater at the facility undergoes the following stages of purification: mechanical sieves (from barley husk), averaging, anaerobic-aerobic biological treatment in anaerobic reactor, aeration tank, separation of treated water and activated sludge in the secondary sludge and further disinfection with sodium hypochlorite solution and discharged into the river.

A semi-production unit was used to perform the research. The scheme of unit, which was incorporated into the technology before the disinfection step, is shown in Fig. 1. For the wastewater supply, a system consisting of the following elements was used: existing tray 1 for wastewater disposal after the secondary settling tanks; centrifugal pump 2; waste water tank 3; overflow tube 4; connection tube 5. The flow rate was established and regulated by a dispenser 6 in the range from 18 to 76 l/h. The 225.6 l bioreactor 7 consists of 4 rectangular tanks measuring 1 410×250×250 mm with water level h = 160 mm. The tubes are connected in series with tubes 8. In the first container cartridges are installed with fibrous carrier 9 in the amount of 12 pcs. size 160×160 mm each. The

Malt plant wastewater composition

Values	pH	COD, mg/l	SS, mg/l	NH_4^+, mg/l	NO_2^-, mg/l	NO_3^-, mg/l	$P_2O_5^{3-}$, mg/l	SO_4^-, mg/l	$Fe_{gen.}$, mg/l
Before treatment	6.8	2850	290	32.0	0.08	—	61	121	2.2
After treatment	7.0	115	24	1.5	0.02	30	10	34	0.9

Note: SS — suspended solids.

scheme is shown in Fig. 2. Natural light was used for the cleaning process.

The installation works as presented in Fig. 1. The waste water from the tray 1 was pumped by the pump 2 into the tank 3. The excess water was returned to the tray 1 through the overflow tube 4, thus ensuring a stable water level in the tank 3. From there, water through the wastewater tube 5 with a dispenser 6 was fed into the bioreactor 7 with a given steady flow rate. In the bioreactor 7, the wastewater passes through sequentially connected tanks, contacts the carrier 9 with the microbial organisms immobilized therein, and duckweed growing in the surface layer. Wastewater is treated in the bioreactor. Water is diverted by pipeline 10 into tray 1 for further disinfection.

Sampling of wastewater was performed from tank 3; at the outlet of the bioreactor 7 with fibrous media by means of a siphon directly from the reservoir after the media cassettes, to investigate the effect of the purification process parameters on the degree of decrease in the concentration of ferrum compounds. Also, the samples were taken at the outlet of the pipeline 10. During the course of the study, wastewater flow rates were changed to 19, 28 and 75 l/h and biomass values were 12 and 25 per g/l of water in the bioreactor.

Determination of the ferrum concentration in wastewater was performed according to the standard colorimetric method with ammonium thiocyanate. ULAB 102 spectrophotometer was used. The method is suitable for measuring iron concentrations in the range of 0.05 to 4.0 mg/l. According to the method, the following steps were performed: 50 ml of sample water is transferred into a heat resistant conical flask. Then, for removal of interfering organic matter in wastewater sample, 1 ml of concentrated sulfuric and nitric acids are added, stirred and gently evaporated on an electric tile until thick acid vapors have appeared (up to about 5–10 ml sample volume). After cooling, 5 ml of dilute nitric acid (1:9) is added to the evaporated residue and filtered into a volumetric flask. Distiled water is added to bring the volume of evaporated sample to 50 ml. Then 2.5 ml of hydrochloric acid solution is (1:1) added and stirred. Next, 5 ml of 20% potassium rhodanide solution is added, stirred and the optical density is measured at a wavelength of $\lambda = 425$ nm in a 5 cm cell for concentrations of 0.05 to 1.00 mg/l and 10 cm is measured immediately (not later than 3 min) for concentrations of 0.5–4.0 mg/l.

All experiments were performed in triplicates. Statistical data analysis was conducted using Microsoft Excel 2016 software. Difference between two mean values was considered significant at $P < 0.05$.

Results and Discussion

According to the results (Fig. 2) it is obvious that the decrease in the concentration of the ferrum occurs in logarithmic dependence, which indicates the increase of

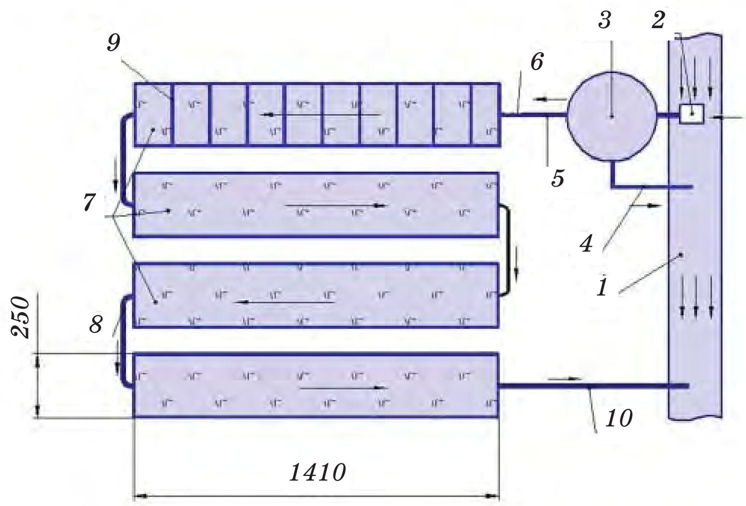

Fig. 1. **Scheme of semi-production experimental unit:**
1 — tray for wastewater disposal after the secondary settling tanks; *2* — centrifugal pump; *3* — waste water tank; *4* — overflow tube; *5* — plumbing tube; *6* — dispenser; *7* — bioreactor; *8* — tubes; *9* — fibrous carrier; *10* — drainage pipeline

the purification effect with the increase of the ferrum in the wastewater at the inlet of the bioreactor. This can be explained by the fact that some of the contaminants are likely to be forms of ferum, which are strongly bound to organic molecules that do not precipitate or are removed by the plants. The sharp increase in the degree of purification at high concentrations of ferrum can be explained by the presence of chelated complexes of ferrum (III), which are restored on the surface of the roots of the plant and sorbed by them [30], and at a significant increase in concentrations — the formation of ferric hydroxide.

Due to the constant change in the concentration of pollutants in the wastewater during the operation of the treatment plant, it is impossible to compare the degree of purification under different technological parameters at certain constant concentrations, but it is possible to compare the approximate graphical dependencies. As it was revealed from the obtained results, there was no significant change in the degree of sewage treatment of the malting plant from ferric ions, while changing the wastewater flow rate and biomass value, as is shown in Fig. 3. It can be seen that all points, despite the change in biomass and duration of the purification process, show same logarithmic dependence with the divergence within the measurement error.

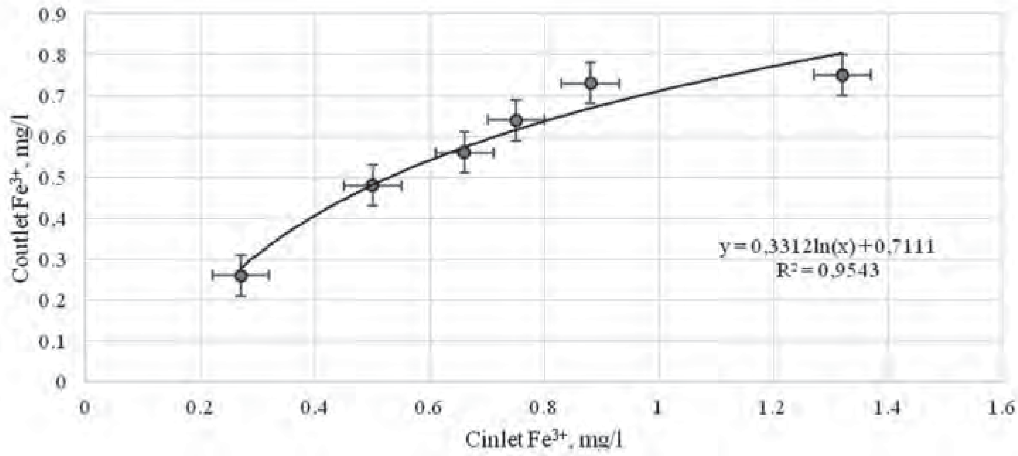

Fig. 2. **Dependence of the ferric iron concentration Coutlet in wastewater after treatment on the ferric iron concentration Cinlet before treatment in experimental bioreactor at the duration of purification t = 12 h and the value of biomass B = 24 g/l**

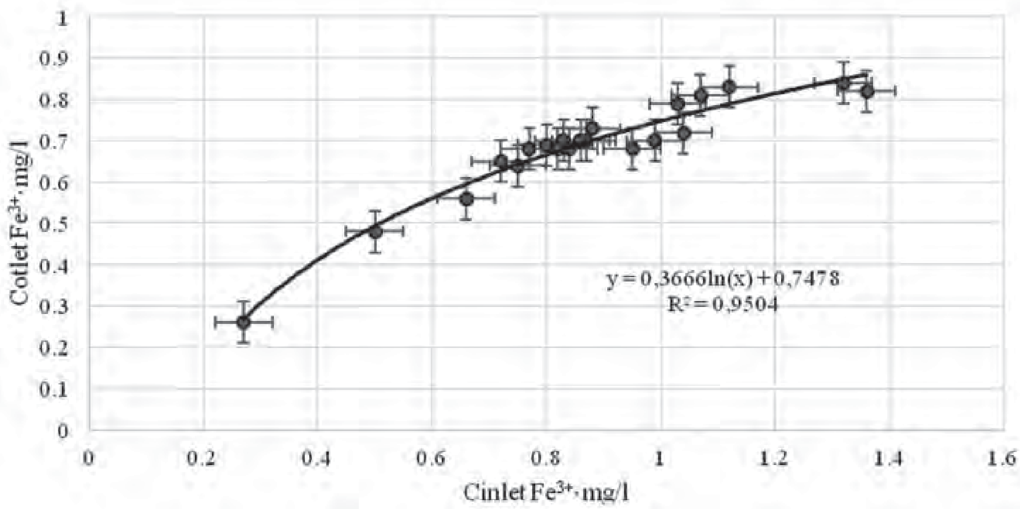

Fig. 3. **Dependence of the ferric iron concentration Coutlet in wastewater after treatment on the ferric iron concentration Cinlet before treatment in experimental bioreactor at at all studied the durations of purification and the values of biomass**

In this case, the question arises: is wastewater treatment related to duckweed biomass? The control experiment without biomass of duckweed (Fig. 4) shows an increase in the concentration of ferum, which can be explained by the result of the flow of the processes of the organic substances decomposition with the recovery of ferric iron into a ferrous form due to the processes of anaerobic respiration of microbiocenosis [31].

Studies of the wastewater treatment plant effluent at a semi-production plant over a 26-day period showed a significant fluctuation in the ferrous content in the inlet wastewater, which is related to changes in wastewater flow rate and their qualitative composition due to the periodic nature of the technological processes (Fig. 5). The minimum concentration of ferrum at the entrance to the plant was 0.2 mg/l and the maximum — 1.4mg/l.

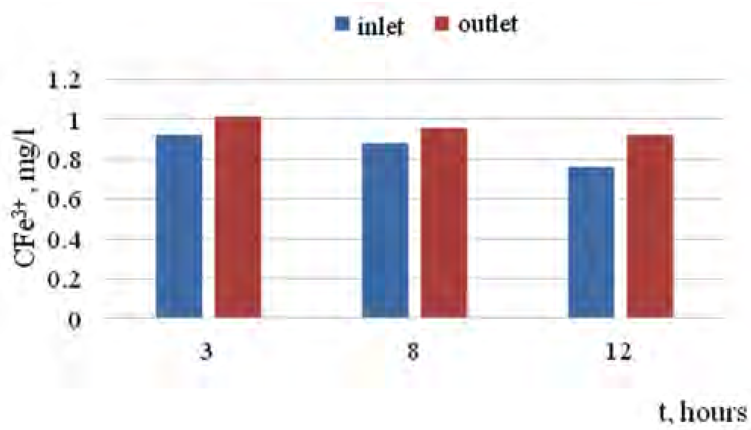

Fig. 4. **Change in the concentration of ferrum (III) in a control experiment without biomass of plants at 3, 8 and 12 h of treatment**

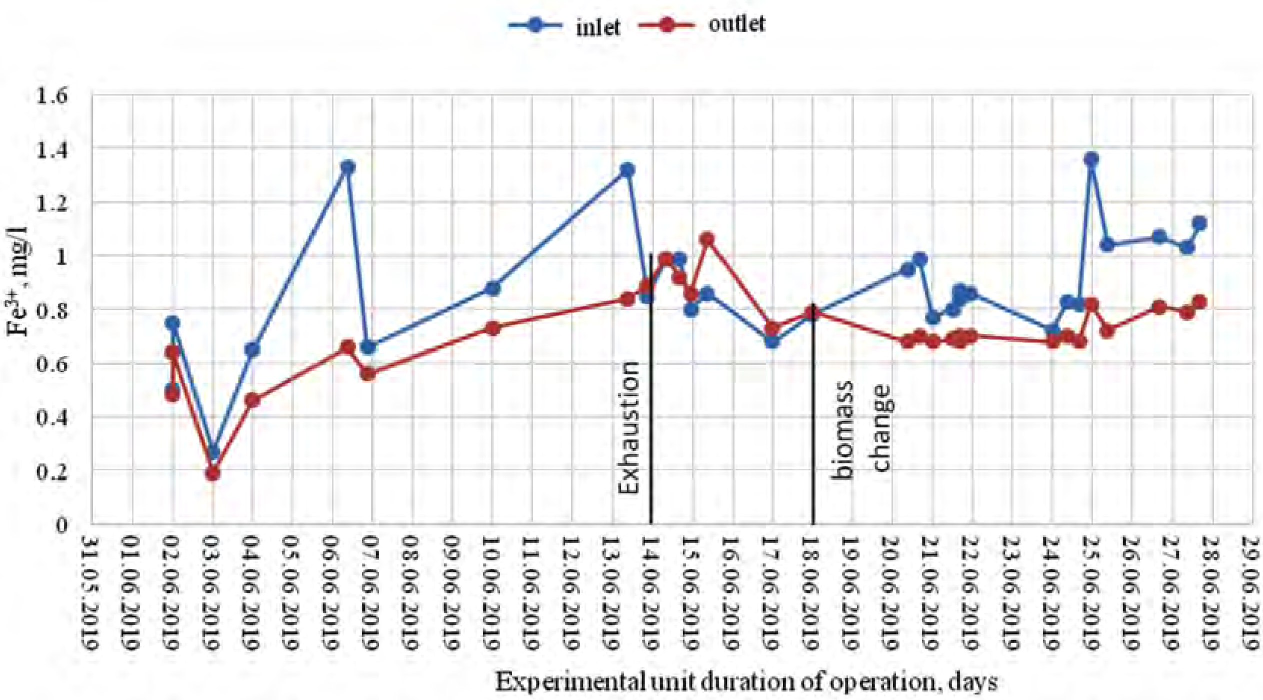

Fig. 5. **Change in the concentration of ferrum (III) in wastewater at the inlet and outlet of the bioreactor throughout experiment**

At the outlet of the experimental unit, the treated wastewater had reduced amplitude of oscillations except for the first three days (as a result of the minimum concentration of the ferrum at the entrance to the installation during that period and incomplete operation efficiency into the working mode due to the period of duckweed and microbiocenosis adaptation period). Also, the phenomenon of duckweed biomass depletion was detected after 13 days from the start of the installation, after which the used biomass was replaced with a new portion — from the cultivator. The biomass replacement process was resumed immediately after biomass replacement.

Conclusions

As a result of research carried out on existing wastewater treatment plants at a semi-production plant for biological treatment of wastewater from ferric compounds, it was first established that the effect of sewage treatment from ferric ions in the bioreactor with *Lemna minor* are up to 40% and *Lemna minor* depends on the initial concentration of the ferrum compounds in water. In the concentration range from 0.2 to 1.3 mg/l, it was found the logarithmic nature of the biological process. Under these conditions, the rational parameters for the purification process are found to be 3—8 h with the *Lemna minor* biomass value not exceeding 12 g/l.

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ВИКОРИСТАННЯ РЯСКИ *Lemna minor* ДЛЯ ДООЧИЩЕННЯ СТІЧНИХ ВОД СОЛОДОВОГО ЗАВОДУ ВІД СПОЛУК ФЕРУМУ

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Метою роботи було визначити раціональні параметри очищення стічних вод солодового заводу від сполук феруму в проточному експериментальному біореакторі із застосуванням ряскових і встановити вплив вихідної концентрації феруму, кількості внесеної біомаси та тривалості процесу очищення на зниження вмісту сполук феруму у стічній воді.

Дослідження проводили на очисних спорудах біологічного очищення стічних вод солодового заводу. Застосовували напіввиробничу установку, яку було введено в технологію перед етапом знезараження.

Як показують одержані результати, в діапазоні концентрацій іонів феруму від 0,2 до 1,3 мг/дм^3 було виявлено логарифмічний характер біологічного процесу. Виявлено, що за цих умов раціональні значення тривалості процесу очищення — 3–8 год за наявності біомаси ряски *Lemna minor* не більше 12 г/дм^3.

У результаті досліджень, проведених на очисних спорудах для очищення стічних вод солодового заводу, які було виконано на напіввиробничій установці для біологічного доочищення стічних вод від сполук феруму, вперше встановлено, що ефект очищення стічних вод від іонів феруму в біореакторі з ряскою *Lemna minor* сягає 40% і залежить від вихідної концентрації сполук феруму у воді.

Ключові слова: стічні води, біологічне очищення, ферум, ряскові, волокнисте завантаження, солодовий завод.

ИСПОЛЬЗОВАНИЕ РЯСКИ *Lemna minor* ДЛЯ ДООЧИСТКИ СТОЧНЫХ ВОД СОЛОДОВОГО ЗАВОДА ОТ СОЕДИНЕНИЙ ЖЕЛЕЗА

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Целью работы было определение рациональных параметров очистки сточных вод солодового завода от соединений железа в проточном экспериментальном биореакторе с применением рясковых и установление влияния исходной концентрации железа, количества внесенной биомассы и длительности процесса очистки на снижение содержания соединений железа в сточной воде.

Исследования осуществляли на действующих очистных сооружениях биологической очистки сточных вод солодового завода. Использовали полупроизводственную установку, которая была включена в технологию перед этапом обеззараживания.

Как свидетельствуют полученные результаты, в диапазоне концентраций от 0,2 до 1,3 мг/дм^3 был обнаружен логарифмический характер биологического процесса. Выявлено, что при данных условиях рациональные значения продолжительности процесса очистки — 3–8 ч при величине биомассы *Lemna minor* не более 12 г/дм^3.

В результате исследований очистки сточных вод солодового завода, которые были выполнены на полупроизводственной установке для биологической доочистки сточных вод от соединений железа, впервые установлено, что эффективность очистки сточных вод от ионов железа в биореакторе с *Lemna minor* достигает 40% и зависит от исходной концентрации соединений железа в воде.

Ключевые слова: сточные воды, биологическая очистка, железо, рясковые, волокнистая загрузка, солодовый завод.