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DETERMINATION OF THE REMOVAL EFFICIENCY OF CHLORAMPHENICOL FROM WASTEWATER DEPENDING ON *LEMNA MINOR* BIOMASS

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Abstract. The article is dedicated to studying the effectiveness of wastewater treatment contaminated with chloramphenicol using *Lemna minor* with a specific biomass of 36 and 50 g/L. Purification of model solutions with an antibiotic concentration of 2–20 mg/L continued for 1–72 hours. The conducted research showed that the degree of chloramphenicol removal depends on the specific biomass of plants and the time of the process. The greatest decrease in the content of the antibiotic was observed during 24–48 hours of the purification process, then the efficiency of its removal decreased and after 72 hours it practically did not change. For concentrations of 2 and 5 mg/L with a specific biomass of *L. minor* of 36 g/L, the purification efficiency in 72 hours reached 23.2 % and 26.8 %, respectively. When the biomass increased to 50 g/L, the efficiency was 17 % and 19 %, respectively. The removal efficiency of chloramphenicol at a concentration of 10 mg/L reached 33 % when the specific biomass of *L. minor* was 36 g/L, and at a concentration of 20 mg/L – 29.5 %. For a specific biomass of 50 g/L, this indicator was 23.6 % with an antibiotic content of 10 mg/L and 21% with a content of 20 mg/L. According to the obtained results, the rational parameters of the cleaning process were established: time 48 hours and specific biomass 36 g/L allowed to achieve 29.4 % efficiency of chloramphenicol removal from wastewater at its initial concentration of 10 mg/L. Further increase in treatment time has a negligible effect on the increase in purification efficiency. An increase in duckweed biomass leads to a decrease in the efficiency of antibiotic adsorption. To process duckweed after its use in wastewater treatment to remove antibiotics fermentation technology in a methane tank can be employed along with other station waste.

Keywords: wastewater, treatment, biological method, duckweed, antibiotics.

1. Introduction

Environmental pollution by pharmaceuticals, particularly antibiotics, has become a significant ecological and societal problem with growing scale and impact. Antibiotic resistance driven by the accumulation of pharmaceutical substances in natural water bodies has already been recognized as a global threat requiring urgent action. Chloramphenicol, a broad-spectrum antibiotic found in wastewater due to medical and veterinary use, can enhance pathogen resistance adversely affect aquatic flora and fauna and penetrate food chains (Ahammad et al., 2021; Ahmed et al., 2017; Ahmed et al., 2017).

The insufficient efficacy of traditional wastewater treatment methods for removing pharmaceutical residues like chloramphenicol necessitates innovative approaches. One such method is biological purification using aquatic plants such as *Lemna minor* (duckweed) which demonstrates high pollutant removal efficiency due to its rapid biomass growth and ability to absorb toxic substances (Ambekar et al., 2000; Balarak et al., 2020).

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Chloramphenicol is an antibiotic capable of inhibiting bacterial infections in animals and humans. However, due to its toxicity and severe side effects its use is strictly controlled or prohibited in veterinary medicine in many countries. Despite such restrictions, chloramphenicol concentrations are still detected in wastewater within a range of 0.1 to 2 µg/L attributed to discharges from medical and veterinary facilities (Ahammad et al., 2021; Busch et al., 2020; Carrales-Alvarado D. H. et al., 2020; Chaturvedi et al., 2021; Chen et al., 2021). Once in water bodies the antibiotic can contribute to pathogen resistance and pose a threat to human health creating a global problem (Busch et al., 2020; Chen et al., 2020; Chen et al., 2020).

Modern wastewater treatment technologies often struggle to remove contaminants like chloramphenicol driving the search for environmentally friendly and cost-effective alternatives. Biological purification using aquatic plants is a promising method as plants can absorb, transform and accumulate toxins (Carrales-Alvarado D. H. et al., 2020; Cheng et al., 2020; Choi et al., 2008; Bhargava et al., 2023). The use of *Lemna minor* for chloramphenicol removal from wastewater relies on its ability to reduce pollutant concentrations through photosynthesis, rapid growth and metabolism (Chaturvedi et al., 2021; Chen et al., 2021; Lai et al., 2009).

Research indicates that the effectiveness of pollutant removal is directly related to the biomass of duckweed in treatment systems. Increasing the biomass of *Lemna minor* enhances its pollutant absorption capacity as a greater contact surface with water results in improved uptake capabilities (Chen et al., 2021; Lai et al., 2009; Leston et al., 2013). Additionally, duckweed produces enzymes that contribute to the breakdown of pollutant molecules. Understanding the relationship between biomass and pollutant removal efficiency is crucial for designing biological purification systems capable of handling high contaminant loads (Chen et al., 2020; Li A. et al., 2020; Li P. et al., 2020).

Aquatic plants and activated sludge are used to remove heavy metals such as chromium, lead and also pharmaceuticals. For example, *Pistia stratiotes* (water lettuce) is successfully applied to reduce heavy metal concentrations in polluted waters; the removal efficiency of 1 mg/L and 3 mg/L of lead and 1 mg/L of chromium is 99.13 %, 79.86 % and 76.25 %, respectively (Zahari et al., 2021). *Eichhornia crassipes* (water hyacinth) helps in the purification of water from phenols and cyanide showing absorption efficiencies of 96.42 % for phenol (300 mg/L) and 92.66 % for cyanide (30 mg/L) (Singh and Balomajumder, 2021). Activated sludge is capable of metabolizing organic compounds, pharmaceuticals and heavy metals due to the activity of microorganisms (Libing & Jianlong, 2023).

The relevance of research on the biological treatment of chloramphenicol with the help of *Lemna minor* is growing in the context of the global problem of antibiotic resistance and water pollution. The relationship between plant biomass and antibiotic removal efficiency forms the basis for developing new wastewater treatment technologies.

The aim of this work is to determine the efficiency of wastewater purification from the antibiotic chloramphenicol using *Lemna minor*, depending on the initial antibiotic concentration, the time of the process and the specific biomass of duckweed.

2. Materials and Methods

The purification process was studied in laboratory conditions on model solutions that simulated wastewater containing chloramphenicol at concentrations of 2, 5, 10 and 20 mg/L. The process was investigated in a static mode in bioreactors with *Lemna minor* biomass at specific concentrations of 36 and 50 g/L with sampling of the purified solutions after 1, 2, 4, 6, 21, 24, 48 and 72 hours.

To determine the chloramphenicol content in the purified solutions liquid chromatography and a calibration curve were used. To confirm the hypothesis that the reduction in antibiotic content occurs specifically due to the action of duckweed control studies were conducted on model solutions without *Lemna minor*.

Chromatographic analysis of the samples was performed using a liquid chromatograph Agilent 1260 Infinity II and results were processed with Agilent OpenLab software.

For the quantitative determination of the content of chloramphenicol the areas of the peaks on the graph were used, the value of which is proportional to the antibiotic concentration in the sample.

To calculate the removal efficiency of chloramphenicol (E, %) from the pharmaceutical wastewater, the following formula was used:

$$E = \frac{C_0 - C_t}{C_0} \cdot 100,$$

where C_0 is the initial concentration of chloramphenicol, mg/L; C_t is the concentration of chloramphenicol in the treated model solutions for t hours, mg/L; 100 is for converting the result into percentage.

3. Results and Discussion

In the control bioreactors (without *L. minor*) all values of chloramphenicol concentrations (2–20 mg/L) remained almost unchanged for 72 hours. This suggests that without the addition of duckweed no reduction in antibiotic concentration occurs (Figs. 1, 2).

The change in the content of chloramphenicol in model solutions depending on the time of purification in bioreactors with *L. minor* biomass of 36 g/L is shown in Fig. 1.

Increasing the purification time in bioreactors with *L. minor* biomass at 36 g/L leads to a reduction in the content of chloramphenicol in solutions for all initial concentrations of the antibiotic. For instance, at an initial concentration of 20 m g/L the antibiotic content decreases to 14.34 mg/L after 72 hour s of purification. Extending the time over 48 hours almost does not change the content of the antibiotic in the purified solutions.

It was established that the dependences of the content of chloramphenicol in solutions purified for 72 hours using *Lemna minor* with a biomass of 36 and 50 g/L at initial values of 2–20 mg/L are almost the same.

A decrease in the content of chloramphenicol was observed in solutions using *L. minor* with a biomass of 50 g/L for all studied concentrations. At an initial concentration of 20 m g/L after 72 hour s of purification the content of the antibiotic decreases to 15.97 mg/L After 48 hours of the cleaning process the content of the antibiotic in the solutions becomes practically unchanged.

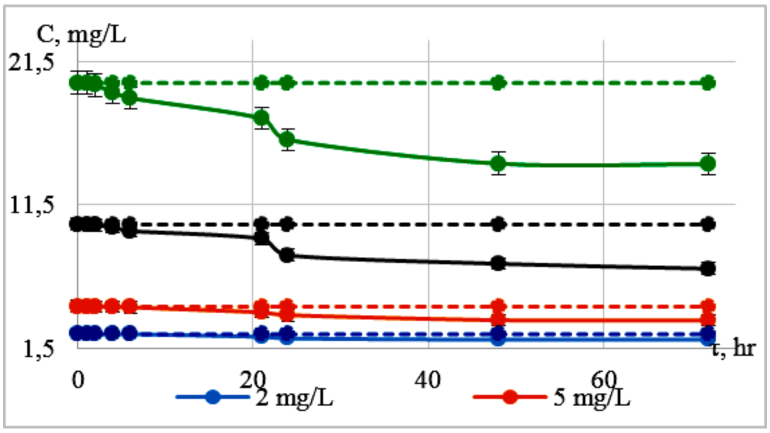

Fig. 1. Dependence of the chloramphenicol content on the time of purification of model solutions using *L. minor* with a biomass of 36 g/L and in control solutions

The change in the content of chloramphenicol in model solutions depending on the time of purification in bioreactors with *L. minor* biomass of 50 g/L is shown in Fig. 2.

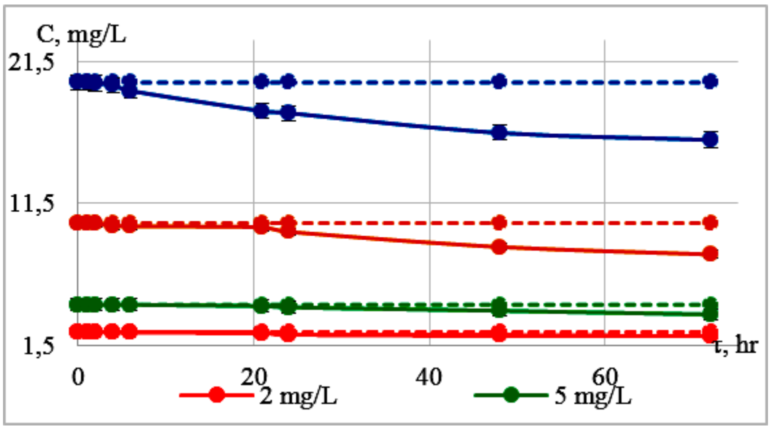

Fig. 2. Dependence of the chloramphenicol content on the time of purification of model solutions using *L. minor* with a biomass of 50 g/L and in control solutions

From Fig. 1 and 2 it is evident that the dependencies of chloramphenicol content on the time of model solution purification using *Lemna minor* biomass at concentrations of 36 and 50 g/L are similar.

At lower antibiotic concentrations of 2 and 5 mg/L the cleaning efficiency at a specific biomass of *L. minor* of 36 g/L reached a maximum of 23.2 and 26.8 %, respectively, after 72 hours. For higher antibiotic concentrations of 10 and 20 mg/L a gradual increase in efficiency was also observed which after 72 hours reached a maximum of 33 % at 10 mg/L chloramphenicol and 29.5 % – at 20 mg/L.

Clearly, reducing the antibiotic content leads to the oxidation of the chloramphenicol molecule through enzymes like cytochrome P450 or other oxidoreductases. These enzymes can alter the structure of chloramphenicol, facilitating its degradation (Bhandari et al., 2021).

At antibiotic concentrations of 2 and 5 mg/L the purification efficiency with a *L. minor* biomass of 50 g/L reached 17 % and 19 % after 72 hours, respectively. For antibiotic concentrations of 10 and 20 mg/L the removal rates were 23.6 % for 10 mg/L and 21 % – for 20 mg/L.

It was found that the antibiotic removal efficiency depends on the time of purification, the initial antibiotic concentration, and the biomass of *L. minor*. The greatest reduction in antibiotic content was observed within the first 24–48 hours after which the process slows and the content remains almost unchanged during the final 72 hours of purification. Thus, using *L. minor* at a specific biomass of 36 g/L contributes to a more effective purification process. Increasing the duckweed biomass to 50 g/L appears to reduce antibiotic adsorption due to the limited contact area between the roots of the upper layers of duckweed and the solution surface. Additionally, the increased duckweed density suppresses photosynthetic activity in the lower layers.

During the purification of model solutions, the leaves of *Lemna minor* turned yellow. This suggests that photosynthesis was disrupted and chloroplasts were damaged due to the antibiotic's effects.

Based on the observed results, an optimal *Lemna minor* biomass of 36 g/L and a purification time of 48 hours were determined.

4. Conclusions

The obtained results showed a decrease in the content of chloramphenicol over time from the start of the process at a specific mass of *Lemna minor* of 36 and 50 g/L.

It was established that the efficiency of antibiotic removal depends on the time of purification of the solutions the initial content of the antibiotic and the biomass of *L. minor*. The greatest decrease in the content of the antibiotic was observed within 24–48 hours after which the process slows down and the content becomes practically unchanged during the 72 hours of cleaning. At lower concentrations (2 and 5 mg/L) the cleaning efficiency using *Lemna minor* with a specific biomass of 36 g/L gradually increases during the first 24 hours and reaches a maximum of 23.2 and 26.8 %, respectively, after 72 hours for a specific biomass of 50 g/L – in 17 and 19 %. At higher concentrations (10 and 20 mg/L) a gradual increase in efficiency was also observed reaching a maximum of 33.0 % at 10 mg/L chloramphenicol and 29.5 % – at 20 mg/L after 72 hours using *Lemna minor* specific biomass of 36 g/L. For a specific biomass of 50 g/L these values are 23.6 and 21 %.

It has been proven that in control studies the content of chloramphenicol remains unchanged during the time of cleaning (72 hours) which indicates that there is no decrease in the content of the antibiotic without *L. minor*.

As a result, rational purification parameters were established that can be used for the design of engineering facilities: a time of 48 hours and a specific biomass of *Lemna minor* 36 g/L which make it possible to achieve the removal efficiency of chloramphenicol from wastewater of 29.4 % at its initial concentration of 10 mg/L.

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