I. Summary

More and more researches are reporting the presence of pharmaceuticals, especially non-steroidal anti-inflammatory drugs in the natural environment. One of them, naproxen is not fully degraded in the human body. Additionally, wastewater treatment plants are not adapted to its utilization. In recent years, bacterial strains which are characterized by increased naproxen degradation potential have been isolated and described. Therefore, the aim of the doctoral dissertation was to immobilize bacterial strains capable to degrade naproxen. The characteristics of the carrier used and bacterial biofilms formed on its surface were made. The effect of immobilization on the course of drug degradation in monocultural conditions, as well as in the presence of autochthonous microflora of the trickling filter was determined. In addition, changes in the activity of enzymes involved in naproxen degradation as a result of immobilization were investigated.

Studies on the effects of immobilization on the biodegradation of naproxen began with the optimization of the immobilization process. However, to correctly assess the physiological state of immobilized bacteria in the biofilm, the method based on the fluorescein diacetate hydrolysis was modified. The modification was to omit the detachment of the biofilm from the carrier and conducting the test on the intact biofilm together with the carrier. The developed procedure assumed shaking of samples in phosphate buffer with a pH in the range of 7.4-7.6 for 1 hour and the result was expressed as total metabolic activity (TEA). The sensitivity assay, during which changes in TEA as a result of starvation were measured, allowed to determine the minimal endogenous metabolism of the *Bacillus thuringiensis* B1(2015b) which was equal to 161-170 μg/g dry weight per hour. It was also observed that nutrient deficiency induced biofilm formation by B1(2015b) cells on the surface of polyurethane foam.

As a result of optimization of the immobilization of the strain *Planococcus* sp. S5 on Loofah sponge, it was observed that the highest TEA values (1250.26 ± 87.61 μg/g dry weight per hour) were achieved during 72-hour incubation in mineral salt medium (MSM; pH 7.2) additionally supplemented with glucose, NaCl and $MnSO_4$, shaken at 90 rpm at 30°C and with high cell concentration. Strain *Bacillus thuringiensis* B1(2015b) immobilized on the Loofah sponge showed the highest TEA values (790.14 ± 40.60 μg/g dry weight per hour) after 48-hour incubation in HTC medium (pH 8), supplemented with glucose and $MnSO_4$, shaken at 110 rpm at 20°C with low cell concentration.

Analysis of naproxen degradation by *Planococcus* sp. S5 strain showed an inhibitory effect at a concentration higher than 12 mg/L on free S5 cells. It was observed that free S5 cells were able to completely degrade the drug in a concentration of 6, 9 and 12 mg/L in 38, 44 and 62 days, respectively. The degradation of the drug proceed in two phases. The first phase, lasting 29 days, was characterized by a slower

naproxen degradation rate. During the second phase, drug degradation was twice as fast. Immobilized S5 cells on Loofah sponge were able to completely degrade the drug in all analyzed concentrations, and the degradation rate was constant, independent of the day of incubation and similar to degradation during phase II performed by free cells. Studies on the course of repeated cycles of naproxen degradation at the lowest analyzed concentration showed that as a result of immobilization, *Planococcus* sp. S5 cells maintained full degradation capacity for 55 days, during which degraded 3 doses of the drug. Additionally, during naproxen degradation, S5 cells secreted significant amounts of exopolysaccharides to increase the protective barrier against naproxen. Studies on the impact of the *Planococcus* sp. S5 immobilization on the activity of enzymes involved in naproxen degradation have shown that immobilization did not change the degradation pathway. However, significant changes were observed in the values of these activities. It was shown that the enzymatic activity in the first phase of drug degradation by free S5 cells was much lower than in the faster degradation phase. Despite the similar rate of drug degradation by free S5 cells in phase II and by immobilized S5 cells, the activity of the analyzed enzymes of immobilized cells was significantly higher than that of free cells.

The naproxen biodegradation by *Bacillus thuringiensis* B1(2015b) immobilized on the Loofah sponge was monitored in a trickling filter augmented with autochthonous microflora from the Imhoff tank flow chamber in Krupski Młyn – Ziętek. Analysis showed that immobilized B1(2015b) cells degraded 70% of naproxen at a concentration of 1 mg/L in the trickling filter without autochthonous microflora. However, in the presence of indigenous microflora, immobilized B1(2015b) cells at the same time degraded 90% of the drug. Obtained results showed synergistic interactions between the autochthonous microflora of the trickling filter and introduced B1(2015b) cells, which resulted in acceleration of naproxen biodegradation. By analyzing the bacterial V3-V5 regions of the 16S rRNA gene using denaturing gradient gel electrophoresis (DGGE), it was confirmed that the introduced *Bacillus thuringiensis* B1(2015b) was able to survive and multiply in the trickling filter after the process of naproxen degradation. In addition, an analysis of the qualitative changes of bacterial and fungal communities of autochthonous microflora after exposure to naproxen, as well as after the introduction of immobilized B1(2015b) was performed. Naproxen has been shown to cause a significant reduction in bacterial microflora biodiversity. Fungal strains were less sensitive to the drug. However, as a result of the introduction of immobilized B1(2015b) cells, which were able to quickly eliminate the drug, an increase in the biodiversity of bacterial and fungal microflora was observed.