SUMMARY

Given the intensive development of industrial activity, pollution of the water environment is becoming an increasingly serious issue. One of the ways the pollutants are introduced into the environment is as a result of the discharge of untreated or insufficiently treated wastewater. In this context, special attention has been given to the presence of micropollutants in the environment, among them synthetic dyes. The widespread use of these has caused a significant environmental problem, resulting not only from the toxicity of the dyes themselves but also from the transformations that these compounds can undergo in the environment. Conventional physical and chemical methods for removing dyes from wastewater have disadvantages and a number of limitations. Therefore, there is an urgent need to improve existing treatment methods and develop new technologies in water and wastewater management, as well as to seek technologies based primarily on bioprocesses and biocatalysis, as a direction that represents one of the key sectors of research toward innovation.

The necessity to protect the environment, the improvement of the public's environmental awareness, as well as the increase in the stringency of legal requirements have drawn my attention to the development of green technologies aimed at the use of enzymes in technological processes, particularly in wastewater treatment technologies. Despite the significant potential for industrial application of enzymes, there are still several limitations associated with their use in technological processes, due to their low stability in native form and their sensitivity to varying reaction environment conditions. Thus, there is a need for further research into the immobilization of enzymes as a method aimed at increasing their stability under often unfavorable process conditions, while allowing recovery and reuse of the biocatalyst.

Based on a literature review, I identified biocatalysis-based processes for the removal of pollutants, including dyes from wastewater, as a promising research direction in the field of water and wastewater management. A high potential in this area is demonstrated by laccase, an enzyme belonging to oxidoreductases, characterized by low substrate specificity and the ability to degrade a wide range of pollutants, including dyes. Hence, within the framework of the current thesis, I have undertaken research in the area of technology for the immobilization of laccase on biopolymer carriers for the removal of dyes from wastewater. I conducted the immobilization of laccase using sodium alginate and chitosan - biopolymer carriers characterized by easy biodegradability, lack of toxicity and biocompatibility. As part of the work, I developed the technology of immobilization of laccase using biopolymer carriers and characterized the geometric parameters of beads with immobilized laccase as well as beads

without the enzyme. I confirmed the effective immobilization of the enzyme using Raman spectroscopy, determined the effect of pH on the activity of free and immobilized laccase, and tested the effect of storage conditions on the enzymatic activity of the developed beads. Determining the effective dose of immobilized enzyme and the optimal temperature for performing the decolorization process was also an important part of the work.

Based on these results, I concluded that while it is important the biopolymer carrier acted as a protective barrier for the enzyme, it is also crucial that it allowed the process of substrate diffusion, ensuring contact between the laccase and the contaminant to be biodegraded. An important parameter for balancing these processes is the cross-linking of the biopolymer carrier. In order to adequately balance the two processes of sorption and biocatalysis under decolorization, while adequately protecting the enzyme under the adverse conditions of the reaction environment, I found it is important to investigate the issue of the properties of the carrier itself, including, in particular, its viscosity.

Following the scientific objective of the work, which has been to analyze the feasibility of using biopolymers with different viscosities as carriers for the immobilization of laccase, the main research objectives included the study of sodium alginate with different viscosities (high, medium and low viscosity) as a carrier for the laccase immobilization for use in the decolorization process of indigo carmine (anionic dye), methylene blue (cationic dye) and their mixture. The performed tests allowed the determination of the viscosity parameters of the immobilization carrier. The results of the decolorization tests enabled the evaluation of the efficiency of the process, as well as the identification of the mechanisms (sorption, biocatalysis) of decolorization of the tested dyes and their mixture, depending on the viscosity of the alginate used as a carrier for the immobilization of laccase. Regardless of the viscosity of the alginate, the decolorization of indigo carmine (anionic dye) occurred mainly by biocatalysis, while during the decolorization of methylene blue (cationic dye), the sorption process dominated. Based on the obtained results, it was determined that the viscosity of alginate affects the efficiency of enzymatic degradation of methylene blue, as the highest total efficiency of removal of this dye was obtained using alginate-based enzyme beads with the highest viscosity, where decolorization occurred by both mechanisms - biocatalysis and sorption. The use of low-viscosity alginate-based enzyme beads resulted in the removal of methylene blue only by sorption, so in the decolorization of this dye, the higher viscosity of alginate promotes removal by biocatalysis. The mechanism of dye removal from the mixture is different from that of decolorization of single dye solutions. The total removal efficiency of indigo carmine from the mixture was lower and decolorization occurred mainly by sorption by the beads, regardless of

the alginate used for immobilization. The use of high-viscosity alginate-based beads also enabled the removal of this dye from the mixture by biocatalysis, with the highest total removal efficiency of the dye compared to medium and low-viscosity alginate-based beads. Decolorization of methylene blue in the mixture occurred solely by sorption. As a part of the study, I also evaluated the toxicity of the dye solutions and their mixture, as well as the solutions after the decolorization process.

Based on the research conducted and the obtained results, I confirmed that it is possible to decolorize dyes and their mixture using immobilized laccase on a biopolymer carrier of varying viscosity, and considering the process occurs through different mechanisms and with varying efficiency. Thus, I verified and confirmed the research hypothesis. The appropriate choice of the carrier depending on the dye that is being removed has an impact on the decolorization mechanism and, specifically the processes of sorption and biocatalysis. The developed technology for the immobilization of laccase on biopolymer carriers, for the removal of dyes from wastewater, represents an important contribution to the development of novel methods of wastewater treatment. The results obtained have a significant impact on the development of research in the field of immobilized enzymes on biopolymer carriers, with particular attention to the properties of the biopolymers used for immobilization. The results of the work also provide an opportunity to determine the further direction of research in order to maximize the efficiency of dye removal using immobilized enzymes and, in the long run, the potential application as an environmentally friendly method of dye-containing wastewater treatment.