Wydział Inżynierii Środowiska i Energetyki
Katedra Biotechnologii Środowiskowej

# ROZPRAWA DOKTORSKA

# Badania ekotoksyczności wybranych leków cytostatycznych i możliwości wykorzystania grzybów do ich usuwania

**mgr inż. Marcelina Jureczko**

Promotor:
dr hab. inż. Wioletta Przystaś, prof. Pol. Śl.

Gliwice, 2021

## Abstract

The increased use of anticancer drugs leads to a higher level of cytostatics released to the environment, hence their presence in surface water, groundwater, as well as potable water. They may constitute an environmental hazard as they affect the stability of genetic material. These compounds are resistant to biodegradation in natural waters, and they are not effectively removed by wastewater treatment plants. Therefore, research into the elimination of anticancer drugs from water constitutes a necessity. Technologies based on white-rot fungi are worth particular attention in this respect. These organisms have found application in the removal of many compounds with the aromatic structure, thanks to the synergistic action of numerous extracellular and intracellular nonspecific enzymes and non-enzymatic strategies. For this reason, wastewater treatment with the application of fungi seems to be a promising alternative when addressing the problem of the presence of anticancer drugs in water.

A relevant literature study allowed to formulate two research theses, according to which: 1) cytostatics can be toxic to organisms which are not target of their medicinal action, hence the need to eliminate them from wastewater; 2) fungi, which decompose many xenobiotics with a ring structure due to the production of a number of low-specificity enzymes, may effectively remove anticancer drugs of the same structure, as well. These theses gave rise to the main objectives of the project: obtaining complex comparative data on ecotoxicity of selected cytostatic drugs and their mixtures in the water environment, and assessment of the applicability of fungi in the removal of these substances from the environment.

The research objectives were achieved through a number of experiments and a study of relevant literature. First, reports on anticancer drugs acting as water pollutants and their harmful effect on the natural environment were collected. Subsequently, ecotoxicity of selected cytostatics: bleomycin and vincristine was evaluated, along with ecotoxicity of mixtures thereof towards representatives of all the three trophic levels of water organisms (plant *Lemna minor*, crustacean *Daphnia magna,* and bacterium *Pseudomonas putida*). The next step focused on the elimination of these compounds from the environment. White-rot fungi were selected and isolated (*Fomes fomentarius* (strain CB13), *Hypholoma fasciculare* (strain CB15), *Phyllotopsis nidulans* (strain CB14), *Pleurotus ostreatus* (strain BWPH), and *Trametes versicolor* (strain CB8)), and their applicability in the removal of the cytostatics in question was determined. First, the possibility of growth of the fungi in the presence of the drugs studied was tested, depending on the nutrient content in the medium. Subsequently, the

efficiency and mechanism of the removal of the tested substances by individual strains was assessed, along with an analysis of the effect of physicochemical conditions on the process of drug elimination by the mycelium. This was achieved in cytostatic removal tests based on physical sorption and biological degradation. The biodegradation experiments were accompanied by the verification of the enzymatic potential of selected strains of fungi and evaluation of the ecotoxicity of samples after the process of removal of anticancer drugs, supplemented with cytotoxicity tests on mouse fibroblasts L929.

Review of the subject literature and the author's research allowed to conclude that cytostatics may constitute a considerable threat for the environment, due to their toxicity towards organisms which are not the target of their action. The analyses performed allowed to classify bleomycin and vincristine as a very toxic and toxic water pollutant, respectively. In ecotoxicity tests, both pharmaceutics present in the mixture demonstrated an antagonistic effect, which signifies that basing on the effects obtained for individual substances may lead to an incorrect environmental risk assessment. The results of the removal of bleomycin and vincristine in both processes: sorption and biodegradation, demonstrated that white-rot fungi may constitute an effective tool in the elimination of the anticancer drugs studied. Despite the fact that the effectiveness depends on the process type, the cytostatic drug, the fungal strain applied, the biomass type and physicochemical parameters of the process, this study allowed to obtain the highest and/or the quickest elimination of the drugs, as compared to other biological and non-biological methods of eliminating these substances presented worldwide. For vincristine, a 97 % elimination of the drug was achieved in the process of degradation (after 4 days of the experiment with the mycelium of the strain CB15), and 26 % in the sorption tests (after 3 hours of contact with living biomass of the strain CB14). The removal of bleomycin reached 36 % (on the $9^{th}$ day of tests of biodegradation) and 59 % (after 3 hours in the sorption process), respectively, with the use of the strain CB8 mycelium. Tests of biodegradation of the investigated cytostatics also demonstrated a drop in the toxicity of samples after the process and a significant activity of the enzyme laccase. Such promising experiment results open up space for further research into the application of fungi and enzymes thereof in the elimination of pharmaceuticals hitherto regarded as degradation-resistant from the environment.