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Treatment of Agricultural Wastewater Containing Pesticides by Hydrophytic Method as a Preliminary Method of Water Recovery

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Abstract: The purpose of this study was to determine the feasibility of using a hydrophytic method to degrade selected pesticides (pyraclostrobin and boscalid) from agricultural waste (wastewater) and to evaluate their removal efficiency. This will allow the recovery of water and raw materials from agricultural wastewater. In addition, a biopreparation was used to increase the efficiency of the process and the rate of degradation and reduce the half-life of the compounds in wastewater. Pesticides, which are commonly used in agriculture, were selected for the experiments. The study was conducted on a pilot scale, in two identical hydrophytic treatment systems supported and not supported by microorganisms. At the same time, in order to identify pesticides, an analytical method based on liquid chromatography-tandem mass spectrometry was optimized, enabling the determination of applied pesticides in wastewater with satisfactory sensitivity, accuracy and precision. The kinetics of pesticide decomposition in the hydrophytic bed were determined on the basis of mathematical models and equations of the dynamics of pesticide disappearance in wastewater. The parameters of the DT_{50} half-life of pesticides in wastewater and the parameters of theoretical time to reach the concentration level of 0.01 mg/L were determined. The use of three different layers (0.15, 0.40 and 0.15 m) of chemically inactive granulometrically differentiated (2 ÷ 8, 8 ÷ 20 and 20 ÷ 80 mm) filter material influenced the high treatment efficiency. During the entire experimental period, the wastewater feeding the bed was treated satisfactorily. The determined parameters of the pesticide half-life in DT_{50} wastewater ranged from 2.33 to 3.29 for microorganisms and 3.42 to 3.79 without microorganisms. The determined parameters of the theoretical time to reach the concentration of 0.01 mg/L $t_{0.01}$ were about 22 and 38 days for microorganisms and 33 and 44 days without microorganisms. Thus, it can be unequivocally concluded that the biopreparation has influence on reducing the half-life of the tested pesticides

Keywords: hydrophytic method; pesticide decomposition; biopreparation bio aqua pur; constructed wetland bed

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1. Introduction

In recent years, more and more attention has been paid to the principles of sustainable development in various areas of life. As a result of these activities, the concept of sustainability is gradually being introduced into many fields of environmental engineering. In addition, the challenge facing environmental engineering today is not only to solve a wide range of technical problems, but also to integrate social and economic aspects. This also applies to issues of water, sewage and waste management, including technologies

for treating wastewater containing, among other things, pesticides in rural non-urbanized areas. The main source of environmental contamination by pesticides is point source contamination during the repeated activities of preparing crop protection product spray liquid and filling and washing sprayers at the same location on the farm. Farms are not equipped for the direct elimination of pesticides from wastewater. Therefore, in the absence of a sanitary sewer system, this type of wastewater should be stored in leak-proof, non-drainage tanks and then transported to a wastewater treatment plant. Unfortunately, because of the high cost of emptying such tanks, they are deliberately unsealed, or wastewater containing pesticides is disposed of in fields, forests and roadside ditches, causing the danger of groundwater contamination [1–4]. The scale of leaks in the liquid waste management system is difficult to estimate, mainly due to the varying volume of so-called non-revenue water consumption [1]. The diverse nature of activities carried out on rural farms means that determining the amount of non-revenue water consumption requires taking into account local factors, such as livestock, watering greens and crops, washing agricultural vehicles and machinery, and keeping the farmyard clean [2]. According to Heidrich and Stanko [3], the rate of non-revenue water consumption in rural areas ranges from 5 to 10%, and according to Bergel and Kaczor [4], it ranges from 14 to 24%.

Pesticides are one of the many groups of chemical pollutants in wastewater [5–8]. In recent years, there has been an increase in the use of pesticides in agriculture. Therefore, it can be assumed that the problem of contamination of water, soils and wastewater with them will also grow [9–12]. The adverse effects of contaminated wastewater generated as a result of rapidly expanding agriculture on the soil and water environment are becoming an increasing problem. The removal of pesticide contaminants from wastewater at the farm level poses a formidable challenge, especially in view of declining water resources. The problem of water pollution from local sources and the need for safe management of working fluids after crop protection treatments, especially contaminated water after filling and washing sprayers, has been recognized and communicated to the wider agricultural community [7,11,13]. Currently, there is a growing interest in biological degradation of liquid residues after crop protection treatments. This allows the recovery of water that can be reused on the farm.

There are many methods for treating wastewater containing pesticides, but these are procedures dedicated to single compounds [14–19]. Among biological methods, we can distinguish the activated sludge method, biological beds and hydrobotanical wastewater treatment, which involve the use of certain plant species with high bioremediation capacity. Constructed wetland beds are becoming increasingly popular [11,13,20,21]. Such solutions with the participation of plants are proven worldwide. A high purification effect is achieved with low expenditures for construction and operation. During wastewater treatment in constructed wetland beds, processes such as filtration, sorption, precipitation and chemical and biological transformations, among others, take place. What is important in this solution is the non-use of chemical compounds used in classical treatment plants and a lack of the generation of waste characteristics of the activated sludge method. The efficiency of the hydrophytic method in autumn and winter decreases slightly. Therefore, it is widely used to treat domestic wastewater including agricultural areas where, due to the dispersion of development, it is more economical to build individual treatment plants rather than collective treatment plants which require large investments in sewage infrastructure. There are well-known applications of hydrophytic systems for treating wastewater from livestock farms, especially in the United States and Japan.

The purpose of this study was to determine the feasibility of using constructed wetland beds to degrade pyraclostrobin and boscalid from agricultural wastewater and to evaluate their removal efficiency. In addition, a biopreparation was used to increase the efficiency of the process and the rate of degradation and reduce the half-life of compounds in wastewater.

The results of research on the efficiency of the biobed-assisted treatment of farm wastewater containing pesticides used in agricultural crop protection in subsurface vertical-flow constructed wetland beds and the determined half-life of pesticides can be used to construct wastewater treatment plants on crop production farms. Such solutions can support existing biobed designs for pesticide liquid preparation, sprayer filling and washing, or function independently. As a result, such measures will help prevent point pollution of surface water and soils, as well as the recovery of water from the application liquid which, undoubtedly, fits in with the Green Deal strategy announced by the European Union.

2. Materials and Methods

2.1. Technological Procedure

The experiment was conducted in a facility that consisted of a retention tank and two parallel systems of constructed wetland beds with the common reed *Phragmites australis*. The constructed wetland beds, with a diameter of 0.50 m and a depth of 0.70 m, were placed in a 120 L tank made of PE plastic, which is characterized by its durability. The facility was equipped with a drain tap, drainage and ventilation. A cross-section of the test facility's bed is shown in Figure 1. The constructed wetland beds were constructed in a system with subsurface vertical flow of wastewater. All of them consisted of three layers of fill from the top with different granularities (Table 1).

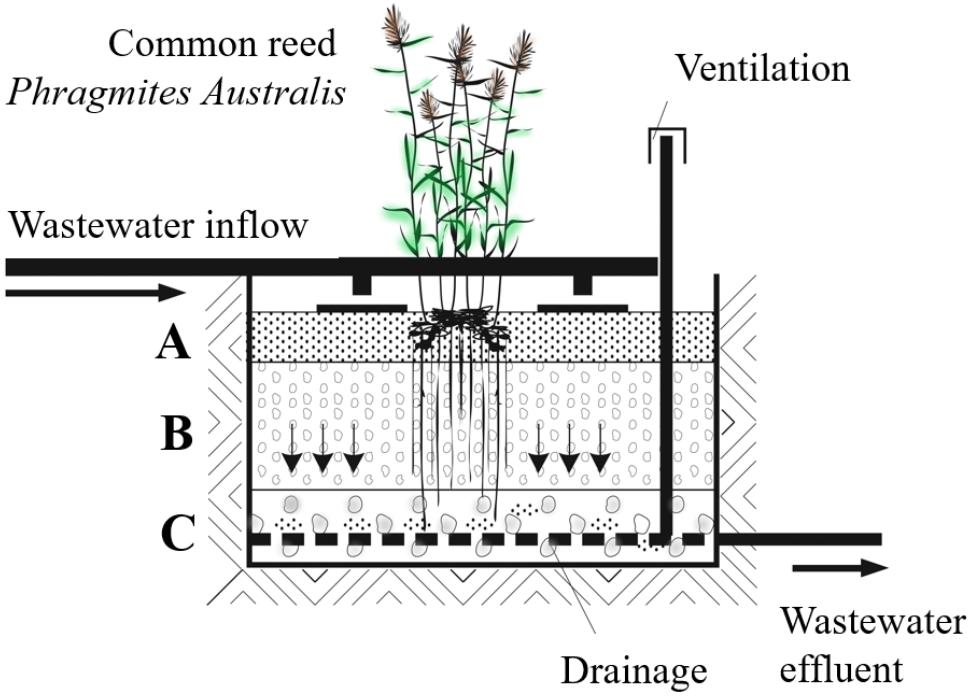

Figure 1. Cross-section of a vertical flow constructed wetland bed (layer: A—0.15 m, B—0.40 m and C—0.15 m).

Table 1. Characteristics of the bed filling.

Lp.	Layer	Granulation	Height
		φ (mm)	H_n (m)
1	A	2 ÷ 8 (gravel)	0.15
2	B	8 ÷ 20 (gravel)	0.40
3	C	20 ÷ 80 (stones)	0.15

Vertical-flow constructed wetland beds were designed and constructed based on the experience of Ozimek and Dabrowski [13], Cooper [20], Brix and Arias [20], Langer-

graber [20] and Obarska-Pempkowiak et al. [20]. The assumed filling thickness of vertical flow deposits was aimed at good oxygenation. Based on the grain size curve, an average grain diameter of d_{50} = 0.45 mm and a grain size uniformity factor of d_{60}/d_{10} = 2.6 were determined. Material with such parameters is recommended as a substrate for reed planting [13,20,22]. For the laid gravel layer, the average grain diameter was d_{50} = 3.5, while the grain size uniformity index was d_{60}/d_{20} = 2.2.

During the first period of working the deposits, according to the guidelines of Troesch et al. and Kinsley and Croll [17,18], in order to acclimatize the plants well, the deposit was fed with treated wastewater, followed by raw wastewater. During the period of deposit development, the biopreparation BIO AQUA was added to the treated wastewater. The formula consists of selected microorganisms immobilized on mineral supports, particularly active against compounds resistant to natural biological degradation in wastewater treatment plants. BIO AQUA PUR significantly reduces the chemical oxygen demand of COD. The product enables better biodegradation of pollutants in municipal wastewater, improves the quality of microorganisms, improves treatment efficiency and reduces odors and the amount of excess sludge generated. It enriches the microorganisms of biological deposits to improve their degradation properties. This preparation is formed by selected microorganisms immobilized on mineral supports, particularly active against compounds resistant to natural biological degradation in wastewater treatment plants. According to the manufacturer's recommendations, the biopreparation BIO AQUA PUR to reactivate the biological process is applied weekly, either at the sewage inlet or in the bioreactor. The biopreparation BIO AQUA PUR should be activated before application by dissolving it in warm water and pouring it into the tank after 10 min. During the period of one year of bed operation, the biopreparation was added once a week to the incoming wastewater. Wastewater was dosed in a hydrophytic treatment plant at a bed load of 0.1 $m^3/m^2/day$. The parameters of the constructed wetland beds are given in Table 2.

Table 2. Parameters of constructed wetland bed of the research facility.

Lp.	Parameter	Symbol	Unit	Value
1	Diameter	D	(m)	0.5
2	Surface area	F	(m^2)	0.2
3	Depth	H	(m)	0.7
4	Hydraulic load	H_L	($m^3/m^2/d$)	0.1
5	Organic compound load	O_C	(g $BZT_5/m^2/d$)	57–730

The pesticides most commonly used in agriculture from the fungicide group containing the active substances boscalid and pyraclostrobin were selected for the study. The toxicological parameters of boscalid and pyraclostrobin are shown in Table 3. After the deposits were worked for a year, the previously prepared mixture of wastewater and fungicides was dosed into the retention tank. Domestic wastewater was used to evaluate the efficiency of pesticide removal in the constructed wetland bed. To enrich the wastewater with fungicides, solutions were prepared corresponding to the parameters of agricultural working fluids: boscalid 30.564 mg/L and pyraclostrobin 8.124 mg/L. Appropriate tests were conducted on a two-year cycle mapping the application dates of sprays on agricultural crops. Wastewater was dosed daily at a rate of 20 L once per day for each bed. Periodically, the wastewater was retained in the bed for a period of 14 days to determine the curves of fungicide distribution dynamics, half-life and the time to reach concentrations of 0.05 and 0.01 mg/L.

Table 3. Toxicological parameters of boscalid and pyraclostrobin.

Active Substance (Chemical Group)	BFC (L/kg)	LD_{50} (mg/kg) Mammals	LC (mg/L) Fish	DT_{50} * (days)	Kfoc ** (mL/g)	EC_{50} *** (mg/L)	CT_{50} (days)
Boscalid Carboxamide (Anilid)	21 Low limit	721	21.3	118	772	5.33	0.42
Pyraclostrobin Strobilurin (Methoxycarbaminian)	- Low limit	884	100	32	9315	0.016	-

BFC—bioaccumulation factor, LD_{50}—lethal dose, * soil degradation half-life DT_{50}, substances with DT50 > 20 are considered to be stable; ** soil adsorption and mobility coefficients Kfoc are defined as the ratio of the active substance in the soil to the concentration of the substance in the equilibrium water phase: if <500 mL/g, substance is considered to be elutable; *** EC_{50}—toxicity determined as toxin effective concentration in the environment, which effects 50% of *Daphnia magna* population.

2.2. Analytical Procedure

Analytical studies were carried out at the Plant Protection Institute—National Research Institute using a Waters liquid chromatograph apparatus and AB SCIEX mass spectrometer using validated test procedures for the determination of pesticide residues in wastewater and plant material [9,23]. Chromatographic analysis (Figure 2)was performed using an Eksigent Ultra LC-100 liquid chromatograph coupled to a QTRAP 6500 mass spectrometer (AB Sciex Instruments, Foster City, CA, USA). Separation of analytes was performed on a KINETEX C18 column (100 mm × 2.1 mm, 2.6 µm) maintained at 40 °C. The volume of the injected sample was 10 µL. The mobile phase was 0.5% formic acid solution with 2 mmol ammonium formate in water (phase A) and in methanol (phase B). Analysis was carried out using gradient elution, with the following program: 0–1 min 1% phase B; 1–13 min 1% to 90%phase B; 13–23 min 90% phase B; 23–25 min 90% to 1% B; and 25–30 min 1% phase B. Detection was carried out using electrospray ionization (ESI) in the positive ion formation mode characterized by the following parameters: voltage applied to the electrode (IS) 5000 V; ion source temperature 400 °C; sputtering assist gas pressure 60 psi; auxiliary gas 50 psi; and shielding gas 30 psi. Nitrogen was used as sputtering gas, auxiliary gas and shielding gas. The optimized parameters of instrumental analysis in the mode of monitoring multiple fragmentation reactions in parallel (MRM) for each pesticide are given in Tables 4 and 5. To ensure the reliability of the results, validation was carried out based on the SANTE/11945/2015 guide. During the validation process, parameters such as linearity, recovery, precision, limit of detection (LOD), limit of quantification (LOQ), matrix effect (ME) and expanded uncertainty of the method (U) were determined. Precision calculated as relative standard deviation (RSD) was less than 22%. The effluent and plant matrix effects for most compounds did not significantly affect the attenuation or amplification of signals.

Table 4. Optimized LC-MS/MS system operating parameters for analyzed pesticides.

Pesticides	Retention Time (min)	Quantitative/Qualitative Determination			Qualitative Determination			DP (V)	EP (V)
		Fragmentation Reaction (*m*/*z*)	CE (V)	CXP (V)	Fragmentation Reaction (*m*/*z*)	CE (V)	CXP (V)		
Boscalid	10.33	343 > 307	27	16	343 > 140	25	8	116	10
Pyraclostrobin	10.66	388 > 194.1	17	12	388 > 163.1	33	10	41	10

DP—declustering potential, EP—entrance potential, CE—collision energy, CXP—collision cell exit potential.

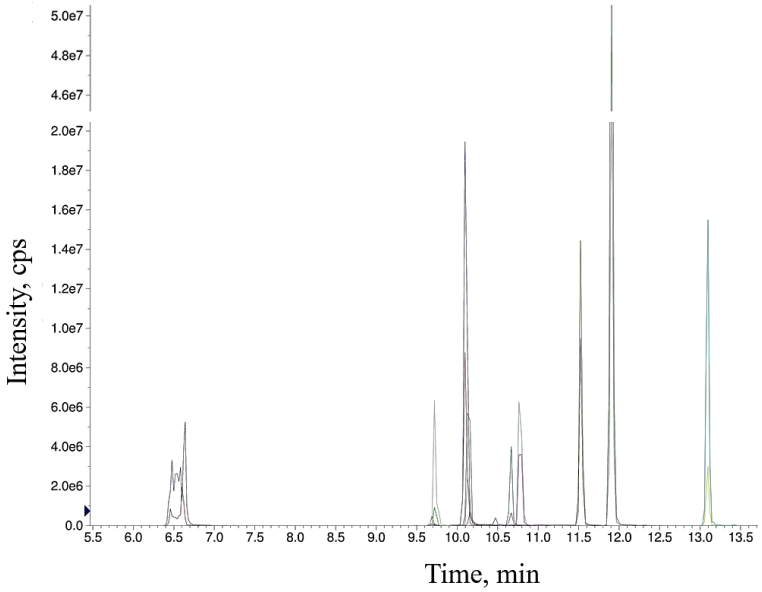

Figure 2. Chromatogram of a mixture of standards with a concentration of 0.50 mg/L.

Table 5. Validation parameters of the method.

Pesticides	R^2	0.001		0.05		0.5		10.0		100.0		U	ME
		(mg/L)											
		R	RSD	R	RSD	R	RSD	R	RSD	R	RSD		
		(%)											
Boscalid	0.99	89	11	93	6	90	11	116	8	100	11	13	10
Pyraclostrobin	0.99	102	9	101	7	106	3	106	7	102	14	11	11

U—extended measurement uncertainty, ME—matrix effect, R—recovery, RSD—relative standard deviation, R^2—coefficient of determination.

In the analyzed concentration range (0.0001–0.30 mg/L), satisfactory linearity of the method was achieved with a determination coefficient of $R^2 > 0.99$. The limit of quantification (LOQ) was set equal to 0.001 mg/L, and the limit of detection (LOD) was set at 0.00003 mg/L. The expanded measurement uncertainty averaged between 8% and 22%.

To describe the persistence of pesticides in wastewater, a mathematical model recommended by the European Union was used to assess the behavior of compounds in soil. Based on the obtained individual concentrations of pesticide active substances in runoff wastewater collected over time, the half-life and rate of disappearance of active substances were calculated. The dynamics of pesticide disappearance in wastewater samples enriched with microorganisms (MIK) and without microorganisms were described by a first-order reaction kinetic equation.

$$C_t = C_0 \cdot \exp(-k \cdot t) \quad (1)$$

where C_t—concentration at time t (mg/L), C_0—initial concentration at time t = 0, (mg/L), t—duration of experiment (d) and k—process rate constant (1/d).

From the knowledge of the mathematical equation and the rate constant, the half-life was calculated, DT_{50} (degradation time), defined as the decay/degradation time, which is the time required for 50% of the mass of a given compound to disappear. Disappearance time values (DT_{50}) for individual pesticides were calculated from a linear equation obtained from a regression between the natural logarithm of pesticide concentration to time:

$$DT_{50} = \ln(2)/k \quad (2)$$

From the knowledge of the mathematical equation, assuming a C_t of 0.05 mg/L and a C_t of 0.01 mg/L, the theoretical disappearance time of the substance to these $C_{0.05}$ and $C_{0.01}$ levels was calculated.

3. Results and Discussion

The half-life of boscalid in soil is difficult to determine. DT_{50} in clay, loam and silt soils ranges from 100.0 to 255.3 days, and in loamy sands from 133 to 348 days. A number of works indicate that boscalid is commonly detectable in various environmental elements. Smalling [24] analyzed residues of boscalid in riverbed sediments and colloidal suspensions of American streams. The researchers found that boscalid was the second most frequently detected pesticide (53% of samples), with a concentration of 0.445 mg/kg. Reilly [25] determined boscalid in 72% of groundwater and surface water samples of US regions. Boscalid was detected in 50% of surface water samples of streams of the Rhineland Palatinate (Germany) and exceeded the normative value for drinking water of 0.001 mg/L in water samples tested [26]. This demonstrates the widespread use of boscalid and its penetration into environmental elements.

In our study, boscalid (BOS) was dosed at 33.4 mg/L in August. The determined concentration of the active substance in the wastewater after thorough mixing was 30.564 mg/L, which confirms the correctness of the analytical method, and the difference is within the error of the method. The first sample was taken after 24 h, and a concentration of 27.451 mg/L was recorded. On the second day, the concentration decreased by 5 units, and on the next day by 4 units to a value of 18.54 mg/L. On the seventh day, the BOS value was 7.214 mg/L, and on the 14th day it reached 1.325 mg/L. After 33 days, the BOS concentration was at 0.084 mg/L, and after 46 days it reached 0.011 mg/L. At day 56, the BOS concentration was not determined (below pgo). When microorganisms were used, the initial decrease in concentration was comparable. After day 1, the concentration was 26.451 mg/L. On the second day, the concentration decreased by 6 units (20.321 mg/L), and on the next day by 3 units to a value of 17.545 mg/L. On the seventh day, the BOS/MIK concentration value was 6.124 mg/L, and on the 14th day it reached 1.124 mg/L. After the 33rd day, the BOS/MIK concentration was at 0.054 mg/L, on the 36th it reached 0.007 mg/L and on day 46 the BOS/MIK concentration (pgo) was no longer determined.

The mass depositions, m_{rp}, expressed as % BOS mass in the wastewater passed through the bed without microorganisms (BOS) and enriched with microorganisms (BOS/MIK), are shown in Figures 3 and 4.

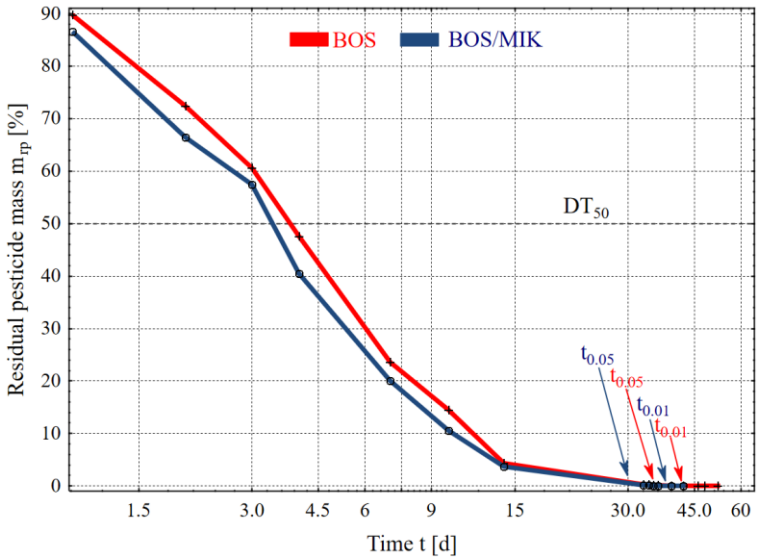

Figure 3. Mass deposition (% residual BOS mass) in non-microbial (BOS) and microbial-enriched (BOS/MIK) wastewater passed through the hydrophytic bed throughout the study period.

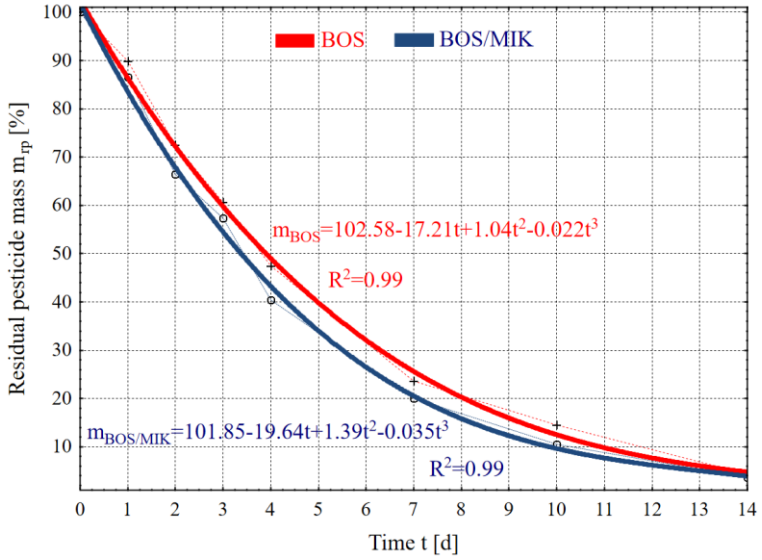

Figure 4. Mass deposition (% residual BOS mass) in wastewater without microorganisms (BOS) and enriched with microorganisms (BOS/MIK) passed through a constructed wetland bed at 14 days.

After the first day after application, the removal efficiencies of BOS were 10.2% without MIK and 13.5% with MIK, respectively; after the 2nd day, they were 27.6% and 33.6%, respectively. A more than 50% pesticide removal efficiency was observed after the 4th day: 52.6% and 59.7% (without MIK additive and with MIK additive). After the 14th day, the efficiencies were 95.7% and 96.5%, respectively. Based on the measurement points shown in Figure 4 of the change in mass deposition values (% residual BOS mass) in wastewater without microorganisms (m_{BOS}) during the 14 days of the experiment, the coefficients of the polynomial equation were calculated: $m_{BOS} = 102.58 - 17.21t + 1.04t^2 - 0.022t^3$. On the other hand, in the bed with the added biopreparation (BOS/MIK), the equation takes the following form: $m_{BOS/MIK} = 101.85 - 19.64t + 1.39t^2 - 0.035t^3$. The high value of the coefficients of determination ($R^2 = 0.99$) confirms the good fit of the obtained graphs to the measurement points and the existence of a strong relationship between the studied parameters—independent (t) and result variables (m_{BOS} and $m_{BOS/MIK}$).

On the 46th day, 100% of the compound was effectively removed from the wastewater/MIK, and without the additive on day 56. These differences indicate the involvement of microorganisms in the decomposition of boscalid. A study discussed the removal efficiency of boscalid from rinsing water produced during the cleaning of pesticide spraying equipment using pilot-scale horizontal-subsurface-flow constructed wetlands (CWs) [27]. The CWs were planted with common reeds (*Phragites australis*) in cobbles (CO-R). The results showed that the removal in the system ranged from 49% to 100%, indicating the efficiency of HSSF systems in treating boscalid-polluted agricultural equipment rinse water in the agricultural area. Agudelo [15–17], studying chlorpyrifos removal in a constructed wetland bed with subsurface flow of overgrown reeds (*Phragmites australis*), found an average removal efficiency of 96.2%, with values ranging from 92.2 to 97.4%. Ignatowicz [11] confirmed the high removal efficiency of selected pesticides from wastewater in a reed (*Phragmites australis*) overgrown treatment plant with subsurface horizontal flow. The pesticides azoxystrobin, boscalid, epoxiconazole, fenarimol, fenazaquin, nicosulfuron, procymidone, pyraclostrobin, thiacloprid and trifloxystrobin at varying constructed wetland bed loading from 0.01 to 0.03 $m^2/m^2/d$ were removed with an average efficiency of 99.8%. Georgios D. Gikas stated that the CW planted with *Phragites australis* showed the highest terbuthylazine removal capacity of up to 73.7%, while the CW planted with *Typha latifolia* reached a maximum removal capacity of 58.4% [28].

To determine the half-life of the pesticides, the SFO model (single first order) was used. The choice was dictated by literature data describing pesticides as degradable substances that can be described by a first-order mathematical function. In addition, there is a lack

of data describing the degradation of pesticides using models of greater complexity that can be fitted to the data without significant limitations or large uncertainties. Based on the results of chromatographic analyses, the relationships between the concentration of the active substance and the theoretical time of its degradation were determined. The correctness of the used model of decay curves described by first-order kinetic reaction equations is confirmed by the high value of the coefficient of determination (R^2). Such a choice of reaction order in their works is also described by other authors, including Fenoll et al. [29] and Gajbhiye et al. [30]. The trends of changes in effluent concentrations of active substances were used to determine their half-life (DT_{50}) [23]. The determined exponential equations were used to determine the theoretical time required to reach the concentration level of 0.01 mg/kg ($t_{0.01teor}$).

The dynamic decay curves of boscalid in wastewater without microorganisms (BOS) and enriched with microorganisms (BOS/MIK) passed through a constructed wetland bed are shown in Figure 5.

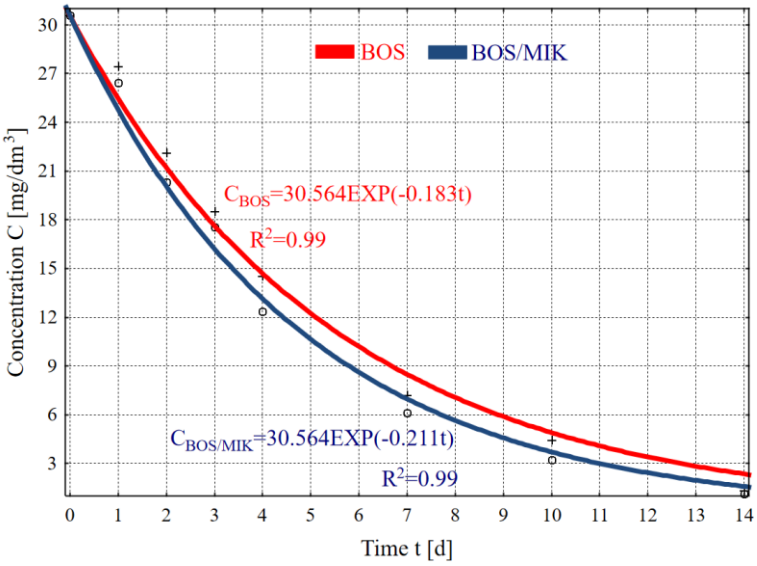

Figure 5. Boscalid disappearance dynamics curve in wastewater without microorganisms (BOS) and enriched with microorganisms (BOS/MIK) passed through a constructed wetland bed.

The dynamics of the disappearance of boscalid without the addition of microorganisms is described by the equation $C_{BOS} = 30.564e^{-0.183t}$ ($R^2 = 0.99$), with the addition of MIK $C_{BOS/MIK} = 30.564e^{-0.211t}$ ($R^2 = 0.99$). The parameters of boscalid decay curves in wastewater without microorganisms (BOS) and enriched with microorganisms (BOS/MIK) passed through the constructed wetland bed are shown in Table 6. The first-order decay rates were 0.183 on day^{-1} for boscalid and 0.211 on day^{-1} for boscalid with microorganisms. The determined half-life was about 4 days without MIK and 3 days after the addition of microorganisms, and the theoretical time for BOS to reach a concentration of 0.05 mg/L was shorter by about 5 days in the MIK-enriched wastewater and was about 30. The concentration of 0.01 mg/L without MIK and with MIK were about 44 and 38 days, respectively. According to Krone-Davis [31], the credible intervals (CIs 95%) for the three pesticide decay parameters were positive and did not span zero, supporting the postulation that the wetland removed these pesticides to some extent. The first-order decay rates were 0.097–0.289 on day^{-1} for diazinon, 0.068–0.232 on day^{-1} for methomyl, and 0.068–0.246 on day^{-1} for acephate. According to Tao Lyu [32], the removal of the pesticide tebuconazole was also fitted with an area-based first-order kinetics model in both unsaturated and saturated CWs. The removal rate constants were in the range of 2.6–10.9 cm on d^{-1} and higher in planted CWs (range of 3.1–10.9 cm d^{-1}) than in unplanted CWs (range of 1.7–2.6 cm on d^{-1}). The low levels of sorption of tebuconazole to the substrate (0.7–2.1%) indicate that the major removal pathways were biodegradation.

Table 6. Values of the coefficients of the equations of the dynamics of disappearance of boscalid with and without the addition of microorganisms (MK), half-life times of pesticides in DT_{50} wastewater and theoretical times to reach concentrations of 0.05 and 0.01 mg/L.

Pesticides	C_0	k	DT_{50}	$t_{0.05}$	$t_{0.01}$
Boscalid	30.564	0.183	3.79	35.04	43.87
Boscalid/MK	30.564	0.211	3.29	30.42	38.05

Our own research showed that a more than 50% efficiency of BOS removal was achieved after 96 h. Microorganisms had little effect on the rate of disappearance. More than 95% was removed after 14 days. The use of the fungus *Trametes versicolor* grown in rice husk (RH) was studied as an option of lignocellulosic bioaugmented substrate for potential application in biomixtures for pesticide carbofuran (CFN) elimination. Rice husk supported growth and activity of the fungus, as evidenced visually and by laccase activity. The system was able to degrade 55.1% of carbofuran in 34 d, with a half-life of 29.9 d [33]

Pyraclostrobin is widely and used in agricultural fields long term. Chen's [34] study of pyraclostrobin degradation in soil by microorganisms isolated from Hawaiian soils, *Pseudomonas Proteobacteria* and *Bacteroides*, proved that the *Pseudomonas consortium* degraded more than 99% of the pesticide (C_0 = 100 mg/L) within five days. Pyraclostrobin has a poor water solubility of 1.9 mg/L at 20 °C and the highest log Pow of 3.99 (20 °C, 99.8%) among the pesticides tested. In our study, pyraclostrobin (PYR) was dosed at 8.4 mg/L in August. The determined concentration of the active ingredient in the wastewater after thorough mixing was 8.124 mg/L, which confirms the correctness of the analytical method, and the difference is within the error of the method. The first sample was taken after 24 h, and a concentration of 6.237 mg/L was recorded. On the second day, the concentration decreased by 1.4 units, and on the next day it also decreased by 1.4 units, to a value of 4.216 mg/L. On day 7, the PYR value was 2.451 mg/L, and on day 14 it reached 0.874 mg/L. After 33 days, the PYR concentration was at 0.024 mg/L, and after 46 days, no PYR concentration was determined (below the limit of quantification). When microorganisms were used, the initial decrease in concentration was slightly faster than without MIK. After day 1, the concentration was 5.458 mg/L. On day 2, the concentration decreased by 1.6 units (3.845 mg/L), and on the next day by about 0.5 units, to a value of 3.354 mg/L. On day 7, the PYR/MIK value was 1.645 mg/L, and on day 14 it reached 0.654 mg/L. After day 33, PYR/MIK concentrations were not determined (pgo).

The mass depositions expressed as % of PYR mass in wastewater passed through the bed without microorganisms (PYR) and enriched with microorganisms (PYR/MIK) are shown in Figures 6 and 7.

In the case of PYR, there were 23% and 33% reductions in the concentration in MIK-free and MIK-enriched wastewater during the first day, and 40 and 53%, respectively, after day 2. On the fourth day, the concentrations of PYR without MIK and with MIK were reduced by 57% and 69%, respectively. On the 10th day, the efficiency of PYR removal from wastewater was 83%, and that enriched with microorganisms was 87%. These differences confirm the activity of decomposition of the compound by the addition of microorganisms. Based on the measurement points shown in Figure 7 of the change in mass deposition values (% residual PYR mass) in wastewater without microorganisms, m_{PYR}, during the 14 days of the experiment, the coefficients of the polynomial equation were calculated: $m_{PYR} = 97.7 - 20.71t + 1.86t^2 - 0.059t^3$. On the other hand, in the bed with the added biopreparation (PYR/MIK), the equation takes the following form: $m_{PYR/MIK} = 95.24 - 26.57t + 2.93t^2 - 0.106t^3$. The high value of the coefficients of determination (R^2 = 0.99) confirms the good fit of the obtained graphs to the measurement points and the existence of a strong relationship between the studied parameters—independent (t) and result variables (m_{BOS} and $m_{BOS/MIK}$).

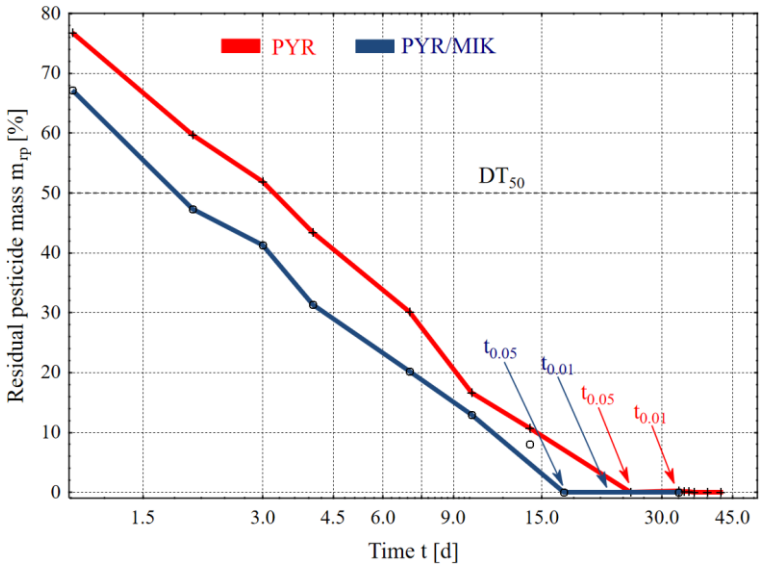

Figure 6. Mass deposition (% residual PYR mass) in microbe-free (PYR) and microbe-enriched (PYR/MIK) wastewater passed through the constructed wetland bed throughout the study period.

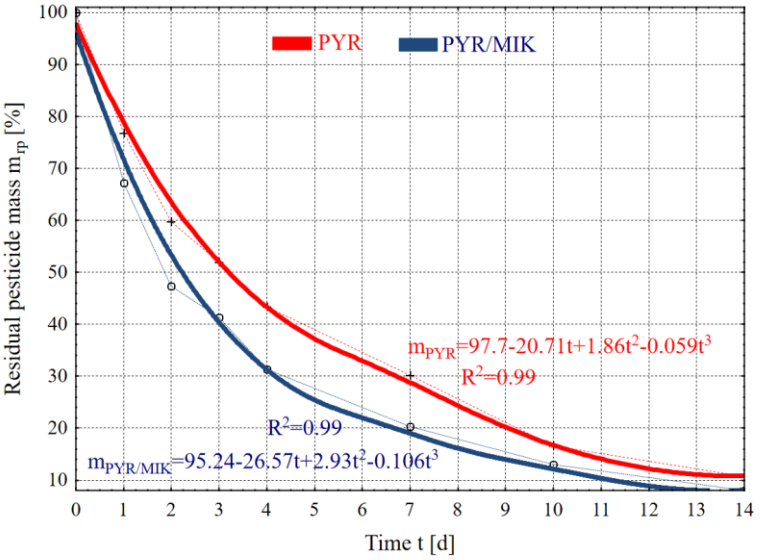

Figure 7. Mass deposition (% of residual PYR mass) in wastewater without microorganisms (PYR) and enriched with microorganisms (PYR/MIK) passed through a constructed wetland bed at 14 days.

On the 33rd day, 100% of PYR/MIK was effectively removed from wastewater, and from wastewater without enrichment on the 46th day. These observations were confirmed by other researchers. Studies conducted by Ignatowicz, Puchlik and Łozowicki [11,12,22] using a vertical-flow constructed wetland bed on a semi-technical scale to treat wastewater containing pesticides and insecticides proved the high removal efficiency of these compounds. Azoxystrobin was removed in 93% and ethyl chlorpyrifos in 98% of beds. According to Felis, the removal of benzothiazole in the vegetated treatment wetlands was 99.7%, whereas the removal of benzotriazole was 82.8%. The vegetation positively affected only the removal of benzothiazole. The integration of the sunlight-induced processes (with TiO_2) with subsurface-flow treatment wetlands caused further elimination of the compounds (42% for benzotriazole and 58% for benzothiazole) [35].

The dynamics curves of pyraclostrobin disappearance in wastewater without microorganisms (PYR) and enriched with microorganisms (PYR/MIK) passed through the constructed wetland bed are shown in Figures 7 and 8. The dynamics of pyraclostrobin disappearance without microorganisms are described by the equation $C_{PYR} = 8.124e^{-0.203t}$

(R^2 = 0.99), with MIK $C_{PYR/MIK} = 8.124e^{-0.298t}$ (R^2 = 0.99). The parameters of pyraclostrobin decay curves in wastewater without microorganisms (PYR) and enriched with microorganisms (PYR/MIK) passed through the constructed wetland bed are shown in Table 7. The first-order decay rates were 0.203 on day^{-1} for pyraclostrobin and 0.298 on day^{-1} for pyraclostrobin with microorganisms. These values are higher than those obtained by Krone-Davis for the selected pesticides [31] and in our own studies for boscalid. Thus, it can be thought that the lower toxicity of the pesticide has the effect of increasing the rate of degradation (Table 3).

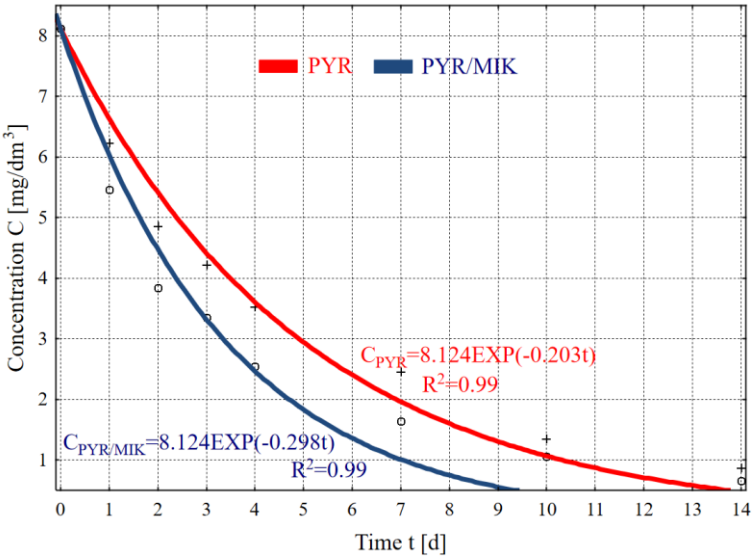

Figure 8. Boscalid disappearance dynamics curve in wastewater without microorganisms (PYR) and enriched with microorganisms (PYR/MIK) passed through a constructed wetland bed.

Table 7. Values of the coefficients of the equations of the dynamics of disappearance of pyraclostrobin with and without the addition of microorganisms (MK), half-life times of pesticides in DT_{50} wastewater and theoretical times to reach concentrations of 0.05 and 0.01 mg/L.

Pesticides	C_0	k	DT_{50}	$t_{0.05}$	$t_{0.01}$
Pyraclostrobin	8.124	0.203	3.42	25.08	33.01
Pyraclostrobin/MK	8.124	0.298	2.33	17.08	22.48

In our own study, PYR by means of microorganisms present in the wastewater was already reduced by more than 30% after 24 h and more than 50% after 48. A 92% reduction was achieved after 14 days. The theoretical half-life of PYR decomposition with MIK is estimated at about 2 days, and without MIK at about 3. The time at which PYR reaches 0.01 mg/L was calculated at 33 days without MIK and 22 days with MIK.

4. Conclusions

Based on the research conducted, the following can be concluded:

1. Constructed wetland beds operating in a system with subsurface vertical effluent flow can be used with great success to recover water by decomposing selected pesticides applicable when spraying agricultural crops.
2. The use of three different layers (0.15, 0.40 and 0.15 m) of chemically inactive granulometrically differentiated (2 ÷ 8, 8 ÷ 20 and 20 ÷ 80 mm) filter material influenced the high treatment efficiency. During the entire experimental period, the wastewater feeding the bed was treated satisfactorily.
3. The determined parameters of pesticide half-life in DT50 wastewater ranged from 2.33 to 3.29 for microorganisms and 3.42 to 3.79 without microorganisms. Thus, it can

be unequivocally concluded that the biopreparation has influence on reducing the half-life of the tested pesticides.

4. The determined parameters of the theoretical time to reach the concentration of 0.01 mg/L $t_{0.01}$ were about 22 and 38 days for microorganisms and 33 and 44 days without microorganisms. Thus, it can be unequivocally concluded that the biopreparation has influence on reducing time of the tested pesticides.
5. The applied biopreparation increased the efficiency of pesticide removal from wastewater and the DT_{50} decomposition time was reduced by up to about 1 day, while the $t_{0.01}$ time was reduced by 11 days. This will make it possible in the future to design disposal stations for the working liquid of pesticides used for spraying agricultural crops, and thus recover water.
6. Based on the results, a biobed system can be designed in the future to recover water from wastewater with pesticides.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BFC	bioaccumulation factor, L/kg
BOS, PYR	without microorganisms, -
BOS/MIK, PYR/MIK	with microorganisms, -
C_0	initial concentration at time t = 0, mg/L
$C_{0.01}$	theoretical times to reach concentrations of 0.01, mg/L
$C_{0.05}$	theoretical times to reach concentrations of 0.01, mg/L
C_t	concentration at time t, mg/L
CT_{50}	half-life days
CE (V)	collision energy,
CXP (V)	collision cell exit potential,
D	diameter, m
d_{10}, d_{20}, d_{50}	average grain diameter, mm
DP (V)	declustering potential,
DT_{50}	degradation time, days
EC_{50}	toxicity determined as toxin effective concentration in the environment, which effects 50% of *Daphnia magna* population mg/L
EP (V)	entrance potential,
φ	granulation, mm
F	surface area, m^2
H	depth, m
H_L	Hydraulic load, $m^3/m^2/d$
H_n	height bed filling, m
k	process rate constant, 1/d
Kfoc	soil adsorption and mobility coefficients, mL/g
LC	lethal dose, mg/L
LD_{50}	lethal dose, mg/kg
ME	matrix effect,
m_{rp}	mass deposition, %

O_C	Organic compound, g $BZT_5/m^2/d$
R	recovery,
R^2	coefficient of determination, -
RSD	relative standard deviation,
t	duration of experiment, d
U	extended measurement uncertainty,

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