Enzymatic Biocatalysts Applied for Pharmaceutical Pollutants Degradation

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Both the growth of the world's population and the associated active use of various pharmaceutical compounds (PCPs) (antibiotics, hormones, cardiovascular, analgesics, , anti-inflammatory and antiepileptic drugs, etc.) have led to the problem of their contamination of water and the environment. PCPs are found in various concentrations in the wastewater of urban wastewater treatment plants. The physical-chemical processes of PCPs removal using membrane filtration, chlorination, ozonation and photocatalytic oxidation, sorption and microbiological degradation ensure the elimination of these pollutants, but have certain limitations in the effectiveness of these processes.Biological treatment has a number of significant advantages, which consist in the use of natural biocatalysts (enzymes, microorganisms) for the destruction of micropollutants. Analysis of recently published studies on the use of soluble and immobilized enzymes as biocatalysts for the biodegradation of various PCPs has shown the effectiveness of these applications.

Keywords: pharmaceutic pollutants ; enzymes ; immobilization ; laccase, peroxidase

1. Introduction

Both the growth of the world's population and the associated active use of various pharmaceutical compounds (PCPs) (antibiotics, hormones, cardiovascular, analgesics, anticonvulsants, anti-inflammatory and antiepileptic drugs, etc.) have led to the problem of their contamination of water and the environment [1]. Many of them can have an adverse effect on human health and are able to form a negative response to the effect they cause, which can be cumulative [2]. PCPs are found in various concentrations in the wastewater of urban sewage treatment plants, while the presence of mixtures of pharmaceutical micropollutants is noted [3][4][5][6]. Sewage treatment plants are the main facilities where PCPs with wastewater from municipal and industrial places enter and where they should be removed. The physical–chemical processes of their removal using membrane filtration, chlorination, ozonation and photocatalytic oxidation, sorption and microbiological degradation ensure the elimination of these pollutants, but have certain limitations in the effectiveness of these processes [3][4][5]. As a result, these substances often "slip through" treatment facilities and enter further into natural water sources (groundwater, rivers, seas), and from there they enter drinking water [4][5][6].

The best results in the removal of pharmaceuticals from wastewater (purification efficiency up to 90%) were achieved using ultrafiltration [7] followed by additional adsorption on a carbon filter [8]. However, the low adsorption capacity, low selectivity, high cost and long duration of the process limit the use of such methods of wastewater treatment with PCPs. In addition, sorbents are ineffective against nanoconcentrations of PCPs [8]. Disposal or recycling of used adsorbents contaminated with PCPs also causes a significant problem. Other technologies for the removal of PCPs using active oxygen forms generated by different methods [9][10], with rare exceptions, are slightly less effective, ensuring the removal of substances at an average of 65–88%. They are characterized by significant energy consumption and the formation of toxic by-products (free radicals, oxidized derivatives of pharmaceutical pollutants and the products of their destruction).

2. Enzymatic Biocatalysts Applied for the Degradation of Pharmaceutical Pollutants

Analysis of recently published studies on the use of soluble and immobilized enzymes as biocatalysts for the biodegradation of various PCPs showed the effectiveness of these applications. Further, researchers decided to consider the results obtained using different forms of enzymes separately in order to highlight the main trends in recent developments.

2.1. Free Enzymes in the Biodegradation of PCPs

Among the soluble enzymes, the use of which has been studied in the bioprocessing of various samples of real and model wastewater contaminated with different PCPs (**Table 1**) [11][12][13][14][15][16][17][18][19][20][21], the most frequent use of laccase isolated from various sources, mainly of fungal origin, should be noted for these purposes. The reason for this is that laccase (EC 1.10.3.2) is one of the most studied extracellular enzymes, which can destroy a wide range of aromatic compounds. Since laccase requires only oxygen as an electron acceptor for the conversion of the substrate and has low substrate specificity, it is a universal enzyme widely used in the biodegradation of various xenobiotics.

Table 1. Enzymes used for degradation of pharmaceutical pollutants in various types of wastewater.

Biocatalyst [Reference]	Pollutant Concentration	Optimal Conditions of Enzymatic Action; Pollutant Degradation Efficiency
Free Enzymes		
Laccase from *Trametes hirsute* [11]	Municipal wastewater with cannabidiol (0.318 µM)	20 °C, 8 h, 135 rpm; addition of 1 mM acetaminophen as mediator; 92.0% degradation of cannabidiol
Laccases from *Trametes pubescens* MUT 2400 [12]	Samples of municipal wastewater after primary sedimentation (W1) and at the end of the process (W2) with total concentration of micropollutants equal to 403.2 µg/L and 349.5 µg/L, correspondently; bis-(2-ethylhexyl)phthalate, diethyl phthalate and ketoprofen were the most notable micropollutants in the mixtures	20 °C, pH 7.7–7.8, 24 h, 100 rpm In W1: 86.3%, 84.9% and 82.4%degradation of bisphenol A, 2-hydroxybiphenyl and 4-t-butylphenol, correspondently; up to 70% degradation of 9 micropollutants and below 50% degradation of other micropollutants; In W2: 63% and 77–81% degradation of ketoprofen and oxybenzone, correspondently
Laccase from *T. versicolor* [13]	Phosphate-citrate buffer with doxorubicin (0.25–10 mg/L)	30 °C, pH 7.0, 24 h, V_{max} = 703 µg/h/L 41.4% degradation of doxorubicin (1 mg/L)
Laccase from *T. versicolor* [14]	Buffer with carbamazepine (1 mg/L)	35 °C, pH 6.0, 24 h 95.0% degradation of carbamazepine
Laccase from *T. hirsuta* [15]	17β-estradiol in natural water (5 µmol/L) and pig manure (200 µg/kg)	25 °C, pH 5.0 94.4% and 91.0% degradation of 17β-estradiol in water (for 2 h) and pig manure (for 7 days), correspondently
Laccase from *T. hirsute* [16]	0.1 M acetate buffer with chloramphenicol (10 mg/L)	28 °C, pH 5.0, 48 h 100% degradation of chloramphenicol
Laccase from *Bjerkandera adusta* [22]	McIlvaine buffer with acetaminophen, bisphenol A, sulfamethoxazole and carbamazepine (20 mg/L) Mixture contained 250 µM of each compound.	25 °C, pH 6.0, 12 h 100% degradation of acetaminophen and bisphenol A; 20.5% degradation of carbamazepine; 22.0% and 19% degradation of sulfamethoxazole in presence of acetaminophen and other compounds, correspondently
Laccase from *T. versicolor* [17]	Milli-Q water with diclofenac, trimethoprim, carbamazepine and sulfamethoxazole; total concentration of PCPs was 1.25 or 5 mg/L in mixture.	25 °C, pH 6.8–6.9, 48 h, 80 rpm With single PCP: 100%, 95.0%, 82.0% and 56.0% degradation of diclofenac, trimethoprim, carbamazepine and sulfamethoxazole, correspondently With PCPs in mixture: 100%, 39.0%, 34.0% and 49.0% of same PCPs, correspondently
Cu^{2+}-assisted laccase from *T. versicolor* [18]	Phosphate buffered saline with triclosan (10 µM)	25 °C, pH 6.0, 4 h, 3.0 mM Cu^{2+} 95.0% degradation of triclosan
Recombinant laccases from *Pleurotus ostreatus* [19]	Acetate buffer (50 mM) with sulfadiazine, sulfamethazine and sulfamethoxazole (100 mg/L)	25 °C, pH 4.8, 1 h 98.1%, 97.5%, and 97.8% degradation of sulfadiazine, sulfamethazine and sulfamethoxazole, correspondently
Soybean peroxidase [20]	Synthetic wastewater with triclosan, sulfamethoxazole, estrone, 17β-estradiol, 17α-ethynylestradiol, nonylphenol and octylphenol (5–50 mg/L)	0.05–0.5 mM H_2O_2, pH 6.0–7.0, 3 h 80.0% degradation of sulfamethoxazole and 95.0% degradation of all other PCPs

Biocatalyst [Reference]	Pollutant Concentration	Optimal Conditions of Enzymatic Action; Pollutant Degradation Efficiency
Free Enzymes		
Laccase from *T. versicolor* and horseradish peroxidase [21]	**Tap water and secondary wastewater with mixture of bisphenol A, 17α-ethinylestradiol, diclofenac and triclosan (10 mg/L of each compound) supplemented by 2.5% (*v*/*v*) McIlvaine's buffer**	**25 °C, 20 h, 1% H_2O_2 pH 3.5–4.5 and 6.5–8.0 for peroxidase and laccase, correspondently; In tap water: 44.0, 68.0, 42.0 and 61.0% degradation of bisphenol A, 17α-ethinylestradiol, diclofenac and triclosan, was with laccase, correspondently; 83.0, 75.0, 49.0, and 56.0% degradation of same PCPs was with peroxidase; In wastewater: 81.0, 93.0, 38.0 and 72.0% degradation of same PCPs was with laccase; 63.0, 78.0, 17.0 and 54.0% degradation of same PCPs was with peroxidase**

The enzymatic degradation of various PCPs (hormones, antibiotics, cytostatic drugs), as well as pesticides and personal hygiene products, was investigated under the action of laccase in wastewater. Laccase was highly active against many target pollutants. However, in most successful cases, the degradation efficiency was 95–98% [14][17][18][19][20]. At the same time, only in a few variants of the studied media did the concentration of PCPs decrease by 100% for 16–24 h [16][17][22]. This process was particularly effective when using genetically improved variants of mutant laccase [19].

The significantly high initial rates of enzymatic degradation of many micropollutants should be noted [13]. It has been shown that the components of wastewater (micropollutants and solid suspended microparticles) can greatly reduce the activity of the enzyme, acting as inhibitors and sorbents. The loss of enzymatic activity can reach 66% [12].

The effectiveness of pollutant degradation depends on the chemical structure of the target compounds and the pH of the treated medium. For instance, a high percentage of degradation (82.4–86.3%) of bisphenol A, 2-hydroxybiphenyl and 4-tert-butylphenol was found, which was explained by the presence of a hydroxyl group in the aromatic structure of these compounds [12]. The conducted assessment of the toxicity of wastewater after its enzymatic treatment using soluble laccase showed a clear decrease in ecotoxicity [12][13]. The degradation of anticancer drugs, such as doxorubicin under the action of laccase was investigated. The highest enzymatic activity was achieved at 30 °C and pH 7, which corresponded to the characteristics of wastewater treatment plants [13].

Carbamazepine (an anticonvulsant and mood-stabilizing drug used to treat epilepsy and bipolar disorder) is one of the PCPs most commonly detected in wastewater, and has adverse effects on human and animal health [14]. Oxidation is an effective method of carbamazepine removal, but it has a number of disadvantages, such as the need for continuous supply of O_3 or H_2O_2 and subsequent removal of toxic oxidation catalysts, such as active radicals. Biocatalytic degradation of carbamazepine using laccase in the presence of redox mediators is a promising approach to its removal from wastewater.

Under optimal conditions of biotransformation, with the use of laccase (35 °C, pH 6.0) and the addition of a mediator (2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) to the medium, the efficiency of the process reached 95%. Moreover, the resulting products of the enzymatic degradation of carbamazepine did not have the effect of estrogenicity [14]. In fact, the removal efficiency of the difficult-to-decompose carbamazepine at water treatment plants is below 20%, which leads to its detection in aquatic environments, including ground and even drinking water. This problem is due to the fact that this substance contains a strong electron acceptor amide group in its structure. When using laccase to influence carbamazepine in various investigations [14][17][22], different degradation results were obtained (from 20% to 95%). The adsorption of this substance on the inner surface of the applied bioreactors turned out to be one of the reasons that worsened its bioavailability for the effects of laccase.

When analyzing the identified possibilities of using fungal laccase for the biodegradation of various PCPs, a number of interesting results were noted. For example, laccase ensured the oxidation of 17ß-estradiol both in natural water and in the complex environment of pig manure [15]. At the same time, this biodegradation efficiency was higher than 91%, while this process took place at pH 5.0, which is characteristic of pig manure due to the increased concentration of organic acids in it. The significance of this result should be noted, since the removal of estrogens during wastewater treatment makes it possible to reduce the number of highly toxic pollutants that cause metabolic disorders and even carcinogenic risks in animals and humans.

Laccase proved to be effective in the biodegradation of a widely used heat-resistant antibiotic, chloramphenicol, in the composition of wastewater [16]. At the same time, as a result of the enzymatic reaction, chloramphenicol aldehyde was formed, which did not show toxicity to a number of bacterial and yeast cells, whereas non-enzymatic acid-base catalysis and hydrolysis of the antibiotic by the amide bond led to dehalogenation and formation of a large number of toxic compounds.

There are a number of PCPs that are regularly found in wastewater, groundwater and drinking water: diclofenac, trimethoprim, carbamazepine and sulfamethoxazole. This is due to their inefficient degradation at wastewater treatment plants using oxidation methods (ozonation, UV photolysis and UV/H_2O_2) [17]. Therefore, the degradation of these compounds under the action of laccase was investigated. It emerged that the biodegradation of these substances individually was more effective than in mixtures under the same conditions created for the enzyme. The obtained products of enzymatic treatment of each pharmaceutical pollutant were recognized as non-toxic [17].

Sulfamethoxazole is an antibiotic with a wide spectrum of antimicrobial action, used for bacterial infections of the urinary tract, bronchitis and prostatitis. This substance is also difficult to decompose and is often found in wastewater. It has been shown that under the action of laccase, it is possible to achieve a fairly successful removal of this substance from wastewater [17][19]. The most effective degradation of sulfamethoxazole was observed in a mixture with acetaminophen, which acts as a mediator for biocatalysis. This result suggests that the decomposition of sulfamethoxazole may be enhanced in the presence of higher concentrations of acetaminophen [22].

It has been shown that the activity of fungal laccase when exposed to triclosan clearly increases in the presence of Cu^{2+} ions in wastewater, which act as a co-factor for this enzyme [18]. Compared with the reaction without Cu^{2+} (67.17%), the efficiency of triclosan degradation increased to 95% in 4 h in the presence of 3.0 mM Cu^{2+}. The analysis of inhibition of the growth of freshwater microalgae (*Chlamydomonas reinhardtii* and *Scenedesmus obliquus*) showed that the products of the enzymatic reaction showed less toxicity toward the microalgae than the original pollutant.

It should be noted that not only laccase but also peroxidase was used to degrade different PCPs: triclosan, sulfamethoxazole and steroids (estrone, 17β-estradiol, 17α-ethynylestradiol) [20][21]. It was found that peroxidase can efficiently decompose (≥95%) all pollutants, with the exception of sulfamethoxazole at a neutral pH value for 3 h in the presence of H_2O_2 [20].

Thus, PCPs can be degraded by enzymes such as laccases and peroxidases. However, a number of problems limit their use; in particular, low laccase activity at neutral pH values or the need to add H_2O_2 as a substrate for peroxidase in wastewater [12]. In this case, preference was given to laccase for use in biodegradation of PCPs, but stabilization of this enzyme was required to ensure its effective and long-term functioning, especially in the case of varying the pH of the treated media. In addition, there is a need for cheap enzymes that can be consumed in large quantities to process wastewater or to obtain biosystems with the possibility of their reuse. The immobilization of the enzymes is oriented toward the overcoming of these problems.

2.2. Immobilized Enzymes in the Biodegradation of PCPs

According to the information performed in **Table 1**, biotransformation of organic compounds using enzymatic biocatalysts is an environmentally attractive addition to traditional wastewater treatment. However, the loss of activity by enzymes in the process of their use is a serious problem.

One of the promising methods of immobilizing enzymes, in particular laccases, is the production of cross-linked enzyme aggregates (CLEAs). This method of enzyme immobilization is well-known and has already proven itself positively, including with regard to laccase for municipal wastewater purification [23]. It consists in binding the amino acid residues of enzymes to each other using a crosslinking agent, and allows the maintenance of high (up to 70%) enzyme activity. CLEAs can be obtained on the basis of pure enzymes and crude proteins, which is a significant advantage in obtaining enzymatic biocatalysts for PCPs degradation in large-scale processes. However, there are a number of problems associated with the low mechanical stability of CLEAs and their fragility, which complicates mass transfer, limits the filterability of media and limits their use in industry [11][23].

An effective solution was to attach laccase to chitosan by forming an imine group between the aldehyde groups of the enzyme and the amine groups of chitosan, and then silanization of the polymer structure was carried out by adding (3-aminopropyl) triethoxysilane (APTES) [11]. Such chemical immobilization of the laccase made it possible to obtain cross-linked aggregates of the enzyme, which maintained stable catalytic activity in the pH range 6–9 and the temperature range 4–60 °C, as well as in the presence of salts ($CaCl_2$, $ZnCl_2$), methanol, ethylene diamine tetraacetic acid and

components of municipal wastewater. After 5 oxidative cycles of PCPs, the stabilized laccase retained 67% of its initial activity.

Graphene materials are known to increase the rate of chemical reactions by improving the kinetics of electron transfer. Due to a highly stable two-dimensional layered structure, large surface area and pore volume, graphene materials were considered as promising carriers for immobilized enzymes, enabling the performance of various practical tasks, including wastewater purification. Pristine graphene is considered to be the most environmentally acceptable in the family of graphene materials. The immobilization of enzymes on this carrier was carried out by the non-covalent π-π stacking and hydrophobic interactions. It was found that the presence of pristine graphene significantly improved the removal of labetalol (β-blocker) from the medium using 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) as a mediator. ABTS can be oxidized by laccase to form a stable cation radical. Since labetalol is not a direct substrate of laccase, the formation of an ABTS cation radical with a high oxidation potential can lead to the transformation of labetalol [24]. The enzyme immobilized on pristine graphene retained up to 60% activity in the pH range 2–10, had increased thermal stability and lost only 10% activity for 40 min at 70 °C. The efficiency of the available labetalol was 100% for 10 working cycles of the immobilized enzyme, and then it decreased by 30% during the two subsequent working cycles of the immobilized laccase. With all the advantages of this biocatalyst, it was found that 6% of labetalol from the medium was sorbed on graphene, which is an obvious drawback in this biocatalytic system.

Discussing the prospects for the use of immobilized enzymatic biocatalysts for wastewater treatment from PCPs, emphasis should be placed on the need to use carriers for enzyme immobilization that can be prepared from affordable and cheap materials with high chemical and thermal stability that are capable of functionalization and ensuring possible reuse. For these purposes, the implementation of synthetic polymers has certain advantages, since such materials have many functional (carbonyl, carboxyl, hydroxyl, epoxy, amine and alkyl) groups that provide functionalization of the polymer surface and effective binding of the enzyme. In this regard, interesting results and rather high levels of biodegradation of various PCPs were obtained using laminated nanofibers from poly(acrylic acid) [25], polyimide aerogels [26], nanofibers from poly(L-lactic acid)-co-poly(ε-caprolactone) (PLCL) [27], poly(vinylidene fluoride) membranes [28][29], polyamide granules activated by branched polyethylenimine [30] and polypropylene beads [31] as synthetic carriers for immobilized laccase.

It is known that magnetic nanoparticles can also be functionalized to immobilize laccase [32]. The experiments performed to remove diclofenac using such an immobilized enzyme were 20% higher than that of soluble laccase; however, this biocatalyst lost activity relatively quickly, and on the fourth working cycle, the biodegradation of this nonsteroidal anti-inflammatory drug was only 19%. Interestingly, when using carriers derived from natural sources, particularly biochar from coniferous wood, for the immobilization of laccase, the enzyme retained 40% activity after five cycles of diclofenac treatment. [33]. It should be emphasized that interest in carriers based on natural materials (bentonites [34], silica and activated carbons, including bio-carbons, which are obtained by pyrolysis of biomass [35]) continues to be high. Covalent immobilization on these laccase carriers makes it possible to obtain fairly active biocatalysts, and at the same time there are no restrictions on the possible obtaining of such biocatalysts on a large scale for wastewater purification from antibiotics.

To increase the efficiency of PCP removal, researchers are trying to combine physical–chemical methods of wastewater treatment with the use of immobilized laccase. In this regard, an interesting solution is the combination of electrooxidation (EO) with enzymatic treatment of wastewater. During electro-oxidation, highly oxidizing hydroxyl radicals are generated using special electrodes. By itself, EO is ineffective for removing PCPs that are present in low concentrations in wastewater. At the same time, high energy costs are required to remove PCPs by this method from the total volume of treated wastewater. A study of the purification of municipal wastewater from triclosan using electrochemical processes in combination with treatment by laccase immobilized on TiO_2 nanoparticles showed that the degradation efficiency of triclosan reached 93% [36]. The TiO_2 nanoparticles used for such immobilization are characterized by high chemical stability and simplicity of functionalization, for which crosslinking agents can be applied [37]. The laccases thus immobilized showed high thermal stability at 50 °C (the half-inactivation period was 45.7 h) and high stability at low pH values of 2 and 3 (the half-inactivation period was 31.8 and 107.1 h, respectively). However, a decrease in the activity of the immobilized enzyme was revealed due to the appearance of F^-, Cl^- or Br^- anions in water, as well as nitrite and cyanide, blocking the access of substrates to the active center of laccase [37].

In another study, membrane filtration was combined with enzymatic treatment of wastewater [38]. Phenol oxidases (laccase and tyrosinase) were used in this combined process. It is known that tyrosinase forms o-diphenol from the initial compound and subsequently releases oxidized, usually highly reactive o-quinone, which slowly polymerizes as a result of auto-oxidative processes. Laccases oxidize phenolic compounds with the formation of corresponding free radicals, which

lead to the formation of molecules prone to polymerization. As a result of polymerization, macromolecules are formed, which are easier to remove from the treated solution. The efficiency of degradation of 14 PCPs from urban wastewater was studied using microfiltration polysulfone hollow fiber membrane with phenol oxidases immobilized on it [38]. As a result, high efficiency of removal (90%) of anti-inflammatory drugs (acetaminophen, naproxen, mefenamic acid, ibuprofen, ketoprofen, indomethacin) from sewage was achieved within 24 h. Removal of acetaminophen and mephenamic acid occurred three times faster (>85% for 8 h).

The use of a combination of enzymes for the degradation of atenolol ensured the high efficiency of the process (100% for 120 h), and the degradation of bezafibrate, cofein, carbamazepine and fenofibrate was 80%, 70%, 40% and 30%, respectively. When using a membrane with enzymes, complete removal of all PCPs was achieved within 5 days. At the same time, the enzymes retained up to 70% of their initial catalytic activity during the entire period of PCP treatment [38].

It should be noted that researchers today are interested not only in developing new options for the immobilization and use of laccases, but also in other enzymes. Thus, in a number of new publications on the destruction of β-lactam antibiotics in wastewater, information has appeared about the successful use of β-Lactamase [39][40]. This enzyme provided the biodegradation of antibiotics in real wastewater containing cefamesin, amoxicillin and ampicillin (50–100 mg/L) simultaneously. The efficiency of the process reached 72.3–92.8% for 20 days.

β-Lactamase covalently immobilized on Fe_3O_4 nanoparticles showed excellent stability and the possibility of reuse for at least 10 batch cycles to degrade penicillin (5 mg/L) for 5 min. After 30 working cycles, the degradation efficiency decreased by 95%; however, the concentration of the antibiotic used in the experiments exceeded the concentration (0.153 mg/L) by 30 times, which is usually determined in the wastewater of the pharmaceutical industry [40].

Chloroperoxidase, immobilized on dendritic silica particles and coated with an amyloid-like protein nanofilm, retained up to 80% of the initial catalytic activity during 20 cycles of enzyme use for antibiotic biodegradation. More than 80% of the levofloxacin and rifaximin present at a concentration of 100 mg/L decomposed under the action of this enzyme within 0.5 h [41].

Another trend that can be noted in studies on the enzymatic biodegradation of PCPs in wastewater is to study the possibility and feasibility of combining immobilized enzymes possessing different “non-selectivity” of action against various substrates; for example, horseradish and lignin peroxidases. This technique expands the range of substrates that can undergo bioconversion and the range of conditions for the implementation of PCPs biodegradation. In particular, the use of immobilized peroxidases in the decomposition of diclofenac, carbamazepine and paracetamol was investigated, and it was shown that horseradish and lignin peroxidases immobilized on Fe_3O_4 nanoparticles and encapsulated in SiO_2-sol-gel retained 43–50% of their activity at 55 °C after 20 consecutive operating cycles for 24 h. A decrease in pH to 3.0 significantly increased enzyme activity, and the efficiency of the degradation of diclofenac, carbamazepine and paracetamol by both peroxidases reached 100%, 100% and 50%, respectively, within 72 h. Meanwhile, the use of only one lignin peroxidase or horseradish peroxidase at pH 5.0 ensured 59%, 60%, 9% and 64%, 68% and 9% destruction of the same PCPs [8].

Thus, the use of enzymes, including in immobilized form, in combination with other enzymes and physical–chemical methods of wastewater processing constitute one of the current directions in the development of effective approaches to the biodegradation of various PCPs in wastewater.

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