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Detection of ampicillin based on the fluorescence of a biocatalytic oxidation product

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Antibiotics used in humans and farmed animals are an essential source of water and soil contamination. Ampicillin is a micropollutant commonly found in water, sludge, food, flora, and fauna. However, the methods used for its detection in environmental samples are often complicated and expensive. Therefore, developing more straightforward strategies to detect well-known target antibiotics is necessary. In this context, enzyme-based detection methods have been demonstrated to be selective, sensitive, rapid, and relatively simple. In this study, a fluorescent byproduct from the ampicillin oxidation using Chloroperoxidase (CPO) enzyme was used as a pointer compound to determine ampicillin concentration in environmental water samples. We oxidized 80% ampicillin for 1h, producing a fluorescent compound with *m/z* 274.2517. A response surface methodology (RSM) based on a central composite design (CCD) was used to evaluate and optimize the effects of hydrogen peroxide, enzyme concentration, and time as independent variables on the maximum fluorescence signal as the response function. The methodology proposes to build a calibration curve that relates the initial concentration of ampicillin with fluorescence intensity after the reaction with CPO, which helps detect ampicillin in the concentration range from 0.035 to 40 μM, with a limit of detection of 0.026 μM. The application of the method to fortified environmental water samples allowed percentages of recovery from 86 to 140%. The formation of the fluorescent compound was not affected by the presence of salts commonly found in wastewater; however, it was affected by other antibiotics. The proposed methodology was tested in the context of water from water bodies, urban, and WWTP effluents.

KEYWORDS

ampicillin, chloroperoxidase biocatalysis, detection method, emerging pollutant, fluorescence

Introduction

Antibiotics are the third best-selling medicines worldwide. Between 2000 and 2010, antibiotic drug consumption increased by 36% (from 54 billion standard units to 73 billion standard units); Brazil, Russia, India, China, and South Africa had a significant increase in this change (van Boeckel et al., 2014; Senadheera et al., 2020). A total of 50%–75% of antibiotics consumed worldwide are, used in veterinary medicine and animal husbandry (Steinfeld et al., 2009) Globally, antibiotic consumption in livestock reached 63,151 tons in 2010, and an increase of 67%–200% is projected by 2030 (Ibrahim et al., 2020; Senadheera et al., 2020) According to a recently reported spatial modeling study, the global antibiotic consumption rate of 9.8 defined daily doses (DDD) per 1,000 population per day in 2000, increased by 46% from 9.8% in 2018 (14.3 DDD). The model incorporated antibiotic usage and consumption data and used geostatistical modeling techniques to estimate the antibiotic consumption of 204 countries from 2000 to 2018 (Browne et al., 2021). Penicillin, macrolides, and fluoroquinolones are the most commonly used in humans, while tetracyclines, penicillin, and sulfonamides are the most widely used drugs in animals (Ma et al., 2021). Drug residues contaminate water, soil, flora, and fauna because of the low drug absorption both in humans and animals, the use of animal excreta as fertilizer, the poor final disposal of expired antibiotics in homes and hospitals, the WWTP's low biodegradability, and the high environmental mobility of antibiotics (Lv et al., 2019; Mejías et al., 2021).

Antibiotic-resistant bacteria are the main consequence of antibiotic contamination, which is a current health problem whose prognosis for the future is a worldwide concern (Uddin et al., 2021; Haenni et al., 2022). Therefore, the proper detection of antibiotic residues in the environment is a very active area of current research. Liquid or gas chromatography coupled with mass detectors is the analysis par excellence due to its accuracy, precision, versatility, and sensitivity (di Rocco et al., 2017; Ghosh et al., 2021; Lakew et al., 2022). However, due to the high cost of analysis, the need for highly trained personnel, and the costly infrastructure associated with it, together with a large number of samples to be analyzed, it has been necessary to develop alternative methods and devices for their detection (Mehlhorn et al., 2018; Yue et al., 2021; Gawrońska et al., 2022).

Ampicillin is a micropollutant commonly found in water, sludge, food, flora, and fauna (Kumar et al., 2019; Peris-Vicente et al., 2022). In recent years several methods and devices have been reported for detecting ampicillin in water or food, with successful results in terms of sensitivity, accuracy, and application in environmental mixtures (Soledad-Rodríguez et al., 2017; Sharma et al., 2019; Sta Ana et al., 2021). However, most reported methods and devices are still technically complex and, primarily based on nanomaterials or composites that require specialized production methods, highly controlled working conditions, or sophisticated equipment (Luo et al., 2017; Shrivas et al., 2017; Modh et al., 2018; Shu and Tang, 2020).

Therefore, there is a need for alternative and simple strategies for ampicillin detection, that maintain performance parameters such as accuracy, precision, and sensitivity to obtain reliable results that are the basis of an environmental diagnosis. Nowadays, several studies have focused on methods based on enzyme activity to analyze the presence of pollutants in the environment (Ejeian et al., 2018; Pachapur et al., 2019; Sarkar et al., 2019; Reynoso et al., 2022; Zhai et al., 2022). Given the nature of enzymes, enzyme-based detection methods are selective, sensitive, rapid, and relatively simple to apply. Enzymes have been used to detect organophosphorus pesticides, phenolic compounds, amine compounds, and pharmaceuticals. Among the reported enzymes, Chloroperoxidase (CPO) is a versatile biocatalyst for multipollutant determination due to its wide substrate variability (Rebollar-Pérez et al., 2016; Morsi et al., 2020; Lin, 2021). In addition, CPO's kinetics and reaction mechanism are known, and its three-dimensional structure is well defined, which may facilitate the eventual elaboration of detection devices.

In this study, a biocatalytic method was developed for detecting ampicillin in environmental water samples using CPO; the procedure was shown to be in the sensitivity range of other reported techniques, accurate, and potentially applicable to the analysis of numerous samples over short times.

Materials and methods

Chemicals

Ampicillin, sulfamethoxazole, tetracycline, and amoxicillin were purchased from Sigma-Aldrich (St. Louis, MO, United States). Chloroperoxidase (CPO) from *Caldaromyces fumago* was purchased from Alltaenzymes (Edmonton, AB, Canada) with an R_Z of 1.4 and specific activity of 22,000 min^{-1} for the halogenation of monochlorodimedone. Buffer salts, potassium chloride, and hydrogen peroxide were purchased from J.T. Baker (Phillipsburg, NJ, United States).

Biocatalytic oxidation of ampicillin

Ampicillin was enzymatically oxidized in a reaction mixture containing 10–50 μM ampicillin, 1 mM H_2O_2, 20 mM KCl, and 0.1 μM CPO in phosphate buffer pH 3.0, 60 mM. Reaction progress was analyzed by steady-state fluorescence (Cary Eclipse fluorescence spectrometer from Varian, equipped with a Xe lamp and Czerny-Turner 0.125 monochromators) with an excitation wavelength of

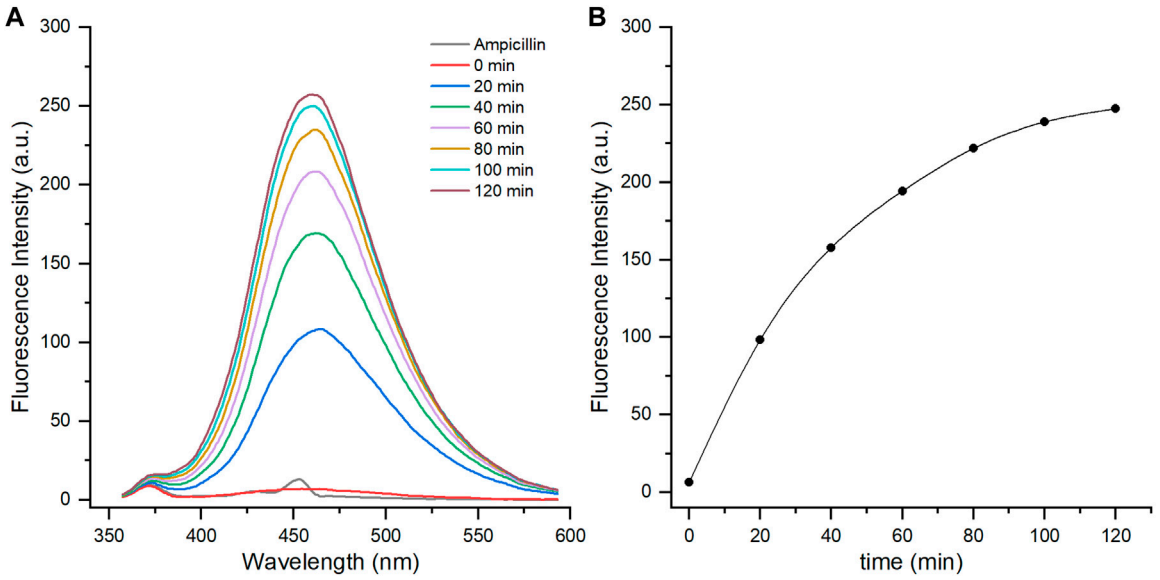

FIGURE 1
(A) Fluorescence emission spectra of a byproduct of an ampicillin biocatalysis by CPO and **(B)** their appearance kinetics.

TABLE 1 Reaction products of CPO-mediated oxidation of ampicillin observed by LC/DAD/MS.

Proposed molecular formula	Observed *m/z*	Calculated *m/z*	Error (ppm)	References
$C_{14}H_{31}NO$	230.2462	230.2478	6.95	this study
$C_{14}H_{31}N_3O_2$	274.2517	274.2495	−8.02	this study
$C_{14}H_{31}N_3O_3$	290.2425	290.2444	6.55	this study
$C_{16}H_{25}N_3O_3S$	340.1723	340.1696	−7.94	this study
$C_{16}H_{29}Cl_2N_3O_4$	398.1590	398.1614	6.03	this study
$C_{32}H_{41}N_6O_9S_2$	717.2354	717.2371	2.37	Li et al. (2014)

330 nm, followed by the light emission of the reaction products at 450 nm. The assays were completed within 1 h under the tested conditions.

Optimization of reaction conditions

A central composite design (CCD) with three independent variables and three levels—enzyme concentration (0.05, 0.075, and 0.1 μM), reaction time (0.5, 0.75, and 1 h), and H_2O_2 concentration (0.5, 1.0, and 1.5 mM)— was applied to obtain the highest fluorescence intensity indicative of the greater production of byproducts. Five central and six axial points were included in the design. A total of 35 experiments were conducted according to the CCD. The experimental results were analyzed through a response surface methodology (RSM), using Design-Expert software (trial version 13.0.11.0, United States). The response was related to the selected variable *via* a mathematical model, and optimization was performed to obtain the highest response values. Optimum values with desirability one were recorded for the variables.

Identification of enzymatic transformation products

Aliquots were collected at different time intervals during the enzymatic oxidation of ampicillin and filtered through 0.2 μm nylon membranes. Then, aliquots were analyzed by

TABLE 2 Central Composite Design for ampicillin oxidation by CPO.

Time h	CPO μM	$[H_2O_2]$ mM	Fluorescence intensity 450 nm
0.5	0.05	0.5	34.59
1.0	0.05	0.5	82.83
0.5	0.10	0.5	28.99
1.0	0.10	0.5	45.43
0.5	0.05	1.5	98.26
1.0	0.05	1.5	137.01
0.5	0.10	1.5	97.23
1.0	0.10	1.5	106.93
0.5	0.05	0.5	34.24
1.0	0.05	0.5	74.79
0.5	0.10	0.5	33.34
1.0	0.10	0.5	50.26
0.5	0.05	1.5	91.10
1.0	0.05	1.5	140.18
0.5	0.10	1.5	101.65
1.0	0.10	1.5	104.95
0.5	0.05	0.5	45.13
1.0	0.05	0.5	84.60
0.5	0.10	0.5	32.84
1.0	0.10	0.5	56.40
0.5	0.05	1.5	95.94
1.0	0.05	1.5	145.67
0.5	0.10	1.5	89.97
1.0	0.10	1.5	111.14
0.75	0.075	1.0	66.93
0.75	0.075	1.0	60.79
0.75	0.075	1.0	76.29
0.75	0.075	1.0	76.23
0.75	0.075	1.0	71.68
0.375	0.075	1.0	66.11
1.125	0.075	1.0	95.84
0.75	0.0375	1.0	87.30
0.75	0.1125	1.0	43.99
0.75	0.075	0.25	18.98
0.75	0.075	1.75	83.89

chromatography using an LC/DAD/MS instrument (Chromatograph Series 1,260 from Agilent Technologies, Santa Clara, CA, United States) to determine ampicillin concentration over time and to observe the byproducts formed. Separation was performed on an Agilent Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm). The mobile phases consisted of water (A) and acetonitrile (B), both with 0.1% (v/v) formic acid. The following elution program was employed: 90%A for the first 2 min; changed linearly to 40% (2–12 min); then, again, 90% A from 12 to 12.5 min. Finally, the column was re-equilibrated for 2.5 min with these initial conditions for a total run time of 15 min. The detection by DAD was at 215 and 330 nm. For the mass detector (ESI-Q-TOF-MS 6520 detector, Agilent), ESI source parameters were as follows: positive and negative ionization mode, fragmentor voltage: 175 V, capillary voltage: 3,500 V, gas temperature: 350°C, N_2 flow: 11 L min^{-1}, nebulizer pressure: 60 psi.

Chromatographic fractions were collected according to the chromatographic peaks' retention time, and analyzed by fluorescence spectrometry to identify products with an emission spectrum equivalent to that observed in the biocatalytic oxidation step.

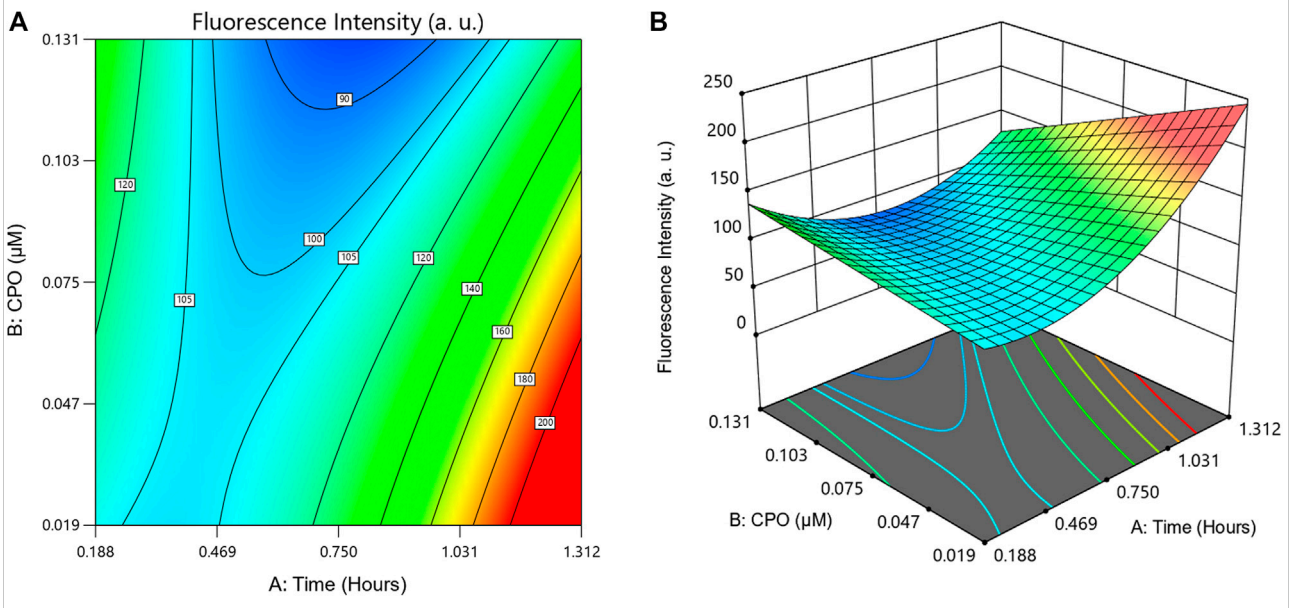

FIGURE 2
(A) Contour plot and **(B)** response surface plot of the interaction among CPO concentration and time reaction at 1.7 mM H_2O_2 in relation to fluorescence intensity of ampicillin byproduct.

Selectivity tests in the presence of interfering compounds

To identify possible individual chemical interferents, the method was tested in the presence of several salts at the maximum concentrations found in the environmental water samples (KH_2PO_4 [0.029 g L^{-1}], NaH_2PO_4 [0.029 g L^{-1}], $NaNO_3$ [0.81 g L^{-1}], NH_4NO_3 [0.081 g L^{-1}] and $CaCl_2$ [0.195 g L^{-1}]. On the other hand, the assay was also performed in the presence of amoxicillin, tetracycline, sulfamethoxazole, and their mixture with a 30 μM concentration for each. The ampicillin oxidation assay was performed as described in the previous section using 30 μM ampicillin.

Assays on environmental samples

Three different ampicillin-enriched water samples were tested to evaluate possible environmental interferences of the sample matrix on ampicillin detection. First, a detection assay was performed on five samples of water spiked with 5 and 10 μM ampicillin. Samples from the treatment plant's secondary effluent, lagoon water, drinking water, groundwater, and river water were used. The samples had previously been filtered through nylon membranes of 0.45 μm and stored at 4°C until use. The determined parameters in water samples are summarized in Supplementary Table S1. The enzymatic assay was performed as described in the previous section.

Results and discussion

Ampicillin biocatalysis with chloroperoxidase

CPO was able to oxidize 70% of the ampicillin in the first 20 min. After that, the reaction rate slowed, reaching 80% conversion in 1 h. Compared to other CPO substrates, the oxidation of ampicillin was lower; for example, the oxidation of tetracycline and sulfamethoxazole reached 70% conversion in 4 min (García-Zamora et al., 2018). Fitting the reaction kinetics to a pseudo-first order equation yielded a reaction rate constant of 0.103 min^{-1} (Supplementary Figure S1); which is similar to that reported for the oxidation of the flame retardant tetrabromobisphenol by CPO (García-Zamora et al., 2019). In addition, some of the oxidation rate or conversion is within the range of physicochemical oxidation, although physicochemical methods have a characteristic that can lead to the mineralization of the compound (Frontistis et al., 2018; Silva et al., 2019; Montoya-Rodríguez et al., 2020).

Ampicillin and its enzymatic oxidation products showed similar electronic absorption spectra (and, in addition, low

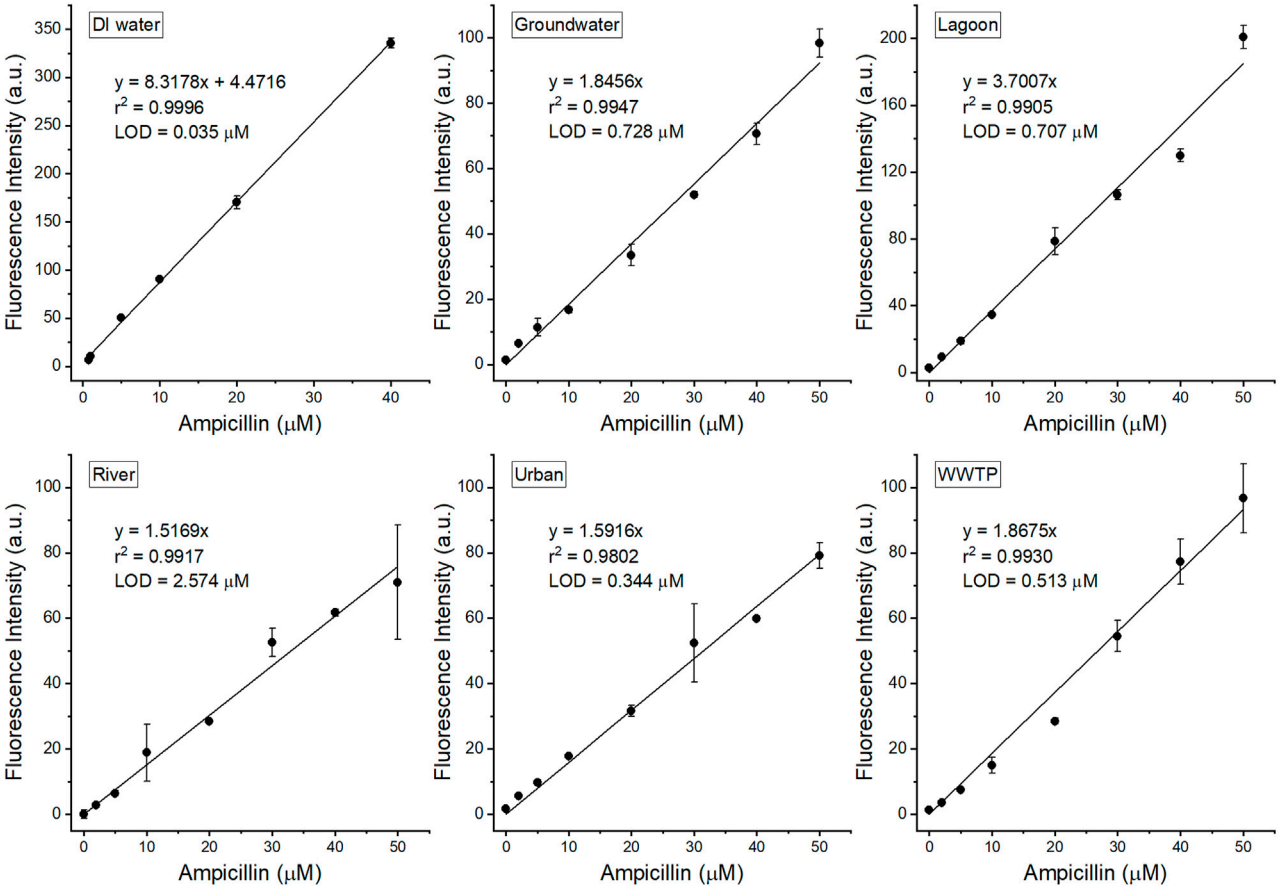

FIGURE 3
Calibration curves and LOD for the detection of ampicillin based on the fluorescence intensity of a byproduct of the biocatalytic degradation of ampicillin by CPO in different environmental water matrices.

light absorption, Supplementary Figure S2); in contrast, the oxidized products exhibit fluorescence, which allows differentiation from the basal one, which has negligible fluorescence at the excitation wavelength (Figure 1). The emission spectrum presents a maximum at 460 nm. After 2 h of the biocatalytic process, fluorescent byproducts were observed. Given the reaction conditions and the mechanism reported for CPO, the reaction products are likely to be chlorinated derivatives of ampicillin, although non-chlorinated compounds of lower molecular weight could also be produced in subsequent chemical reactions (García-Zamora et al., 2018; Wang K. et al., 2019; Undiano et al., 2021). Through HPLC-MS, the mass of some oxidation products was observed, and their molecular formula was proposed with a difference of mass/charge $\Delta_{m/z} \leq 8.02$ ppm (Table 1). As can be seen, most oxidation products have a higher molecular mass than ampicillin, which is not surprising because the peroxidase oxidation mechanism follows a free radical mechanism, which can lead to polymerization of some reaction products (Ortiz de Montellano, 2010; Dunford, 2016). The product responsible for fluorescence was identified with *m/z* 274.2517 and the formula $C_{14}H_{31}N_3O_2$, suggesting a fragmentation of the ampicillin molecule upon oxidation. The oxidation kinetics of ampicillin coincides with the appearance of the fluorescent product, which was monitored by mass spectrometry, and fluorescence (Supplementary Figure S3 and Figure 1), which suggests that it is the first oxidation product from the enzymatic reaction. None of the other reaction products identified showed fluorescence under the tested conditions. Hence, it was considered as a pointer compound to indirectly determine the concentration of ampicillin, measuring the fluorescence of samples after the enzymatic reaction, as will be described later.

TABLE 3 Limit of detection of several methods for ampicillin detection.

N°	Analysis method	LOD (μM)	Linear range (μM)	Matrix	References
1	Fluorescence method based on the fluorescence of a biocatalytic oxidation product	0.035	0.75–40	Wastewater, groundwater, river, lagoon and urban water samples	this study
2	CDs-based fluorescent probe for turn-on selectively by iron ions (Fe^{3+})	0.700	0–60	River water, tap water, and mineral water	Fu et al. (2020)
3	Chemiluminescence reaction using cupric oxide nanoparticles–luminol–H_2O_2	0.261	0.4–4.0	Pharmaceutical preparations	Iranifam et al. (2014)
4	Competitive fluorescent lateral flow immunoassay based on labeled functional nucleic acids	2.6×10^{-6}	2.8×10^{-5}–5.7×10^{-4}	Hospital wastewater samples	Lin et al. (2020)
5	Direct-flow surface plasmon resonance immunosensor on gold plate	1.000	2.5–30,000	Milk, river water and spring surface water samples	Tomassetti et al. (2017)
6	Electrochemical aptasensor based on a MoS_2/polypyrrole nanocomposites cast on a screen-printed electrode	2.8×10^{-7}	1.4×10^{-7}–7.1×10^{-7}	River water samples	Hamami et al. (2021)
7	Electrochemical aptasensor based on endonuclease DpnII activity	3.2×10^{-5}	1×10^{-4}–0.1	Milk and drinking water samples	Wang et al. (2019)
8	Electrochemical aptasensor based on entropy-driven DNA walking machine	9.6×10^{-7}	1×10^{-6}–1×10^{-3}	Drinking water samples	Zhang et al. (2020)
9	Electrochemical aptasensor based on MB-modified aptamer probe on gold electrodes	1.0000	5–5,000	Human serum, saliva and milk samples	Yu and Lai, (2018)
10	Electrochemical aptasensor based on the CDs-In_2O_3-In_2S_3 nanocomposites	1.2×10^{-7}	2.8×10^{-6}–0.859	Lake water samples	Yan et al. (2021)
11	Electrochemical aptasensor modified with MB on gold electrodes	0.0330	0.1–1,000	Pharmaceutical preparations	Xiang et al. (2021)
12	Electrochemical surface plasmon resonance aptasensor on gold chips	1.000	2.5–1,000	Pharmaceutical preparations and river water samples	Blidar et al. (2019)
13	Flow injection chemiluminescence based on luminol–periodate reaction	0.014	0.020–1.0	Pharmaceutical preparations	Li et al. (2003)
14	Fluorescent aptasensor based on functionalized AuNPs using a fluorescently labelled aptamer	0.0260	0.1–100	Urine	Simmons et al. (2020)
15	Fluorescent-colorimetric aptasensor using functionalized AuNPs by 5′-fluorescein amidite-modified aptamers	0.0143	1.4310 × 10-3–0.1431	Milk sample	Song et al. (2012)
16	Nanostructured electrochemical aptasensor of MOF and terephthalonitrile-based COF.	6.2×10^{-10}	2.8×10^{-9} - 5.7×10^{-3}	Serum, river water and milk samples	Liu et al. (2019)
17	Photochemical aptasensor a self-powered using a CdS/Eu-MOF nanocomposites	9.3×10^{-5}	1×10^{-4}–0.2	Milk and lake water samples	Gao et al. (2019)
18	Photoelectrochemical aptasensor based on N-GQDs and $AsBiS_2$ sensitized with Zn/Co oxides	2.5×10^{-7}	5.0×10^{-7}–0.01	Tap water and lake water samples	Yan et al. (2020)
19	POP-based electrochemical aptasensor by the coupled polymerization of 1, 3, 6, 8-Tetraphenylpyrene and α, α′-dibromo-p-xylene	3.8×10^{-9}	2.8×10^{-3}–0.014	Human serum, milk and river water samples	Yuan et al. (2021)
20	Probe displacement electrochemical aptasensor based on a thiolated and MB-modified aptamers	3×10^{-5}	0.2–15000	Urine, saliva, milk and aquifer water samples	Yu et al. (2018)
21	Spectrophotometric determination based on the carboxylic acid groups present in AMP by a mixture of KIO_3 and KI.	0.247	0.715–7.155	Pharmaceutical preparations	Manirul Haque, (2021)
22	Spectrophotometric determination with sulfanilic acid by oxidative coupling reaction	0.688	143.1–858.6	Pharmaceutical preparations	Darweesh et al. (2020)

AuNPs: Gold nanoparticles, *CDs*: Carbon dots, *COF*: covalent organic frameworks, *MB*: methylene blue, *MOF*: metal organic frameworks, *N-GQDs*: Nitrogen-doped graphene quantum dots.

Optimization of the detection method by a fluorescent byproduct of biocatalysis

The detection method proposed in this study is based on the formation of a fluorescent byproduct of the oxidation reaction of ampicillin with CPO. The fluorescence response can be influenced by many experimental parameters that should be optimized to obtain the best performance. CCD was selected because it is a design that includes linear, quadratic, and interaction terms and allows for greater level numbers without performing experiments using every combination of factor levels. The enzyme concentration, reaction time, and hydrogen peroxide concentration were optimized among catalytic factors. These three parameters are significant factors in biocatalysis with CPO (Rebollar-Pérez et al., 2016). Since the fluorescent product is one among several, it is crucial to

determine the right amount of enzyme and reaction time for its maximum production; moreover, the amount of peroxide must be optimized to prevent the enzyme from becoming inactive and generating less product. Table 2 presents the results of the applied CCD. As can be seen, signal intensities vary from 28.9 to 145 AU. These experimental results were fitted to a second-order polynomial equation, resulting in the following mathematical expression:

$$Intensity = 16.38 - 91.84 \times time + 494.62 \times CPO + 57.12 \times H_2O_2 - 1165.06 \times time \times CPO + 157.05 \times time^2 \quad (1)$$

The results of the analysis of variance (ANOVA) of the model are shown in Supplementary Table S2. The model and every term, including interaction time*CPO were statistically significant at the 5% level. Furthermore, the coefficient of determination (R^2) obtained was 0.96, indicating that the equation adequately represents the relationship between fluorescence intensity and the independent variables.

It was possible to build contour graphs using this model. Figure 2 shows the response surface plot of the significant interaction found in the model. As can be seen, to achieve higher intensity values, it is necessary to increase the time and apply low enzyme concentrations. This last result would seem contradictory given that higher enzyme concentrations increase the transformation of ampicillin. However, a high concentration of the enzyme can trigger a subsequent modification of the fluorescent compound based on the detection at 450 nm, which decreases the fluorescence signal.

Response optimization was performed to determine the values of each independent variable that produced maximum fluorescence. Overall desirability is an objective function ranging from 0 (if the optimal values are outside the selected ranges) to 1 (if the goal for the response is reached). Then, optimal values with desirability 1 were 1.1 h, 0.043 μM CPO, and 1.7 mM H_2O_2, resulting in the model's theoretical intensity of 167.87 AU. A confirmatory experiment was performed with the predicted optimum conditions, and the experimental fluorescence intensity obtained was 163.788 ± 2.18 AU, validating the fluorescent compound's production equation to detect ampicillin.

A calibration curve was then generated by applying these conditions at different concentrations of ampicillin (Figure 3, DI water). As can be seen, there is a good fit of the data to a straight line, with a correlation coefficient of 0.99; the line equation, helpful in determining LOD and LOQ is found in Figure 3. The obtained LOD and LOQ of the ampicillin concentration were 0.035 μM and 0.12 μM respectively, and the linear range was from 0.75 to 40 μM. These values are among the best obtained by methods based on a chemical transformation of ampicillin, as shown in Table 3 (analysis methods: 3, 13, 21, and 22); when compared to methods based on nanomaterials, the results are below that performance (analysis methods: 6, 10, 16, and 17 from Table 3). However, the simplicity of the technique confers certain implementation advantages; even so, the LOD value obtained here compares with others produced with more sophisticated methods and devices, such as those based on aptamers (analysis methods: 9, 11, 12, 14, and 15 from Table 3).

The biocatalytic method is simple and does not require extensive training; however, as signal intensity depends on the initial ampicillin concentration, an additional amplification procedure is necessary to improve sensitivity.

TABLE 4 Interferents for the biocatalytic method for ampicillin detection.

Compound	Fluorescence intensity (450 nm)	Interference (%)
None	42.44 ± 0.02	0.00
KH_2PO_4	43.59 ± 1.39	2.71
NaH_2PO_4	39.63 ± 2.12	6.62
$NaNO_3$	45.95 ± 0.81	8.25
NH_4NO_3	40.90 ± 0.17	3.63
$CaCl_2$	42.48 ± 3.34	0.08
Amoxicillin	33.52 ± 0.69	13.36
Sulfamethoxazole	28.07 ± 0.63	27.44
Tetracycline	27.92 ± 3.85	27.83
Antibiotic mixture	21.86 ± 0.93	43.48

Selectivity in the formation of the pointer compound

The selectivity of the assay was tested by determining the catalytic activity effect of CPO caused by salts and other antibiotics (Table 4) and, as a consequence, the formation of the pointer compound. All the salts tested showed less than 10% interference in the ampicillin assay, using the highest salt concentrations found in the environmental samples (see next section). However, in the presence of other antibiotics, catalytic activity was significantly affected because they are also substrates of the enzyme, and CPO could catalyze at the same time as the oxidation of more than one of them, affecting the transformation rate of ampicillin. It has been previously reported that CPO can transform sulfamethoxazole and tetracycline; the conversion of these two antibiotics by CPO was carried out in 4 min under similar conditions, so they are better substrates than ampicillin (García-Zamora et al., 2018). A similar result was reported for determining organophosphorus pesticides by CPO in the presence of some compounds (Rebollar-Pérez et al., 2016). Increasing the reaction time or amount of enzyme did not improve

TABLE 5 Analysis of ampicillin in spiked environmental water samples by the proposed biocatalytic method.

Water sample	Ampicillin added (μM)	Ampicillin found (μM)	Recovery (%)	Coefficient variation (%)
Groundwater	5	6.58	131.58	23.99
	10	9.64	96.42	5.08
Urban	5	5.57	111.43	8.44
	10	10.29	102.94	2.94
River	5	3.71	74.14	13.30
	10	10.89	108.94	1.29
WWTP	5	4.31	86.27	7.40
	10	8.64	86.38	2.85
Lagoon	5	7.22	144.36	19.83
	10	9.93	99.30	0.14
Groundwater	10	9.64	96.42	5.08

the reaction toward ampicillin oxidation. However, the pointer compound was still produced after a specific time, and the detection was not affected by the presence of other antibiotics. Neither sulfamethoxazole and tetracycline nor their reaction products present fluorescence under these conditions.

Analysis of environmental spiked water samples

The assay was applied to several environmental water samples to test the proposed assay's applicability. Supplementary Table S1 shows the physicochemical characterization of water samples collected from different sites. The BOD_5 values for water samples from the WWTP and the lagoon indicate that the water is contaminated; given the COD values, all water samples are heavily polluted. The vast difference in values between BOD_5 and COD is due to the presence of non-biodegradable substances.

The recovery percentage was used to describe the method's analytical performance and the assay's precision. Recovery refers to the amount of analyte measured as a percentage of the amount of analyte initially added to a sample of the appropriate matrix, which contains no detectable level of the analyte or a detectable level. As seen in Table 5, the proposed method adequately predicts the concentration of ampicillin at 10 μM in the different water matrices, and the recovery percent ranges from 86 to 107%. However, interfering effects were observed at a lower-range concentration (5 μM ampicillin) with a recovery of 14,474% and a variation of 7%–23%. The method's accuracy and precision depend on interferents simultaneously present in the matrix, which may have a summative or synergistic effect. To consider this effect, it is advisable to prepare the calibration curve in the environmental matrix in which the analysis will be performed. Figure 3 shows the calibration curves in each environmental matrix used; as can be seen, the fit presents an adequate coefficient of determination, and LOD and LOQ vary slightly, except in the lagoon water matrix, where we can see an effect on fluorescence intensity.

Conclusion

The detection of ampicillin in aquatic environmental matrices is relevant given its occurrence in water bodies, urban, and WWTP effluents. The biocatalytic reaction of ampicillin with the CPO enzyme reached around 80% conversion in 1 h, producing a fluorescent compound useful for detecting ampicillin in water samples. The RSM-CCD method optimized enzyme concentration, hydrogen peroxide concentration, and reaction time for fluorescent product formation. The method's sensitivity allowed ampicillin detection and quantification at the micromolar level (LOD and LOQ of 0.035–0.12 μM, respectively). Enzyme activity was not affected by the presence of salts commonly found in wastewater; however, it was affected by other antibiotics that are even better substrates than ampicillin. Optimizing biocatalysis conditions achieved 86%–140% recovery of spiked ampicillin from different environmental water samples.

Using a straightforward methodology, it was possible to determine ampicillin in different environmental water samples. Detection f compounds of environmental interest using enzymatic methods have a potential application given the mild reaction conditions and simple implementation steps; these methods are also fast and selective. However, there is a need to improve the sensitivity at nanomolar or picomolar levels; this could be achieved by pre-treating the

sample to concentrate it thousands of times or amplifying the enzymatic reaction's response signal.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

JG-Z, Biocatalytic oxidation, identification of reaction products; JA-A, selectivity, detection, and quantification limits; GR-P, Optimization of the detection method, FP-A, Statistical analysis, EG-D, methodology, writing-review, and editing, ET, Conceptualization, supervision, funding acquisition, writing-review, and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.1040903/full#supplementary-material

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