SUPPORTING INFORMATION

Evanescent wave optical fiber sensors using enzymatic hydrolysis on nanostructured polyaniline for detection of beta-lactam antibiotics in food and environment

Pooja Nag [a,b], Kapil Sadani[a,c], Sanjeeb Mohapatra[d], Suparna Mukherji [d], Soumyo Mukherji [a]

[a] Department of Biosciences and Bioengineering, [d] Centre for Environmental Science and Engineering, Indian Institute of Technology Bombay, Mumbai 400076, India

[b] Department of Mechatronics Engineering [c] Department of Instrumentation and Control, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal 576104, India

[#]*Corresponding author. Phone: 91-22-25767767; Fax: 91-22-25723480* mukherji@iitb.ac.in

Abstract:

The supplementary information describes (i) the optical detection assembly used for conduction of experiments; (ii) the method for preparation of polyaniline coated, TEM beta-lactamase immobilized optical fibers; (iii) detailed mass spectroscopic analysis of antibiotics hydrolysis; (iv) the effect of surface coverage of polyaniline on sensor performance; (v) pH sensitivity of polyaniline coated fibers; and (vi) sensor reusability studies. It also includes the transmission electron microscopy characterization of the polyaniline nanofibers, and the results for HR-LCMS analysis of wastewater.

S1. Optical detection assembly

Optical detection assembly used for experiments comprised a cool white light emitting diode (EDEX-11.A5-E1-AB16, 430nm -700nm) as the source and USB 4000, Ocean Optics USB 4000 (200nm to 900 nm) spectrophotometer as the detector. Light was coupled in the optical fiber using a 40X microscope objective (N.A.=0.65) and a X-Y-Z positioner (Newport®, USA) and coupled out using SMA 905 connector with bare fibre adapter (Thorlabs®, USA). Data acquisition was done using Spectrasuite® software, with an integration time of 50ms averaged over 100 samples.

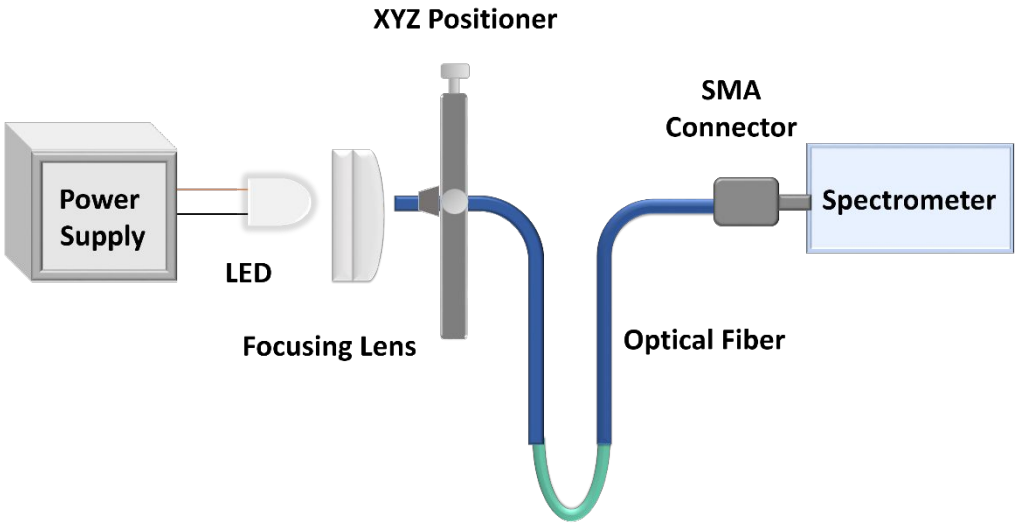

Figure S1. Optical detection assembly

S2. Polyaniline nanofibers coating on optical fibers

Polyaniline nanofibers were prepared via oxidative polymerization of the monomer aniline in 1M HCl with ammonium persulfate (APS) as the initiator. Cleaned U-bent optical fibers were incubated in 50mM aniline (in HCL) followed by addition of 50 mM APS to initiate the polymerization. The polymerization mixture gradually changed colour from pink to blue to green indicating formation of emeraldine salt[1]. Modification of the U-bent optical fiber with polyaniline nanofibers was monitored online and has been illustrated in Figure S2. Polyaniline deposition on optical fiber was done till an OD of 2, after which, the probe was immediately incubated in 1 M HCl to stop polymerization. The OD of 2 was selected in such a way so as to have an optimum density of polyaniline on the sensor surface. A higher density would increase noise while a lower density would reduce sensitivity. Figure S2 depicts the absorbance spectrum and time resolved spectral response due to polyaniline polymerization on fiber. The deposited emeraldine salt form of polyaniline was deprotonated in the subsequent steps of DI wash and glutaraldehyde treatment to form emeraldine base.

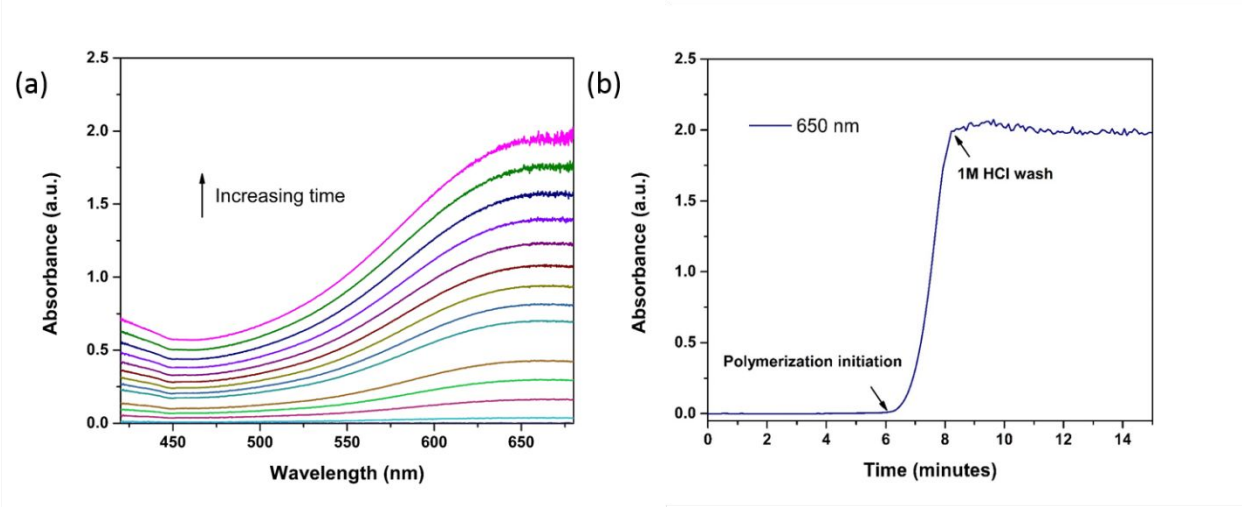

Figure S2. (a) Absorbance spectrum (b) Time resolved spectral response of polyaniline immobilization on optical fiber

S3. FEG-TEM of polyaniline nanofibers

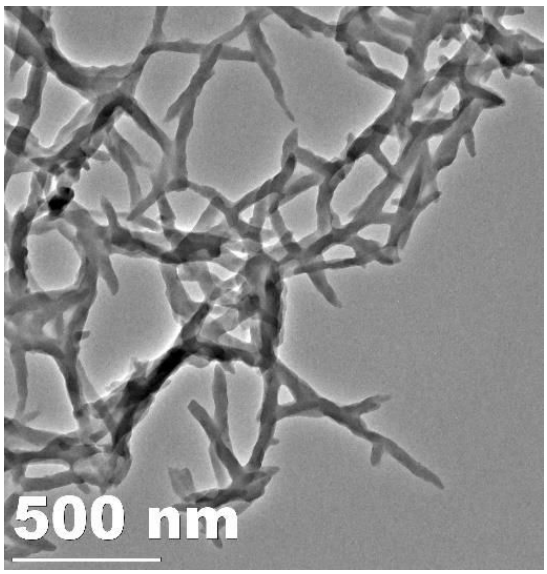

Figure S3. TEM image of polyaniline nanofibers

S4. Preparation of phosphate buffer saline (PBS)

10mM PBS was prepared according to cold spring harbor protocol. Briefly, 1.37 M sodium chloride (NaCl), 27 mM potassium chloride (KCl), 100 mM of disodium hydrogen phosphate (Na_2HPO_4) and 18 mM of potassium dihydrogen phosphate (KH_2PO_4) was added to 800 ml of deionized water. The pH was adjusted to 7 with HCl followed by addition of water to a final volume of 1L.

S5. Immobilization of beta-lactamase and BSA

Polyaniline coated optical fibers were modified with glutaraldehyde for covalent immobilization of the enzyme via amine cross-linking. TEM beta-lactamase enzyme was reconstituted in deionized water to a concentration of 120 IU/ml prior to use. Immobilization of enzyme on polyaniline coated fiber surface resulted in an increase in absorbance with peak at 600 nm (Figure S4). The remaining free amine sites were blocked with 2 mg/ml bovine serum albumin (BSA), reconstituted in 10 mM phosphate buffer saline, pH 7.

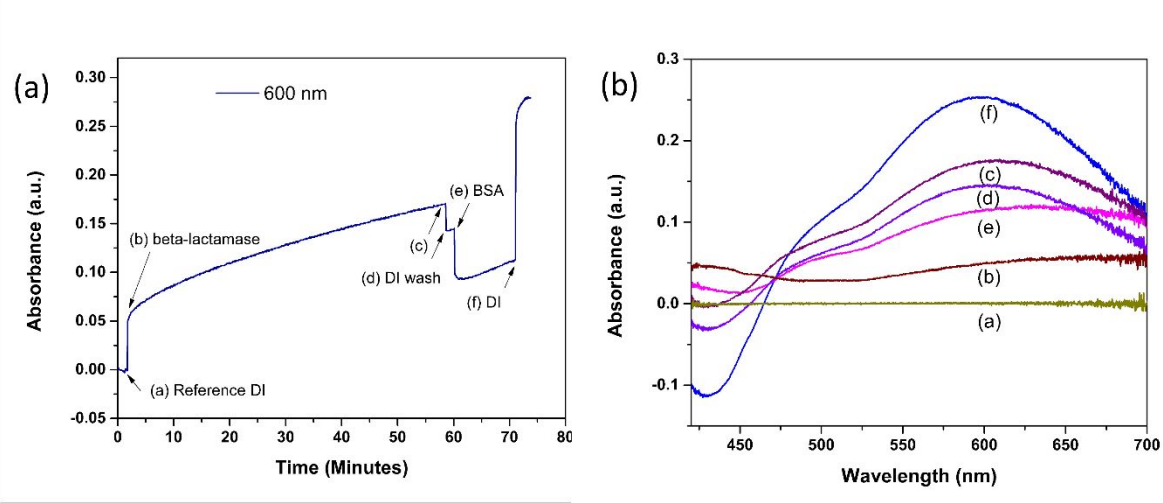

Figure S4. (a) Time response (b) Change in absorbance spectrum due to binding of enzyme beta-lactamase and BSA on polyaniline coated optical fiber

S6. Confirmation of enzyme activity with mass spectroscopy

Hydrolysis of the antibiotics, used for generation of calibration curves and validation studies of the sensor (ampicillin, amoxicillin, cefotaxime and ceftazidime), were confirmed through quadrupole time of flight mass spectroscopy (Q-TOF MS), Agilent Technologies, USA, in electrospray ionization mode. The antibiotics were dissolved in deionised (DI) water to a concentration of 1 mg/ml and incubated in the enzyme beta-lactamase for 1.5 hours. Comparison of m/z peaks in the mass spectrum of the antibiotics in DI water, and that incubated in enzyme, confirmed hydrolysis of the said antibiotics as discussed below.

Ampicillin

The mass spectrum of ampicillin (Molecular weight 349.4 Da) dissolved in DI water revealed the peak of the hydrogen $[M+H]^+$ and sodium adducts $[M+Na]^+$ of ampicillin at m/z 350.1 and m/z 372.1 respectively (Figure S5(a)). Incubation of the antibiotic with enzyme resulted in vanishing of the molecular peak at m/z 350.1 indicating hydrolysis of ampicillin. Hydrolysed forms of beta-lactam antibiotics have a mass shift of +18 Da. In the case of certain antibiotics, enzymatic hydrolysis is succeeded by decarboxylation of the hydrolyzed products, the peak for which appears at -44 Da. For ampicillin, these peaks were observed at m/z 368.1 $[Mhyd+H]^+$ and m/z

324.1 [Mhyd/decarb+H]$^+$ respectively. Additional peak at m/z 390.1 correspond to the sodium adduct of the hydrolyzed form of ampicillin [M_{hyd}+Na]$^+$ (Figure S5(b)).

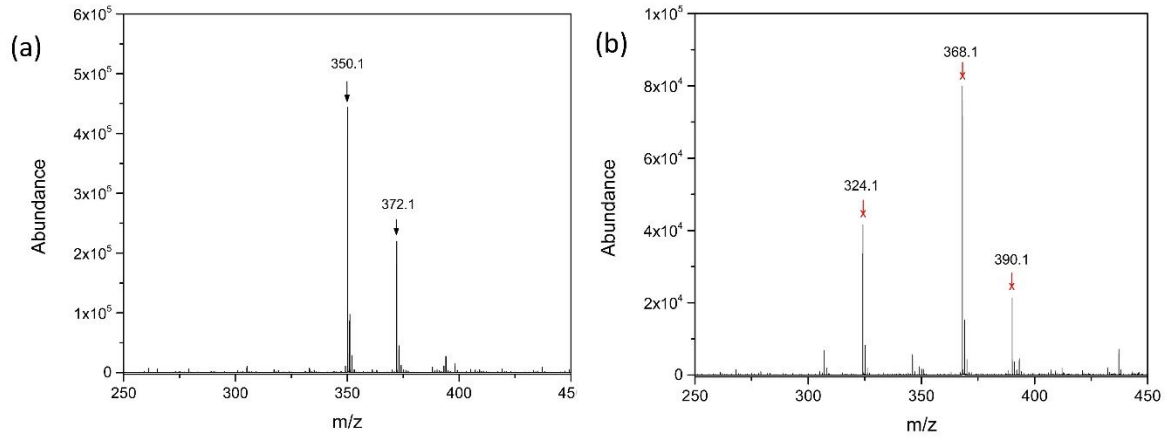

Figure S5 (a) Mass spectrum of (a) ampicillin (b) enzyme treated ampicillin (Red arrows indicate hydrolyzed peaks)

Amoxicillin

The molecular peak of amoxicillin (Molecular weight 365.4) at m/z 365.4 was not detected in the MS spectrum, but a peak at m/z 350.1 appeared due to NH_2 loss [M-NH_2+H]$_+$. The peak at m/z 372.1 correspond to the sodium adduct [M-NH_2+Na]$_+$ of the NH_2 eliminated molecular ion (Figure S6(a)). Incubation of the antibiotic with the enzyme beta-lactamase resulted in disappearance of the peak at m/z 350.1 confirming enzymatic hydrolysis (Figure S6(b)). Additional peaks due to hydrolysis were observed at m/z 368.1 and m/z 390.4 corresponding to hydrogen [M_{hyd}-NH_2+H]$_+$ and sodium [M_{hyd}-NH_2+Na]$_+$ adducts of the hydrolyzed NH_2 eliminated molecular ion. A peak at 324.1 can be attributed to the decarboxylated form of the hydrolyzed NH_2 eliminated molecular ion.

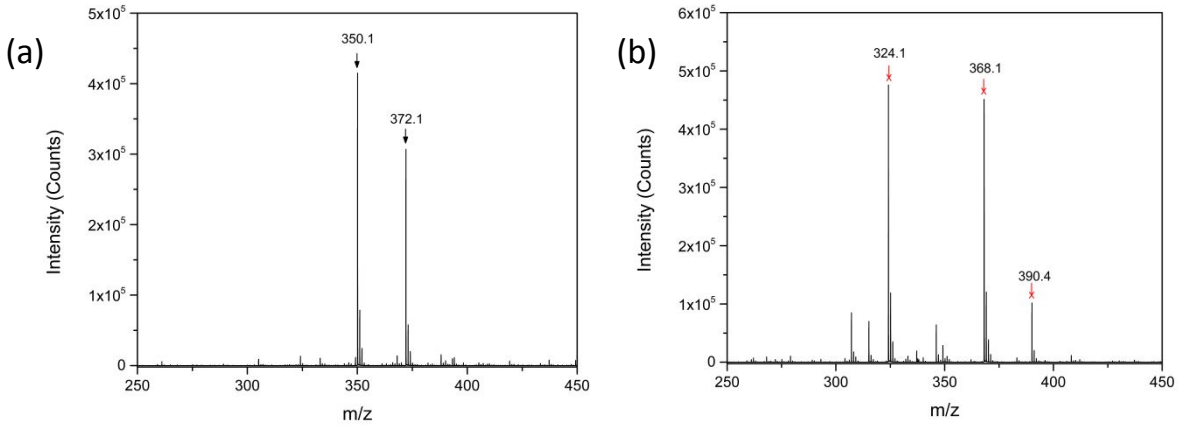

Figure S6. Mass spectrum of (a) amoxicillin (b) hydrolyzed amoxicillin (Red arrows indicate hydrolyzed peaks)

Cefotaxime

The protonated molecular peak of cefotaxime (molecular weight 455.47) $[M+H]^+$ and its sodium adduct $[M+Na]^+$ were detected at m/z 456 and 478 respectively (Figure S7(a)). Incubation of the antibiotic in beta-lactamase enzyme resulted in vanishing of the peaks and appearance of newer peaks at m/z 414 and 370 for $[M_h\text{-}X+H]^+$ and $[M_{h/d}\text{-}X+H]^+$ confirming enzymatic hydrolysis. Additional peak at m/z 243 was also observed due to hydrolysis of the antibiotic

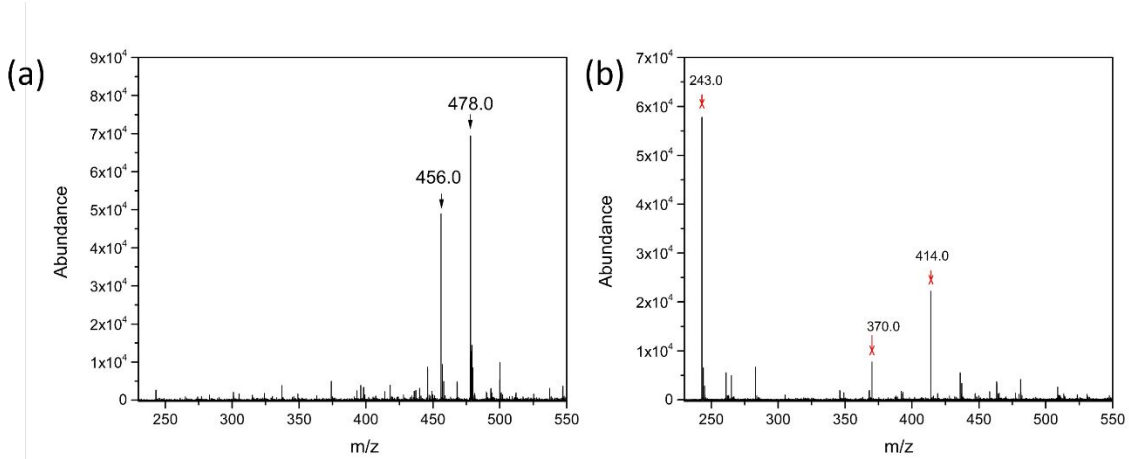

Figure S7. Mass spectrum of (a) cefotaxime (b) hydrolyzed cefotaxime (Red arrows indicate hydrolyzed peaks)

Ceftazidime

The molecular peak of ceftazidime (molecular weight 546.58 Da) $[M]^+$ dissolved in DI water was observed at m/z 546.9. A larger peak at m/z 468.0 appeared in the MS spectrum which can be attributed to the pyridine (molecular weight 79.1 Da) eliminated form of ceftazidime $[M\text{-}Pyr+H]^+$ (Figure S8(a)). Incubation of the antibiotic with beta-lactamase enzyme resulted in reduction of the molecular peak at m/z 547.1 and pyridine eliminated peak at m/z 468.1. Two additional peaks corresponding to hydrolysis of ceftazidime were observed at m/z 442.1 and 486.2 which corresponded to hydrolyzed form of pyridine eliminated ceftazidime $[M_{hyd}\text{-}Pyr+H]^+$ and decarboxylated and hydrolyzed $[M_{hyd/decarb}\text{-}Pyr+H]^+$ form of pyridine eliminated ceftazidime. (Figure S8(b)).

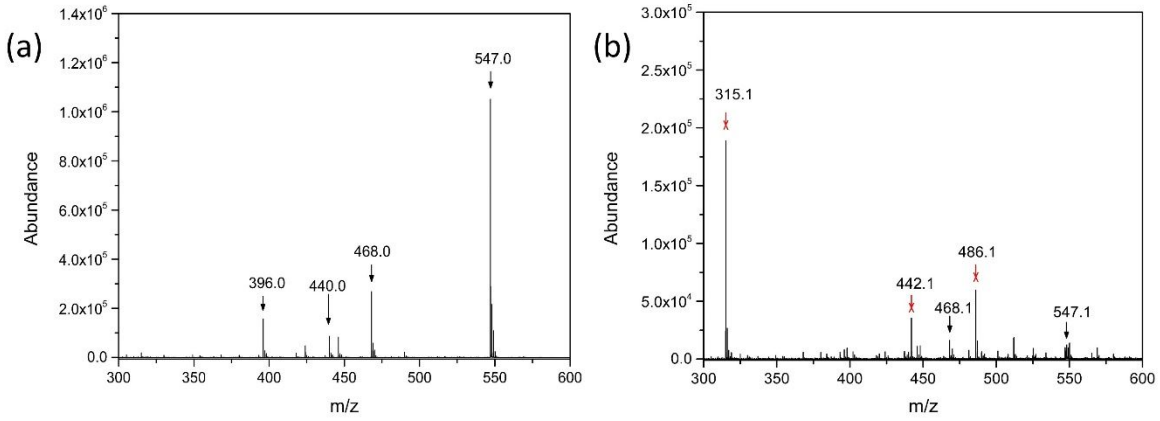

Figure S8. Mass spectrum of (a) ceftazidime (b) hydrolyzed ceftazidime (Red arrows indicate hydrolyzed peaks)

S7. Effect of surface coverage of polyaniline on sensor performance

The optical density of polyaniline deposited on the sensor surface plays an important role in sensitivity of the sensor. Lesser coverage of polyaniline on the sensor surface leads to lower sensitivity whereas a higher coverage of the nanofibers increases the sensitivity due to a larger overall surface area available for protonation. However, with increase in the OD of polyaniline, the amount of signal as received by the photodetector at the wavelength of interest reduces and hence the signal to noise decreases. The response of the sensor with different ODs of polyaniline to 1.8 nM of ceftazidime in deionized water is illustrated in Figure S9.

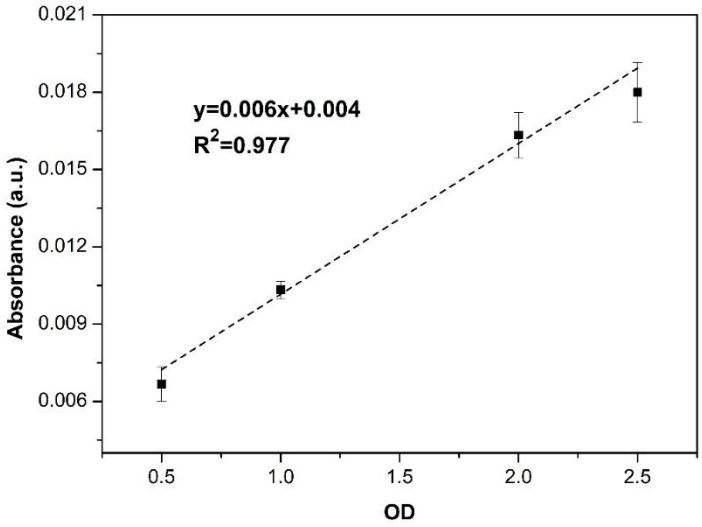

Figure S9. Response of sensors coated with different optical density of polyaniline, to 1.8 nM of ceftazidime in deionized water

S8. pH sensitivity of polyaniline coated optical fibers

The sensitivity of polyaniline coated optical fibers in the acidic range of pH 7 to 4 was studied as change in absorbance in the visible spectrum of 430 nm to 750 nm. 1X PBS solutions of pH 7 to 5 at intervals of 0.2 units were prepared. The optical fibers were referenced at pH 7 and the change in absorbance with respect to the reference was recorded (Figure S10(b)). Decrease in pH resulted in an increase in absorbance in the range 430 to 460 nm and a decrease in the rest (Figure S10(a)). Fig. shows the time resolved spectral response at 440 nm with different pH solutions.

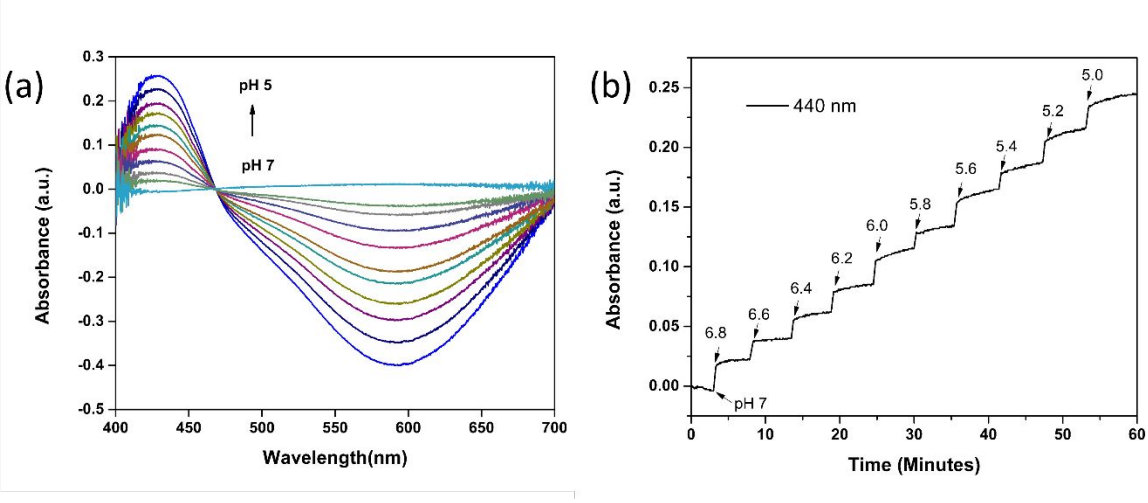

Figure S10. (a) Change in absorbance spectrum (b) Time resolved spectral response at 440 nm with different pH solutions

S9. Presence of antibiotics in wastewater

Table S1. The list of β-lactams along with their mass error, matched score with the database, formula, molecular ion (m/z), and retention time (RT)

Antibiotics	mass error (ppm)	Score	Formula	m/z	RT (min)
Amoxicillin	1.36	58.87	$C_{16}H_{19}N_3O_5S$	366.1134	7.88
Cefadroxil	-4.94	64.67	$C_{16}H_{17}N_3O_5S$	364.0944	51.52
Cefathiamidine	-0.16	52.62	$C_{19}H_{28}N_4O_6S_2$	495.135	29.09
Cefazolin	2.27	51.41	$C_{14}H_{14}N_8O_4S_3$	477.0207	21.29
Cefixime	3.55	87.05	$C_{16}H_{15}N_5O_7S_2$	454.0499	21.35
Meticillin	-3.8	54.64	$C_{17}H_{20}N_2O_6S$	381.1102	41.91
Penicillin V	1.44	66.15	$C_{16}H_{18}N_2O_5S$	373.0832	37.53

S10. Detection of antibiotics in waste water

To test the applicability of the sensor for detection of beta-lactam antibiotics in antibiotics-contaminated wastewater, the sensor response was recorded in triplicate (Figure S11). The calibration curve for tap water was used to estimate the cumulative concentration of beta-lactam antibiotics in the wastewater, and was found to be 32.4 nanomoles.

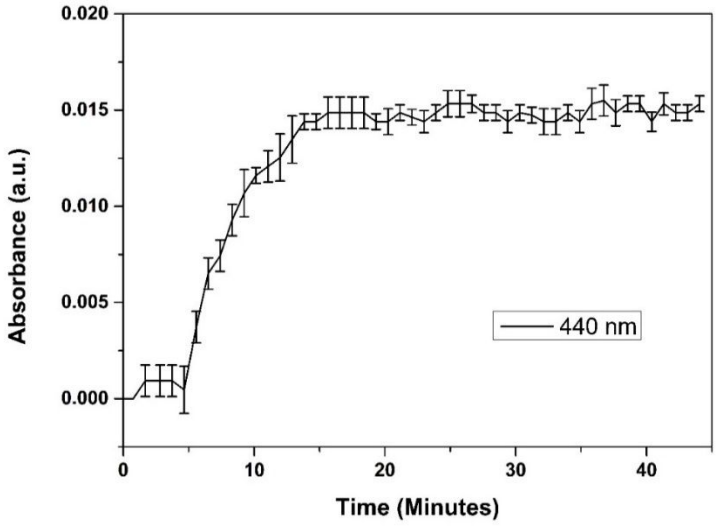

Figure S11. Time varying response of the sensor to beta-lactam antibiotic contaminated wastewater.

S11. Sensor reusability

Table S2. Errors associated with sensor reuse

No. of uses	Error w.r.t initial reading (%)	Coefficient of variation
Use 1	NA	4.88
Use 2	3.45	10.1
Use 3	13.79	5.66
Use 4	37.93	15.71

Reference

1. Boeva, Z. A., Sergeyev V.G., Polyaniline: Synthesis, Properties, and Application, *Polym. Sci. Ser. C*, **2014**, 56, 144–153