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Research Paper

Paper-based PANI/Enzyme Biofilter Development for Phenol Removal

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Abstract: Phenol compounds are used in many industrial areas. Due to their high toxicity and stability, phenol compounds are carcinogenic to humans and animals even at low concentrations during their production and use. For this reason, the removal of phenol contaminants is both necessary and beneficial. Water pollution caused by phenols is one of the most serious problems globally, threatening both people and the environment. Increasing industrial and human activities have led to an increase in wastewater discharge into water resources. These phenolic chemicals are harmful, and although there are different methods used, it is very important to find new materials and effective methods to remove these pollutants from water. This study aimed to convert the phenols purified from water using tyrosinase paste to a less harmful state by making an enzymatic biofilter for phenol removal, thanks to the polyaniline structure we formed on the filter paper, to ensure phenol retention. While this process took place, FeCl3 solution was used as the reactor material, and aniline was turned into polyaniline with FeCl3 solution in HCl. While these processes are being carried out, it is aimed to prepare the most efficient biofilter by using the components that make up the experiment at different concentrations. By calculating the % efficiency of the catechols, absorbance values were measured before and after filtration. It was revealed that the highest percentage of biofilter activity was formed using 0.15 M aniline, 10 KU tyrosinase enzyme, and 1% chitosan concentrations.

Keywords: phenol removal, polyaniline, biofilter, biotechnology

1. Introduction

Water pollution is one of the world's most serious issues, posing a threat to humans and the environment. Increased industrial and human activities have resulted in a rise in wastewater discharge into water resources. Phenolic chemicals from various industrial processes, such as refineries, herbicides, insecticides, pharmaceuticals, and so on, are among the most common water contaminants. These chemicals are harmful and degrading them is challenging; hence, discovering materials and effective methods for removing these pollutants from water is critical [1][2]. According to the Environmental Protection Agency (EPA), the wastewater's phenol concentration should be less than 1 mg/mL. Before being released into the receiving big bodies of water reservoirs, wastewater containing phenols and other hazardous substances must undergo thorough treatment. The most popular techniques for eliminating phenol and phenolic chemicals from wastewaters include electrochemical methods, solvent extraction, chemical oxidation, activated carbon adsorption, and biological treatment [3].

Many phenolic compounds may be efficiently removed using traditional procedures such as extraction, distillation, chemical oxidation, electrochemical oxidation, and adsorption, among others. Modern therapies, on the other hand, use less chemical reagents than older procedures, but they come at a significant energy expense [4][2][5]. The use of tyrosinase or peroxidase to treat

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wastewaters containing low concentrations of phenol has garnered recent attention for enzymatic approaches for the removal of phenol. The usage of peroxidase has the drawback of requiring stoichiometric levels of H_2O_2 as the oxidant, as opposed to tyrosinase, which simply needs O_2. Tyrosinase (EC 1.14.18.1) is a polyphenol oxidase found in a variety of organisms, notably the fungus Agaricus bisporus, that has tremendous promise as a biocatalyst for phenol biomodification and phenol-polluted water bioremediation [6][7][8]. Researchers have reported on the efficacy of immobilized tyrosinase in the breakdown of phenolic compounds, and it has been utilized to remove phenol. However, due to their exceptional biocompatibility, encapsulations on natural polymers have attracted a lot of attention as a means of enhancing the activity and stability of the enzymes. The polymer polyaniline (PANI) stands out among other polymers due to its special qualities, which include good environmental stability, ease of doping, and excellent redox recyclability, which allow for the construction of polymers with significant differences using straightforward acidic or basic treatments [9].

Polyaniline (PANI) is also an excellent material for adsorption of organic dyes and other pollutants because of its unique electrical properties and a number of other advantages, including ease of synthesis, low cost, excellent environmental stability, a simple acid-base doping/dedoping process, reactive NH– groups, and tunable properties [10]. PANI polymers feature several amino and benzene ring groups, which allow them to absorb organic and inorganic contaminants through - and electrostatic interactions. PANI has shown to be effective in removing heavy metal ions and organic pollutants by adsorption [11][12]. PANI Composites containing nanostructures have been investigated for a variety of applications including the removal of harmful chemicals. Recent investigations have shown that PANI-based adsorbents may remove drugs [13], insecticides [14], bisphenol A (BPA) [15], personal care products [16], endocrine disrupting compounds (EDCs) [17] at relatively high pH values (e.g. 5–7).

A combination of PANI with enzymes as a phenol removal biofilm could be a new option in the field. Thus, in this study, we developed an enzymatic biofilm by polymerizing aniline, thereby providing immobilization and using the tyrosinase enzyme. While we retained phenols with polyanilines, we also converted phenols with the tyrosinase enzyme. In this way, we have purified phenol, a harmful substance, from water.

2. Experimental Methods

2.1. Materials

40 WhatmanTM filter paper (210 μm of thickness and 8 μm of pore size), HRP enzyme type II (210 U/mg), Tyrosinase from mushroom (Tyr, EC:1.14.18.1), iron(III) chloride (FeCl3), hydrochloric acid, aniline monomer, catechol (≥ 99%), 4-aminoantipyrine (4AAP), hydrogen peroxide, ethanol, and acetic acid were purchased from Merck (Darmstadt, Germany). Phosphate buffer solution (PBS) was prepared using di-potassium hydrogen phosphate and potassium dihydrogen phosphate. All the chemicals were used under the laboratory grade.

2.2. Biofilter Preparation

In the first step of biofilter preparation, 3 M FeCl3 in 2% HCl was prepared as the reactor material. This iron solution was applied to the filter paper with the help of a brush and dried. Then 0.15 M of aniline prepared 2% HCl was placed in a beaker as a polymerization solution. Filter paper covered by FeCl3 was thrown into this beaker and shaken. As a result, the reactor material FeCl3 polymerized the aniline monomer, and polyaniline was formed due to chemical polymerization. As a result, aniline polymerization occurred on the paper's surface, and the filter paper was coated with

polyaniline. Then the filter paper was washed with ethanol and DI water. After drying the polyaniline-covered paper, a solution of 2% acetic acid and 0.25% chitosan solution was prepared and injected onto the polyaniline-covered paper surface. Chitosan modified filter paper dried again and got ready for the enzyme immobilization step. 10 KU of Tyrosinase enzyme was prepared in PBS and dropped onto the filter paper surface. Chitosan, a porous material, provided the immobilization of the tyrosinase enzyme. After drying the filter paper again, the enzymatic biofilter was ready for phenolic compounds removal from wastewater samples. The preparation steps for the PANI/Enzyme biofilter are represented detailed in Figure 1.

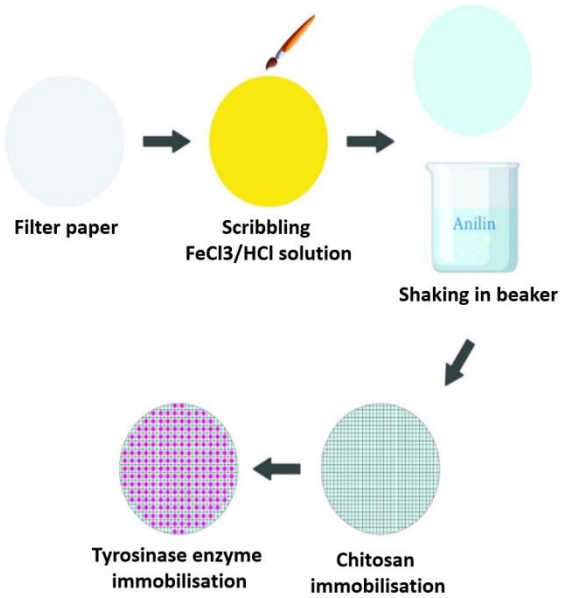

Figure 1. Biofilter preparation steps include chemical polyaniline preparation and enzyme immobilization

2.3. Phenol Removal

Catechol, a phenol-based substance, was used as a sample compound for phenolic contaminants in wastewater samples. Catechol solutions of different concentrations were prepared in PBS and filtered through the developed biofilter. In this filtration process, the polyanilines on the surface of the biofilter retained the phenol from the wastewater and allowed the phenol to be purified from the water samples (figure 2). The tyrosinase enzyme, which immobilized chitosan, a porous material, also transformed phenol into a less harmful form (O-quinone) (figure 2). The biofilter we prepared is used for phenol removal and degrades phenolic compounds to less harmful products.

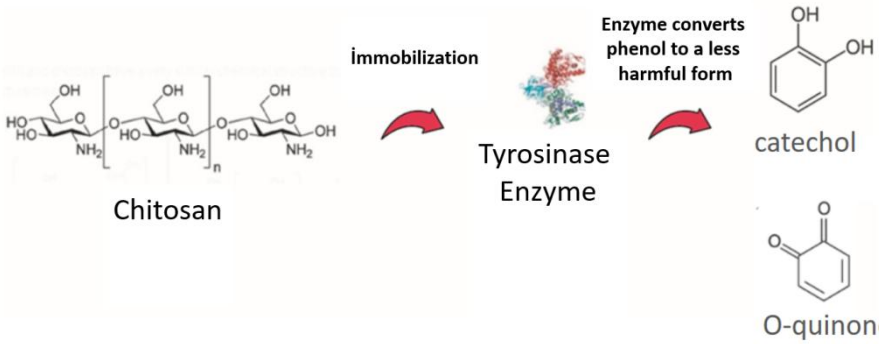

Figure 2. Chemical representation of the Chitosan – Tyrosinase enzyme - Phenol relationship

2.4. Calorimetric Detection of Phenol

Typically, the colorimetric analysis of phenolic compounds can be accomplished by monitoring the target's oxidative coupling with 4-aminoantipyrine (4-AAP). In detail, the colorless 4-AAP can react with phenolic compounds to procedure a colored quinone imine molecule. To enable the chromogenic reaction, H2O2 and enzymes are frequently used to rapidly analyze phenol in diverse conditions. Recently many groups described a colorimetric platform with horseradish peroxidase (HRP) for the visual detection of phenolic compounds [18][19][20]. Figure 3 presents the colorimetric reaction of phenolic compounds and 4-AAP that catalysis by HRP in the presence of H2O2. While the approach displays a good phenol monitoring performance, there are still plenty of opportunities to enhance the colorimetric method.

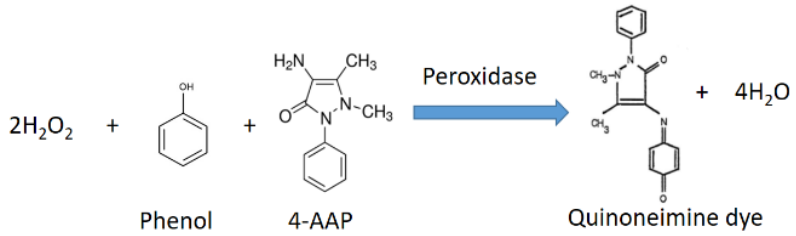

Figure 3. The chemical reaction of 4-AAP and phenolic compound catalyzed by HRP in the presence of peroxide, on the colorimetric detection method

In this part, phenol detections of different concentrations of catechol were performed. Three samples were prepared for each catechol concentration using 96 ELISA plates, and a colorimetric phenolic compounds detection procedure was applied. Different catechol samples of 10 mM, 7.5 mM, 5mM, 2.5 mM, 1 mM, 0.5 mM, and finally, an unknown catechol concentration as a filtration sample were prepared. For colorimetric detection of these samples, 40 µL 4-aminoantipyrine (4AAP), 30 µL hydrogen peroxide (H2O2), 10 µL HRP enzyme (peroxidase) were mixed with 100 µL of each catechol sample. The samples were kept at 37 °C. As a result, a color change was observed. Thermo Scientific™ Varioskan™ LUX was used for measuring the absorbance on 96 ELISA plates. Absorbance values were measured at wavelength 504 nm.

As a result of these values, it was aimed to draw a calibration curve depending on the color change due to the addition of the HRP enzyme. This graph analyzed the absorbance values of catechol at unknown concentrations after and before the filtration using the prepared biofilter. In addition, % phenol removal was calculated according to this graph, and it was revealed how successful the phenol removal was.

2.5. Biofilter Optimization

In this step of the project, the concentrations of the biofilter materials were optimized, and we tried to find the most efficient combination. The biofilter preparation procedure was repeated with three different concentrations for each Chitosan, Tyrosinase enzyme, and Aniline value. Firstly, the optimum value of aniline was found using 0.15 M, 0.30 M, and 0.60 M aniline. Then, only tyrosinase enzyme values were changed, and 1 KU, 5 KU, and 10 KU tyrosinase enzymes were used. Finally, an experiment was conducted using 0.5%, 1%, and 2% chitosan concentrations to find the most appropriate chitosan value. Filtering was performed again from these prepared biofilters, and according to the results, the optimum values of each material whose concentrations were changed for the most successful biofilter were found.

3. Results

3.1. Biofilter Preparation

Four different filter papers prepared for analysis were observed in Scanning Electron Microscopy (SEM), and their images at a 10.0 µm scale were examined. The first filter paper (figure 4, a) was an untreated plain filter paper. Then, a chemical aniline polymerization was carried out by shaking the filter paper in a beaker containing aniline and HCl, and the surface of the paper was coated with polyaniline (figure 4, b). After coating the third filter paper with polyaniline, an extra porous material, chitosan, was added (figure 4, c). A biofilter was created at the fourth step by adding the extra tyrosinase enzyme to the filter paper after chitosan (figure 4, d).

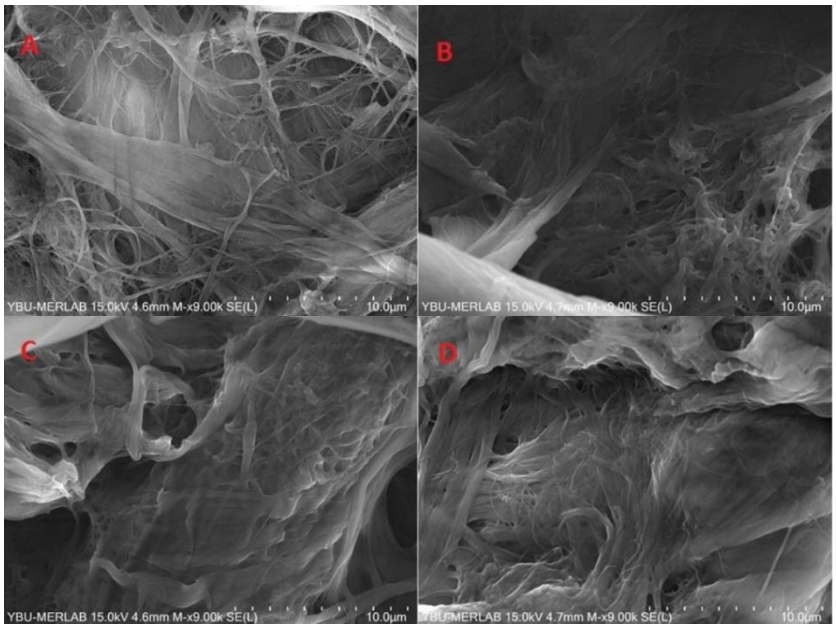

Figure 4. SEM images of different filter papers. (a) plain filter paper, (b) polyaniline coated filter paper, (c) chitosan-coated filter paper, and (d) tyrosinase enzyme coated filter paper

It was determined from figure 4 that the blank filter paper (a) had a much plainer fibrous structure than the other filter papers. It was also analyzed that cellulose fibers were much more definite, and it was observed that there was a finer fiber structure. It was observed that the polyaniline-coated filter paper (b) had a fibrous structure that had cover around them and was thicker than the first ones. Finally, it was analyzed that filter paper with chitosan and tyrosinase enzyme added (c and d) had a thicker fibrous structure and aggregated porous structure in which thicker fibers were formed.

3.2. Calorimetric Detection of Phenol

As a result of this process, a standard curve was obtained depending on the color change resulting from the measured absorbance values. According to the results of this experiment, the average absorbance values of three repetitions were measured. Considering these values, the standard deviation was calculated. According to the results, it was seen that consistent data were obtained as a result of an acceptable deviation. According to the curve created, the increase in the absorbance value was confirmed as the catechol concentration increased. This curve was graphed, formulated, and then turned into a linear graph (figure 5). It was observed that the catechol at different concentrations increased linearly according to the generated curve. This means that catechol with higher concentration was obtained with a darker color, and it was revealed that this catechol had

higher absorbance values. The independent experiments' absorbance values for each catechol concentration and their average O.D. values are represented in Table 1.

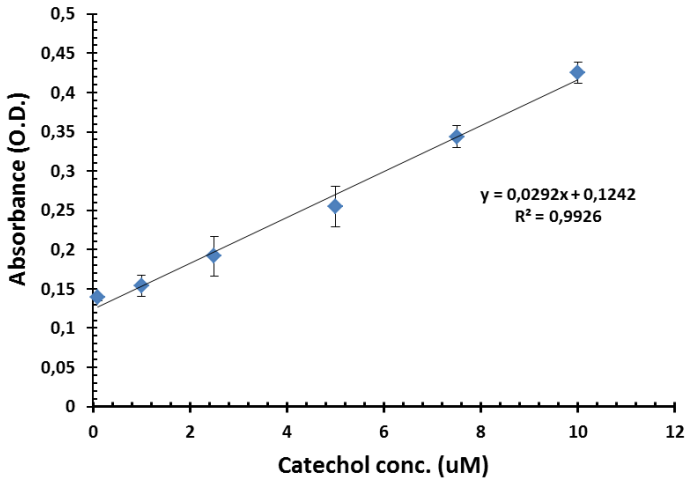

Figure 5. Calibration graph of change in absorbance value depending on catechol concentration

Table 1. Absorbance values of catechol for (a) unknown concentration before filtration and (b) after filtration were detected by the colorimetric method

Catechol (µM)	1st sample	2nd sample	3rd sample	average absorbance (o.d.)
(a) Before filtrate	0,43	0,47	0,47	0,46
(b) After filtrate	0,26	0,24	0,26	0,25

In addition, absorbance values of three samples of catechol with unknown concentrations were taken before filtering through the biofilter, and three samples taken after filtration was measured. The average absorbance values were calculated using the calibration curve. As expected, the absorbance value of the filtered samples was lower than the absorbance values before filtration since there was phenol removal after the filtration. As a result, it was revealed that there was successful phenol removal by the developed biofilter.

3.3. Biofilter Optimization

In this part of the work, we observed the effects of aniline, chitosan, and tyrosinase enzymes used in three different concentrations on the phenol removal process by the developed biofilter. The absorbance values of three samples prepared for each material were measured, and their average was calculated. According to the mean found, catechol concentrations were calculated from the standard curve, and their standard deviations were determined. It was tried to reveal consistent phenol removal according to the acceptable standard deviation results. Accordingly, the catechol concentrations before and after the filtration process were calculated at the 0.15 M, 0.3 M, 0.6 M aniline concentrations prepared, and the percent catechol removal was found. According to these results, it was observed that the most successful phenol removal was achieved for aniline at a concentration of 64.28% and 0.6 M. Percentage of removal and standard deviation results for all samples were represented in Table 2.

Table 2. Results with the phenol removal by the biofilter have aniline at different concentrations

	Phenol conc. in water sample (µM)	Phenol conc. after removal (µM)	% of Removal	Standard deviation
0.15 M Aniline	11,56	4,53	60,79	3,9
0.3 M Aniline	7,60	4,51	40,61	4,1
0.6 M Aniline	7,12	2,54	64,28	2,9

Secondly, the effect of tyrosinase enzyme at different concentrations on phenol removal was observed. The absorbance values of this enzyme at 1 KU, 5 KU, and 10 KU concentrations before and after filtration were measured, and their average was taken. It was revealed that there were consistent results due to the standard deviations calculated accordingly. Phenol concentrations were obtained by calculating the found averages from the standard curve, and percent phenol removals were calculated. Accordingly, the most efficient concentration was found for the tyrosinase enzyme with 63.94 % phenol removal from wastewater at a concentration of 10 KU, as represented in Table 3.

Table 3. Results with the phenol removal by the biofilter have Tyrosinase enzyme at different concentrations

	Phenol conc. in water sample (µM)	Phenol conc. after removal (µM)	% of Removal	Standard deviation
1 KU Enzyme	10,54	7,61	27,73	4,9
5 KU Enzyme	10,54	4,39	58,26	3,9
10 KU Enzyme	10,54	3,80	63,95	2,8

Finally, 0.5%, 1%, and 2% chitosan were tested on biofilter preparation to find the most efficient concentration for phenol removal. Absorbance values of catechol were measured before and after filtration from three different biofilters prepared with these concentrations. Three samples were prepared in the same way from the catechol before and after filtration. The mean and standard deviations of the measured absorbance values were calculated. In the light of this data, the most accurate data was tried to be obtained. Concentrations of catechol were calculated using the calibration curve. It was observed which chitosan concentration percent phenol removal occurred the most among these calculated concentrations. According to the results in table 4, it was seen that the highest phenol removal efficiency was determined at 1% and 2% chitosan, with quite close results (66.54 % and 66.58 %). Thus, we concluded that 1% of chitosan usage was enough to remove efficient phenol.

Table 4. Results with the phenol removal by the biofilter have chitosan at different concentrations

	Phenol conc. in water sample (µM)	Phenol conc. after removal (µM)	% of Removal	Standard deviation
% 0.5 Chitosan	9,79	5,06	48,31	4,5
% 1 Chitosan	9,79	3,27	66,55	3,6
% 2 Chitosan	9,79	3,27	66,58	2,9

According to these results, it was found that aniline, tyrosinase enzyme, and chitosan, which were tested at different concentrations, should be used in the most efficient concentrations for phenol removal. Accordingly, as is seen in Figure 6, the biofilter prepared using 0.15 M aniline, 10 KU tyrosinase enzyme, and 1% chitosan concentrations is the most efficient biofilter in terms of phenol

removal. It was observed that a biofilter prepared at these concentrations would remove the most phenol by percentage.

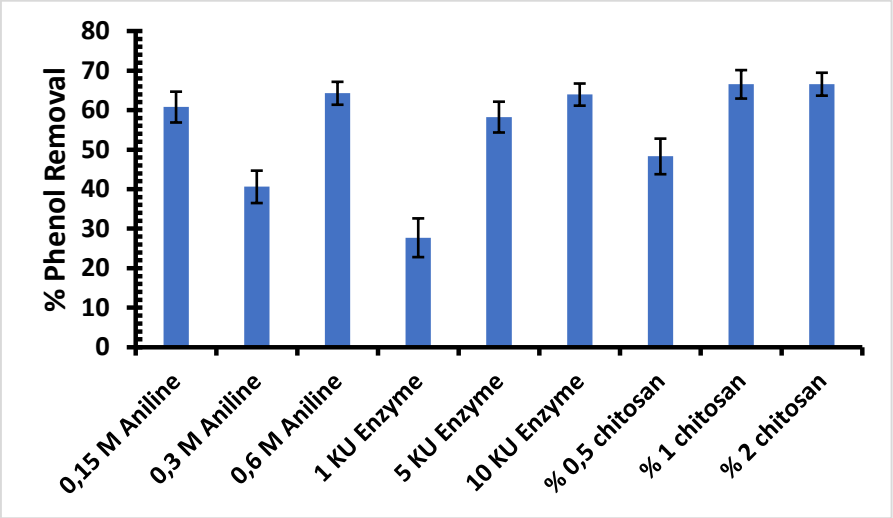

Figure 6. Graph of percent phenol removal in the presence of aniline, Tyrosinase enzyme, and chitosan at different concentrations

4. Discussion

In this work, Tyrosinase/PANI based biofilter was fabricated and each component concentration were optimized for efficient phenolic compounds removal from wastewater. The sufficient amount of chitosan was indicated as 1% while 0.15 M aniline and 10 KU tyrosinase enzyme combination gave the maximum removal efficiency. With the optimum amount of each component, we could reach 66 % of phenol removal which close to the activated carbon/ceramic based methods [21] and even higher than most of the biomaterials-based removal methods [22]. A combination of PANI, which is one of the mostly used biocompatible polymer, with enzymes as a phenol removal biofilm was also a new and unique option in the field. While we retained phenols with polyanilines, also converted phenols to less harmful compound with the tyrosinase enzyme. With this way, we have purified phenol, a harmful substance, from water samples.

5. Conclusions

The removal of phenols, which cause water pollution, is very important for both the environment and human health. An enzyme-based biofilter was fabricated and characterized to remove phenolic compounds from wastewater. In order to create the most efficient biofilter, the materials in the most suitable concentrations were tried to be brought together. In terms of phenol elimination, the biofilter made with 0.15 M aniline, 10 KU tyrosinase enzyme, and %1 chitosan concentration were the most effective. A biofilter constructed at these doses removed the most phenol in terms of percentage. The phenol removal efficiency was measured by detecting the amount of phenol in the unknown catechol concentration before and after filtration. It has been revealed that this small-scale laboratory study can be applied efficiently in real life under appropriate conditions and conditions, and results can be obtained in phenol removal.

Authors' contributions

NYT and NBA designed the structure. NYT grew the sample according to the specifications. NYT and NBA fabricated the device, carried out the experiments work, and wrote up the article.

Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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