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Characterization of Fiber Optic Biosensors Based on Chitinase Immobilization on Chitosan Film-Tofu Solid Waste: Metal Ions Monitoring in Water

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Abstract: A fiber-optic biosensor based on the immobilization of chitinase enzyme on chitosan-solid waste tofu film has been proven to be effective for analyzing the presence of Cd (II) ions in the coastal area of Makassar. The working mechanism of the fiber optic biosensor is based on the inhibitory properties of the chitinase enzyme by the metal ion Cd (II). Research objectives determine the optimization of chitosan film by using solid waste tofu immobilized chitinase, characterization of chitosan film - solid waste tofu immobilized kiinase, the characterization of fiber optic biosensors includes inhibition percentage and measurement concentration range, detection limit value, selectivity and reproducibility. The results showed the optimum conditions for chitosan film-solid waste tofu at a concentration ratio of 55%: 45% with an intensityof 32.01, tensile strength of film (tensile strength) of 36.01. The characterization of the biosensor which includes the percentage of inhibition, and the measurement concentration range of Cd (II) ion was obtained at a percentage of 27.126% with a measurement concentration range of 1.0×10^{-5} - 1×10^{-8} M or equivalent to 0.0083 – 8.30 ppm (103 Cd (II) 83.0 ppm). The detection limit value is 6.1 or equivalent to 5.9×10^{-6} M (0.00076 ppm). The fiber optic biosensor is selective for Co (II), Cd (II) and Zn (II) ions with a biosensor reproducibility of 0.1592 + 0.0562. The measurement results for the concentration of metal ion Cd (II) at 4 (four) locations were in the range of 0.1446 – 0.270, the lowest was at the Losari Beach location at 0.1446 ppm while the highest was obtained at the Soekarno Hatta Port location at 0.2194 ppm. The fiber optic biosensor measurement did not differ significantly from the SSA.

Keywords: Chitinase enzyme; Chitosan film-tofu solid waste fiber optic biosensor; Immobilization

Introduction

The presence of heavy metal ions in aquatic ecosystems contributes greatly to the pollution of the aquatic environment (Ali et al., 2019). Agricultural activities, combustion processes in industries that use fossil fuels and transportation systems are the biggest contributors to heavy metal ion pollution in water that can reduce water quality (Tan et al., 2020). Various heavy metal ion pollution in the waters has been determined such as metal ion pollution of Arsenic, Lead and Mercury which results in respiratory system disorders, carcinogenic kidney damage in infants and children and impaired immune function (Witkowska et al., 2021). Furthermore, Cadmium (Cd) compounds enter the human body through the absorption of water, the functioning of human organs is adversely affected by food and air. Cadmium poisoning is measured by the presence of cadmium in per deciliter of blood. Exposure to high concentrations of cadmium poisoning has an impact on humans, namely disruption of the respiratory State the objectives of the work and provide an adequate

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background, avoiding a detailed literature survey or a summary of the results. System, brain damage, and damage to kidney function (Andjelkovic et al., 2019). An enzyme-based biosensor is a method used in the development of biosensors for the analysis of heavy metal pollution in the aquatic environment and has developed rapidly over the last two decades (Ashrafi et al., 2019). The use of the enzyme immobilization technique increases the stability of the biosensor so that it is easy to pair with certain transducers (Polat et al., 2022). The use of biosensors based on enzyme immobilization has both sensitivity and selectivity in determining various toxic pollutants where enzyme activity is inhibited or inhibited by these pollutants (Bucur et al., 2018). Several biosensor systems based on enzyme immobilization using electrometer transducers and conductometers have been proposed for testing glucose and urea levels using glucose oxidase and urease enzymes (Ghourchian et al., 2004; Sakalauskiene et al., 2022). Although the enzyme immobilization method has been widely used in the development of biosensors, in general, these biosensors have a complex construction, both in terms of immobilization and transduction processes (Ali et al., 2019). As a result, it is not economical and less efficient. Additionally, the biosensor is unable to detect the sample if other metals interfere with it or cause it to be interpreted in a certain way, making it unable to assess the metal ions in the water sample as a whole (Elli et al., 2022). An alternative biosensor that has been proven to be effective in analyzing the presence of heavy metal ions, especially in water samples, is a fiber optic biosensor. A fiber-optic biosensor based on the immobilization of the enzyme urease in an ultra-bind membrane has been used to determine blood urea levels (Saeedfar et al., 2013). Furthermore, testing of lycoxanthin and xanthine compounds using the xanthine oxidase enzyme on a fiber optic biosensor has been able to produce a small detection limit value (Ablat et al., 2018). The amperometry urease enzyme-based fiber optic biosensor has also been used for the determination of Hg (II) ions in water samples and glucose compounds (Hermanto et al., 2019). The use of several types of enzymes in the design of fiber optic biosensors still has several weaknesses, namely the stability of the enzyme is still low, most of these enzymes are not resistant to high temperatures and organic solvents (Sharma et al., 2014).The chitinase enzyme is an enzyme that degrades chitin directly. Chitinase is an enzyme that can convert the N-acetomido group (-NH-CO-CH3) at 1.4 N acetyl glucosamine (chitin) into an amine group (-NH2) at 1.4 N glucosamine (Liu et al., 2013). Chitinase enzymes from various microorganisms (bacteria and fungi) generally, chitinase enzymes from microbes are extracellular enzymes that are secreted through the external membrane into the culture (Brzezinska et al., 2012).

The characterization of the chitinase enzyme isolated from thermophilic bacteria showed that the chitinase enzyme has specific activity, high selectivity, and is stableto high temperatures and organic solvents (Chrisnasari et al., 2016). The activity of the chitinase enzyme can also be inhibitedor inhibited by metal ions. Furthermore, there is the formation of a complex between metal ions and theenzyme through the active site or active site of the enzyme (Kuzu et al., 2012). The determination of the value of chitinase turbidity, which is influenced by optical properties, is very high. This indicates that the chitinase enzyme can be coupled to a fiber optic biosensor to analyze metal ion pollution in water. The focus of thestudy is (1) optimization of the chitosan-solid waste tofu film to immobilize chitinase (2). Characterization of the chitosan-solid waste tofu chitinase immobilized film including membrane tensile strength, film surface area, water absorption (3) Characterization of the chitosan-solid waste tofu chitosanfiber optic biosensor including the percentage of inhibition and the range of concentration measurements of detection limit values, selectivity and reproducibility.

Method

Material

$CuSO_4$. $5H_2O$ (99%, Sigma-Aldrich) was diluted in aquods to prepare 1000 mg/L of stock Cu (II) ions aqueous solution. Then, it was diluted in aquods according to the determined Cu (II) ions concentration at the range of 5.5-550 mg/L, and the real concentration was obtained using an Atomic Adsorption Spectrophotometer (AAS, 7000 SAA, Shimadzu Japan). It was 5.52, 26.49, 57.01,115.9, 251.5, and 567.47 mg/L. According to the AAS result and dilution factor, which was still in the range of previous studies.

Optimization of Chitosan-Solid Waste tofu Film Chitinase Immobilization

Several ingredients are mixed with various ratios, consisting of solid waste tofu: chitinase: chitosan in a ratio of 1:1:1; 1:2:2; 1:3:3 and 1:3:3 for a total of 10 grams. Then 20 ml of glacialacetic acid solution was added and stirred evenly. Then 0.1 g of glycerol was added as a plasticizer. Furthermore, the solution is poured on a rectangular plate with the desired thickness of the film, then left in the open air for 24 hours so that the solvent evaporates so that a thin film of chitinase immobilization is formed.

Fiber Optic Biosensor Construction

The construction of the fiber optic biosensor was carried out in which the immobilized chitinase film chitosan -tofu solid waste was attached to pH paper

(both in the form of a circle and then carefully placed into the flow cell, then connected to a column and stainless steel.

Determination of Maximum Wavelength

The maximum wavelength of each Cd (II) solution was measured by measuring the absorbance of the Cd (II) standard solution. Each standard solution was added with a buffer with a pH of 7, then allowed to stand for 30 minutes, and the intensity was measured using a spectrophotometer at a wavelength of 400-700 nm.

Measuring Concentration Range and Percentage of Inhibition

The measurement concentration range of Cd (II) is the lowest detection limit and the highest limit and still shows a straight-line relationship between the percentage of inhibition and -log Cd (II). This is determined by drawing a straight-line relationship between the percentage of inhibition with the lowest concentration limit and the highest concentration limit in molar units (M) by observing the Cd (II) solution between 10^{-9}–10^{-1} M.

Detection Limit Value

Specific analytical methods for the quantitative determination of an element or microscopicmolecule in a sample matrix are often faced with the problem of detection limit, which is expressed by the lowest concentration of a substance that can be determined statistically to have a different value from the analytical blank. According to IUPAC (the International Union of Pure and AppliedChemistry), the detection limit is expressed as a concentration derived from the smallest measurement that can be detected by a certain procedure. Meanwhile, according to ACS (American Chemical Society), the detection limit is the lowest concentration of an analytical stated by an analytical procedure.

Table 1. Characterization of Chitosan Film - Tofu Solid Waste

No	Volume (ml)			Intensity		
	Tofu Waste	Chitosan	Chitinase	S1	K1	R1
1	1	1	1	27.59	0.827	0.945
2	1	2	2	30.19	0.838	0.97
3	1	3	3	32.01	0.819	0.987
4	1	4	4	29.31	0.853	0.963

The detection limit is determined using he IUPAC equation, namely Y (LOD) = YB + 3 SB, where Y (LOD) is the detection limit. YB is the blank signal as the sample concentration using a signal equivalent to the blank plus three times the standard deviation, and SB is the standard deviation blank.

Selectivity

The selectivity coefficient was determined by observing the percentage of inhibition againstvarious standard solutions of the main Cd (II) ions and standard solutions of interfering ions (Mn+). Each observation result was plotted in the percentage inhibition relationship curve with log [M n+] to obtain the measurement concentrations, sensitivity, and selectivity coefficients.

Reproducibility

The reproducibility of the biosensor response can be determined by measuring the measuredinhibition percentage at least three times at each measured sample concentration, and by determining the difference between the highest and lowest percentage inhibition values using the same sample concentration.

Result and Discussion

Morphology Chitosan Film- Solid Waste Tofu

In various comparisons of the concentration of chitosan film-solid waste tofu chitinase immobilization affects the quality of the film, which in turn will affect the intensity value of the biosensor (Bagal-Kestwal et al., 2019). The best film quality for designing chitinase–Cd (II) is the ratio of chitinase: tofu solid waste: chitosan (1:33) where the film has a slope of 32.01 which is close to the theoretical value for the bivalent cation Cd ion. (II) of 31.5 mV/decade with a linear regression value of 0.97 close to a linear regression value of 1.

Chitosan membranes have optimal flexibility and porosity to absorb chitinase in the membrane (Kulkarni, 2021). These two physical properties are the physical properties needed to determine the lipophilicity of the film (Lozano-Navarro et al., 2020). Chitosan biosensor film - a good tofu solid waste must have an active ingredient composition that can bind to the analyte on the surface of the membrane - sample solution tabel 1 with a very fast, reversible and selective reaction (Bautista-Expósito et al., 2020).

Membrane strengh increases with increasing percentage of chitosan in the mixture and reaches its optimal point at a ratio of 55%: 45% as 36.01 followed by a decrease in tensile strength on increasing chitosan concentration in the mixture (Figure 1).

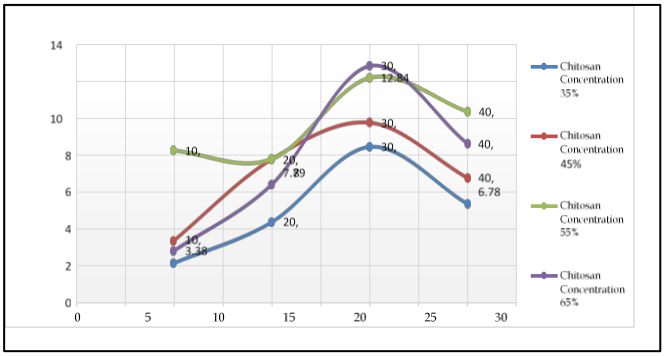

Figure 1. Tensile strength chitosan film-tofu solid waste

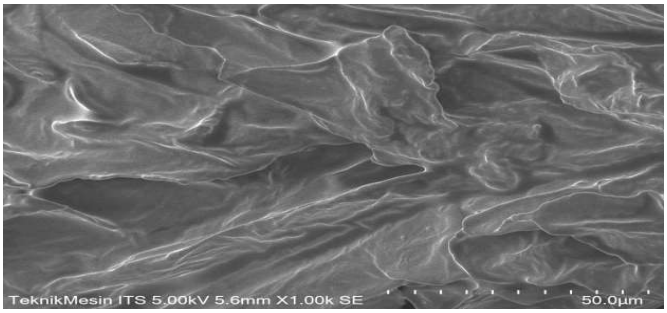

Figure 2. SEM Picture Chitosan-Tofu Flim

Percentage of Inhibition to-log Cd (II)

Determination of the percentage of inhibition and the measurement concentration range of Cd (II) ions.

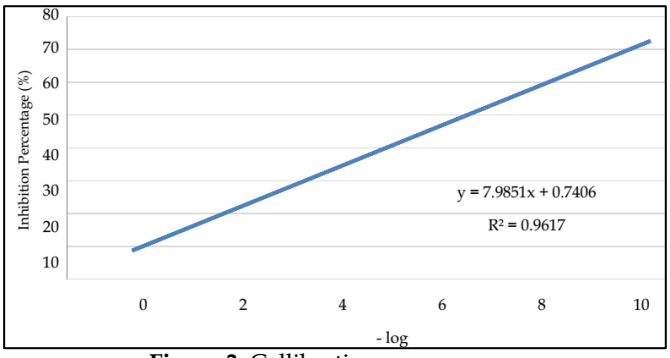

Figure 3. Callibration curve

The figure 3 shows a linear line -log Cd (II) from 5-8 or equivalent to a concentration of 1.0 x 10^{-5} - 1.0 x10^{-8} M having a slope of 0.567 with a linear regression value of 0.9617. The fiber optic biosensor based on theratio of tofu waste: chitosan: chitinase has an inhibition percentage value of 27.126% with a measurement concentration range of 1.0 x 10^{-5} - 1 x 10^{-8} M or equivalent to 0.0083 – 8.30 ppm (10^{3} Cd (II) 83.0 ppm). In this concentration range, it is considered the best for the measurement of Cd (II) ions because the percentage of inhibition is closest to the theoretical value of 20.30%. The intensity value in the linear region increases with the increase in the concentration of Cd (II). Factors that can affect the percentage of enzyme inhibition by metal ions are the number of enzymes trapped in the chitosan membrane, enzyme activity before and after the reaction with metal ions, chitosan membrane composition, enzyme immobilization procedures, properties of membrane-forming materials and membrane stability (Hermanto, 2020).

Determination of Detection Limit Value

From the results of x-axis extrapolation: - log Cd (II) the detection limit was found to be 6.1 or equivalent to 5.9 x 10-6 M (0.00076 ppm). This value is relatively small for the engraving of Cd (II) ions when compared to the SSA method. The measurement range is 10-8 – 10-5 M. A wide range allows for measuring Cd (II) at various sample concentrations

Selectivity

By identifying the other metallic elements present in the analyte and paying close attention to the determination of specific ions, the selectivity level of chitinase immobilization in the chitosan membrane for certain metal ions may be measured. Using chitinase oxidation with a 1:1 ratio for Cd (II) ions and other heavy metal ions, the following observations were made on how other heavy metal ions affected the measurement of Cd (II).

Table 2. Selectivity of Cd (II) Ions on Other Metals

Compound	Intensity
Cd (II)	0
Cd (II) + Co (II)	0.235
Cd (II) + Cu (II)	0.289
Cd (II) + Ni (II)	0.129
Cd (II) + Hg (II)	00.72
Cd (II) + Pb (II)	0.046
Cd (II) + Zn (II)	0.026

Table 2. According to a measurement of the chitin deacetylase-based biosensor's selectivity level based on the aforementioned table, the addition of Zn (II), Pb (II), and Zn (II) ions has no discernible impact on the analysis of Cd (II) ions. The difference in metallic Cd (II) ions is unaffected by the addition of these ions. However, it significantly affects the analysis of Cd (II) ions when heating Co (II), Cu (II), and Ni (II) ions. This is accounted for by the fact that the Ni metal ion's radius is the smallest order relative to those of other heavy metal ions, making it simple to excite Ni (II) ions to enter the immobilization phase with Cd (II) ions. The ability to interfere with heavy metal ions can be seen from the size of the radius which is shown as follows:

Reproducibility

The reproducibility of the biosensor response has been described for both the blank signal (without inhibition and with the inhibition signal). The blank signal was observed by introducing a chitin solution (0.7 M) into the biosensor system. The mean values (mean of

relative reflectance (n = 5) and the standard deviation obtained are quite reasonable, namely 0.1592 + 0.0562. The responsiveness of the degree of instability is obtained. In this case, the percentage of initiation is measured at least three times for each concentration of the sample, which is measured at least three times for each concentration of the sampletaken. The difference in the percentage value of the highest and the lowest value using the same concentration of Cd (II) ions was obtained as large as 2-5% This value describes a good repeatabilityvalue for the determination of metal ions in water samples.

Results of Analysis of Metal Ions Cd (II) in the Coastal Coast of Makassar

The results of the complete analysis of Cd (II) ions found on the Makassar coast can be seen in the following table. The sampling locations were at 4 location points, namely in the Tanjung Bunga area, Losari beach, Sekarbo Hatta harbor and Poetere harbor. The results of the analysis of the metal ion Cd (II) can be shown in figure 4.

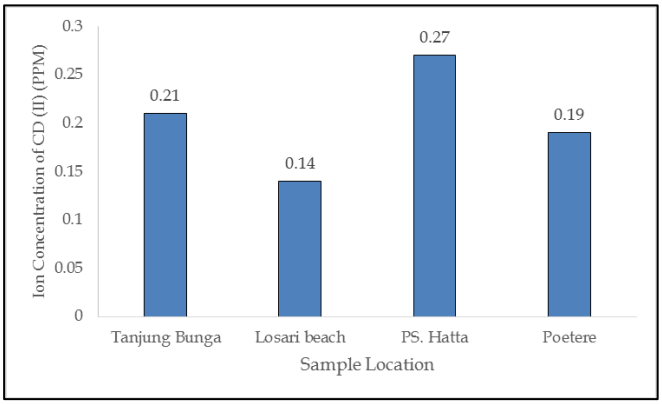

Figure 4. Percentage of Cd (II) metal ion levels in various coastal areas of Makassar

The Losari Beach location had the lowest Cd (II) ion level, 0.1446 ppm, according to the histogram in the figure, while the Soekarno Hatta harbor location had the highest, 0.2194 ppm. This demonstrates how the Soekarno Hatta port region's presence of a metal coating industrial waste disposal facility has averted the pollution of the environment with cadmium metal. Cadmium metal can come from corrosion in ship iron and the zinc plating industry (Chales et al., 2022). The long-term accumulation of this metal, industrial waste from urban areas, electroeltoplasting metal coating, the preservation and canning processes used to preserve fishery products, and household waste are the main contributors to the presence of Cd (II) metal ions in seawater in Makassar coastal waters.

Conclusion

The optimum conditions for chitosan film-solid waste tofu at a concentration ratio of 55%: 45% with an intensity of 32.01, tensile strength of film (tensile strength) of 36.01. The characterization ofthe biosensor which includes the percentage of inhibition, and the measurement concentration range of Cd (II) ion was obtained at a percentage of 27.126% with a measurement concentration range of $1.0 \times 10^{-5} - 1 \times 10^{-8}$ M or equivalent to 0.0083 – 8.30 ppm (103 Cd (II) 83.0 ppm). The detection limit value is 6.1 or equivalent to 5.9×10^{-6} M (0.00076 ppm). The fiber optic biosensor is selective for Co (II), Cd (II) and Zn (II) ions with a biosensor reproducibility of 0.1592 + 0.0562. The measurement results for the concentration of metal ion Cd (II) at 4 (four) locations were in the range of 0.1446 – 0.270, the lowest was at the Losari Beach location at 0.1446 ppm while the highest was obtained at the Soekarno Hatta Port location at 0.2194 ppm. The fiber optic biosensor measurement did not different significantly from the SSA method measuremen. A concise and factual abstract is required (maximum length 200 words). The abstract should state briefly the purpose of the research, the principal results, and major conclusions. An abstract is often presented separate from the article, so it must be able to stand alone. References should, therefore, be avoided, but if essential, they must be cited in full, without reference to the reference list. Non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

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Author Contributions
Conceptualization, Hamsina, Hermawati, Nani Anggraini; Data curation, M. Tang, Ruslan Hasani; Funding acquisition, Nani Anggraini; Methodology, Hamsina, Hermawati, Ruslan Hasani, Ifa Safira; Visualization, Hamsina, Ifa Safira; Writing–original draft, Ifa Safira, Ruslan Hasani, M. Tang; Writing–review & editing, Hamsina, Ruslan Hasani, M. Tang and Ifa Safira.

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Conflicts of Interest
No Conflicts of interest.

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