Review:

Tyrosinase-Based Paper Biosensor for Phenolics Measurement

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Abstract: *Environmental pollution resulting from various industrial activities is still a problem for developing countries. The high content of phenolics such as phenols, polyphenols, bisphenol A, catechol,* m- *and* p-*cresol from industrial activities are discharged into surface water, soil, and air. Periodic monitoring of the impact of these toxic pollutants is needed for proper control and handling. These detrimental chemicals are usually measured using conventional methods with many drawbacks such as expensive analysis costs, long measurement times, requiring competent analysts, and complicated instrument maintenance. However, the presence of tyrosinase-based paper biosensors is now considered the most promising tool in overcoming the challenges mentioned earlier because they can detect these components quickly, precisely, accurately, inexpensively, and can be measured* in situ. *The working principle of this biosensor sees optical changes such as dyes, redox processes, and physicochemical properties (aggregation or dispersion) due to the presence of analytes accompanied by the occurrence of color changes that appear. This biosensor uses a layer-by-layer electrostatic method, which causes the deposition of multi-layered films on solid surfaces. In this paper, we review the development of the tyrosinase-based paper biosensor method for phenolic measurement in water, air, and food that gives better results than the conventional methods.*

Keywords: *paper biosensors; phenolic compounds; phenols; tyrosinase; wastewater*

■ INTRODUCTION

The effects caused by water pollution that occurs in Indonesia cause poor levels of public health and a lack of availability of clean water in line with the increase in the economy and population. In a total of 34 rivers in Indonesia, almost 75% have been polluted [1] due to industrial and household activities in the form of organic and inorganic waste that exceeds the acceptable quality standard, which is 0.5 to 1.0 mg/L [2]. Phenol and polyphenol compounds are widely distributed in the environment, including those from sewage and natural waters. These pollutants tend to last longer and are difficult to degrade, causing severe toxic effects such as phenolic compounds. These compounds are present in many consumer products applied to body parts, such as mouthwash and liniment, including disinfectants for household cleaners. The by-products of these compounds originate from industrial activities such as the manufacture of dyes, plastics, pesticides, medicines, antioxidants, paper, and the oil industry [3]. Phenol liquid waste discharged directly into water bodies without prior processing makes the pollutant content higher, such as motor vehicle washing activities in the North Bekasi area which produce phenol on average 2.7, 1.5, and 3.2 mg/L [4].

In the natural processes, phenolics are often used in perfume manufacturing and are applied in food as antioxidants [5-6]. Likewise, phenol and bisphenol are widely used in hospitals and households as disinfectants. The compound is used in the cosmetic, leather, textile, and paper industry. Exposure to this affects the endocrine system [7], exhibiting estrogenic properties [8]. They are also found in water due to the degradation of natural materials such as dead plants and animals (organic material), industrial activities, and agricultural activities (the use of pesticides, such as

pentachlorophenol that are degraded to chlorophenol). The decomposed materials are then washed and discharged to water bodies. The wide application of phenolics results in drastic ecological problems [9], such as bad odor and taste, irritation problems, and toxic at high concentrations. Their presence in drinking water quality can occur due to contamination in groundwater caused by the release of industrial products or leachate. At concentrations exceeding the threshold in freshwater (0.001 mg/L, as stipulated in the Indonesian Government Regulation No. 82/2001), they are harmful and toxic to human health [10] for the nervous system, heart, kidney, liver, and easily absorbed through the skin and mucous [11], affect embryonic development and sexual differentiation, either by binding to or by blocking hormone receptors that can affect some hormones [12]. They may decrease fertility levels, changes in thyroid hormone content [13-14], changes in liver and immune function, development of heart problems, increased chances of miscarriage or premature birth in pregnant women [15], and increase the risk of diabetes and cancer [16-18]. Various types of inorganic substances in wastewater interfere with the measurement of colorimetric samples, so it is necessary to do some kinds of pretreatment (distillation and extraction) before the actual measurement. The phenolics are generally measured using gas chromatography (GC) or high-performance liquid chromatography (HPLC) equipment to test the water quality. However, this technique has several shortcomings, such as long analysis times, high costs, and competent technicians [19]. One way to overcome these problems requires equipment that can detect quickly and precisely, such as biosensors. Biosensors are one of the most widely applied technologies because of their small size, so they are easy to carry, have good accuracy of results, and are low-cost [20].

■ BIOSENSING TECHNOLOGY AND APPLICATION

A biosensor is an analytical tool to measure the target molecules contained in the sample. The device consists of components to identify molecules such as aptamers, antibodies, and enzymes. Molecular recognition between the recognition element and the target compound provides a biological signal, converted in quantity and then measured by the transducer. These signals can be detected optically (colorimetry, fluorescence, chemiluminescence, surface plasmon resonance) or electrically (voltammetry, impedance, and capacity). The biosensors can be used as a continuous monitoring tool in contaminated areas to detect hazardous chemicals or substances. This technique offers the possibility of rapid *in-situ* monitoring, thus providing real-time information. The principle of the biosensor is that the desired biological material (specific enzyme) is immobilized by conventional methods (physical or membrane retention, non-covalent or covalent bonding). This immovable biological material is closely related to the transducer. The analyte then combines with the biological material to form a bonded analyte, resulting in a measurable electronic response.

In some cases, the analyte is converted into a product associated with the release of heat, gas (oxygen), electrons, or hydrogen ions; then, the sensor converts the product into an electrical signal that can be amplified and measured. In short, biosensors are mini-systems that allow the development of portable sensors used in water quality monitoring [20-21] that can provide very low concentration values such as ppm, ppb, and ppt [22] of a compound. The development of biosensors has received considerable attention in recent years, such as enzyme-based, antibody, aptamer, immunosensor, and piezoelectric biosensors. Electrochemical, fluorescence, nanomaterial, silica or quartz, and microbial biosensors for various biomedical and environmental applications with future technologies have been reviewed [23]. The latest advances in current biosensor design combine nanomaterials with improving performance, such as optical, electrical, mechanical, and chemical properties (Table 1). However, current biosensor technology is quite good at detecting various analytes, with some disadvantages of low specificity and low sensitivity. In addition, the detection time is long, but the development of nanomaterial-based biosensors [24-26] can increase the sensitivity and response speed of biosensors in meeting

Table 1. Tyrosinase-based biosensors over time and their performance

Sensor layer	Analysis	Detection method	Concentration range	Results	LOD	Ref.
TiO_2/MWCNTs/PDDA/Nafi	Determination of bisphenol A in a flow-batch system	Electrochemistry of TYR/TiO2/MWCNTs/PDDA/Nafion biosensor	0.28–45.05 M	Sensitivity of 9137 μA mM^1 cm^2, respond time every 5 min for 45 min, stability 14 days, 25 °C	0.066 mM	[56]
Au/CoP-Tyr	Dopamine (DA) detection	Cyclic voltammetry, AFM, and EIS	2–30 μM	The CoP-Tyr biosensor provides high sensitivity and good stability for up to seven days. Sensitivity 1.22 ± 0.02 μA cm^{-2} μM^{-1}. The standard deviation (RSD) value obtained is up to 4.7%,which indicates the potential use of biosensors in samples (blood or urine). The average achievement for CoP-Tyr is 96%	0.430 μM	[72]
Porous Silicon	Optical monitoring of pyrocatechol	Spectrophotometer UV-Vis	1–100 μM	Sensitivity increases with increasing catechol concentration	0.43 μM	[68]
Tyr/SN-PA/SPE	Phenolic compounds in water	Differential pulse voltammetry (DPV) and amperometry methods	0.01–160 μM and 0.1–300 μM	Phenol detection concentration range of 0.01–160 μM and 0.1–300 μM	0.007 and 0.042 μM	[6]
CNTs	Phenol in wastewater treatment plant	Electrochemistry (Screen-printed electrode)	0.5 mM	The activities of EA, EAC, and EAPC (catechol oxidation process) were 2.4, 4.6, and 25 A394/min per 1 mg CNTs, respectively. The activity increased 10.5 and 5.4 times higher than EA and EAC, respectively. Response time 128 h, 40 days	14 and 35 mM	[63]
Nafion/Tyr/Au/SPCE	Bisphenol A detection	Voltammetry, potentiostat, SEM and XRD	0.5–50 μM	The biosensor has a reproducibility (n = 3) with a relative standard deviation of 11%, and the obtained %RSD (n = 10) yields a value of 0.5% indicating good repeatability. Biosensor is stable in storage time after 6 months with 90% response	0.077 μM	[67]
Ty/Chit/PtNP/GPH-CSPE	L-tyrosine detection in medical and pharmaceutical samples	Voltammetry	0.1–100 μM	The maximum sensitivity and selectivity response of the biosensor was +0.8 V vs. Ag with an optimal pH of 7	0.0475 μM	[69]
Long fiber grating with polyacrylamide gel	Phenol solution concentrations	Fiber-optic sensor	0–1000 μM	The sensitivity of the sensor to catechol, *m*-cresol, 4-chlorophenol and phenol was 0.0088, 0.0021, 0.0018, and 0.0009 nm/μM	7.6 μM	[37]
Graphene oxide	Phenol solution concentrations	Surface plasmon resonance (SPR) spectroscopy	1–20 μM	Sensitivity 0.00234° M^{-1}	1 μM	[70]
Au/pol/Tyr	Epinephrine detection	Chronoamperometry	0.1–50 μM	The sensitivity based on the calculation of the ratio of the slope of the calibration curve to the surface area of the electrode was obtained 3.08.10-7A·μM^{-1}·cm^{-2}, and stability of the biosensor was tested every week using a 20 M EP solution, showing the stability level for 30 days	0.06 μM	[64]
GE/β-CD-AuNPs/Tyr	Drug inhibition	Amperometry	1.56–25 μM	Low RSD values were obtained as 0.11 and 0.10%, respectively, indicating good repeatability (3.31 ± 0.34 A) and reproducibility (3.02 ± 0.04 A). The biosensor was able to maintain 70% activity after the 10^{th} day at 4 °C	0.42 μM	[65]
SiSG-TYR/Fe_3O_4-MWCNT/GCE	Simultaneous detection of catechol and hydroquinone in local tap water	Cyclic voltammetry	1.5–30 and 1.5–40 μM for CC and HQ	The biosensor provides satisfactory repeatability, reproducibility, good stability, and anti-interference performance with low relative standard deviation (RSD) values of 1.38 and 1.18%, respectively	0.055 and 0.057 μM	[62]
Tyr/CSCNT/felt	Determination of hydroquinone in water	Voltammetry, SEM, and AFM	9–545 μM	CSCNT/felt was able to detect the presence of hydroquinone, but there was a significant increase in current when Tyr was immobilized in CSCNT/felt. Measurement of hydroquinone was conditioned in	1.4 μM^{-1}	[71]

Sensor layer	Analysis	Detection method	Concentration range	Results	LOD	Ref.
PA6/PAH@AuNPs/Tyr/FTO	Bisphenol A detection in water	Chronoamperometry, SEM, and Spectroscopy (UV–Vis	0.05–20 µM	0.1 M phosphate buffer, at a maximum pH of 7.0 (R^2 = 0.99777), LOQ was 4.5 µmol L^{-1} A large irreversible peak when 5 M BPA was added to the PA6/PAH@AuNPs/Tyr electrode with (n = 3), an increase in the RSD value of 2.9 to 7.6%	0.011 µM	[73]

the detection requirements of the target analytes [27].

Various biosensor applications from year to year continue to be developed in various aspects such as environmental quality monitoring [28-30], the food sector [31], the agri-food sector [32-33], agriculture [34-35], for diagnostic purposes (discovery of pathogens and drugs), detection of toxins and diseases [36], and detection of hazardous substances in defense sector [37-38]. The research that has been carried out by the author [39] used microorganisms originating from Indonesia (*Lactobacillus plantarum, Uricase*) immobilized on natural zeolites as solid media in the health sector. Uric acid in urine samples could be easily detected by biosensors using carbon paste electrodes at the optimum conditions, namely pH 7.6 and 28 °C, giving a uric acid concentration value of 0.015 mM; the device was stable for up to 18 d. The next work [40] was carried out using the results of endophytic-free, wild dringo bengle (*bangle*, *Zingiber cassumunar*) tissue cultures to determine the characteristics of pancreatic inhibitory compounds. The results were then compared with the activity of Streptomyces, endogenous AEBg12 extracted with ethanol (the best solvent), to obtain pancreatic lipase inhibitor compound with IC_{50} value of ethanol extract 180.83 g/mL. The TLC results showed that the ethanolic extract of Streptomyces AEBg12 had a luminous blue band, indicating the presence of flavonoids, flavanones, flavonols, or isoflavones, due to the inhibitory activity of the microbe. AEBg12 was higher than *bangle* and wild *bangle* tissue culture. Another use is to detect malaria spreading, which generally occurs in tropical and densely populated areas such as Indonesia and the African continent. Nearly 1.2 million deaths each year occur in Africa due to malaria; 70% of malaria infection is located in 11 African countries and the Indian subcontinent [41]. This is a severe threat because it can cause loss of human life and reduce government budgets for developing and underdeveloped communities. Early and rapid detection is needed, as performed by Ragavan et al. [11] by designing a gold nanoparticle biosensor to prevent the spread of malaria. In terms of health, this biosensor technique continues to develop from time to time [42-43]. Enzyme-based biosensors have also been used to monitor beer quality during storage using cobalt phthalocyanine as an electronic mediator [28] and continue to progress in agriculture [35] as well as in the environmental field. Enzyme-based biosensor technology has made this equipment a major trend from 2010 to 2018 due to its high selectivity and sensitivity over antibody-based biosensors [44].

■ TYROSINASE-BASED PAPER BIOSENSOR

Compared to chip-based biosensors, the use of paper-based biosensors in POC testing has received more attention due to their cost-effectiveness, biodegradability, ease of manufacture, functionalization, and modification [45]. Tyrosinase is known to be able to detect phenolic compounds because of its high substrate specificity. This enzyme is a copper-containing monooxygenase found in many microorganisms, animals, and plants [46-47] and catalyzes two main reaction processes: the hydroxylation of monophenolase and L-tyrosine, the oxidation of diphenolase and L-DOPA (3,4-dihydroxyphenylalanine). A tyrosinase-based biosensor is a preferred method and is being developed because it has advantages such as specificity, fast response time, accuracy, inexpensive analysis, and is more environmentally friendly [20]. The main properties of the development of tyrosinase-based biosensors include accuracy and precision, sensitivity, specificity, wide measurement range, test reliability, easy calibration, and long-term stability. These properties are beneficial for their use in food and environmental applications that make researchers continue to develop

tyrosinase-based biosensors in various test analyses with their advantages and disadvantages (Table 2). The tyrosinase-based paper biosensor is one of the many existing techniques because it is more practical without the need for sophisticated tools in the subsequent testing. The function of filter paper in paper biosensors is to facilitate strong biomolecular adsorption and improve morphological properties, strength, and retain moisture.

Table 2. Tyrosinase-based paper biosensors over time and their performance

Sensor layer	Analysis	Detection method	Concentration range	Results	LOD	Ref.
Chitosan & alginate	Phenolic Compounds in Water	Paper Biosensor (optic), Spectrophotometer UV-Vis	1–500 µg/L	The sensitivity of paper biosensors for clean water and river water is 5 g/L	0.86 (±0.102) µg/L	[49]
TYR and MBTH	Polyphenol test in grapes	Paper Biosensor (optic)	0–0.5 mM	Smaller yield than the Winder-Harris assay and Folin-Ciocalteu assay	5 µM	[52]
Chitosan & alginate	Bisphenol A dust household scale	Colorimetric and GC	0.05 to 3.87 µg/g	The average color intensity of 100 µg/g household-scale BPA dust is 112.6 (± 2.082) with an RSD value of 0.018 (n = 3). The biosensor was rinsed using a phosphate buffer solution and tap water. Slightly higher colorimetric yield at 100 µg/g BPA in phosphate buffer (112.6 ± 2.082; n = 3) compared to tap water (110.1 ± 3.055; n = 3)	0.28 µg/g	[57]
Gold nanoparticles, chitosan and alginate	Phenol from the effluent of wine, paper, and plastic industries	Paper biosensor (see the color change that occurs)	0–1 mM	Waste sensitivity from winery (0.991 mM), paper (0.78 mM) and plastic (0.487 mM)	-	[58]
TiO_2/MWCNTs/PDDA/Nafi	Determination of bisphenol A in a flow-batch system	Electrochemistry of TYR/TiO_2/MWCNTs/PDDA/Nafion biosensor	0.28–45.05 M	Sensitivity of 9137 µA mM^1 cm^2, respond time every 5 min for 45 min, stability 14 days, 25 °C	0.066 mM	[56]
Tyr and MBTH	Monitoring of phenolic compounds in wastewater	Paper biosensor	0–0.512 mM	The sensitivity of the biosensor was tested using 4-chlorophenol, catechol, *m*-cresol, and *p*-cresol in a concentration range of 0.001 to 0.512 mM. Sensor stability for 70 days at 4 °C	0.032 mM for 4-chlorophenol and 0.128 mM for *m*-cresol and *p*-cresol	[66]
Nafion/Tyr/Au/SPCE	Bisphenol A detection	Voltammetry, potentiostat, SEM and XRD	0.5–50 µM	The biosensor has a reproducibility (n = 3) with a relative standard deviation of 11%, and the obtained %RSD (n = 10) yields a value of 0.5% indicating good repeatability. Biosensor is stable in storage time after 6 months with 90% response	0.077 µM	[67]
Tyr/SN-PA/SPE	Phenolic compounds in water	Differential pulse voltammetry (DPV) and amperometry methods	0.01–160 µM and 0.1–300 µM	Phenol detection concentration range of 0.01–160 µM and 0.1–300 µM	0.007 and 0.042 µM	[6]

Hydrogen bonding and electrostatic adsorption occur between filter paper and chitosan due to the charged surface and structural similarities [48] (Fig. 1). The tyrosinase catalyzes the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones (Fig. 2).

A paper biosensor made from cellulose is used as a supporting matrix in maintaining enzyme activity, and tyrosinase identifies samples on the paper's surface in maintaining the enzyme activity. The paper biosensor assembly is formed using a layer-by-layer (LbL) technique so that the solution of chitosan and alginate polyelectrolytes can settle to the surface layer of filter paper. The principle of this paper biosensor is to look at the color formation that occurs, which can then be measured by colorimetry [49].

Paper biosensors assembly using the electrostatic LbL method allows for the uniform deposition of

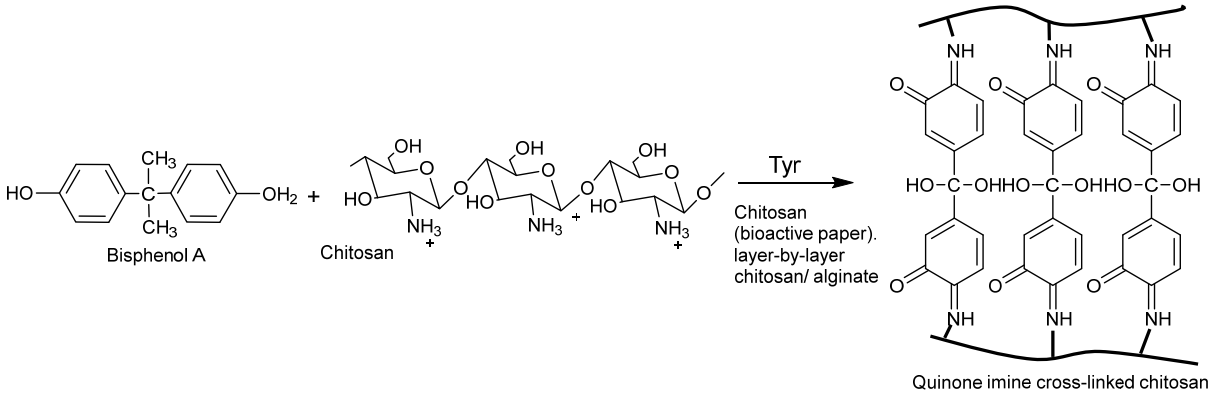

Fig 1. Mechanism of tyrosinase-based paper biosensor for phenolics

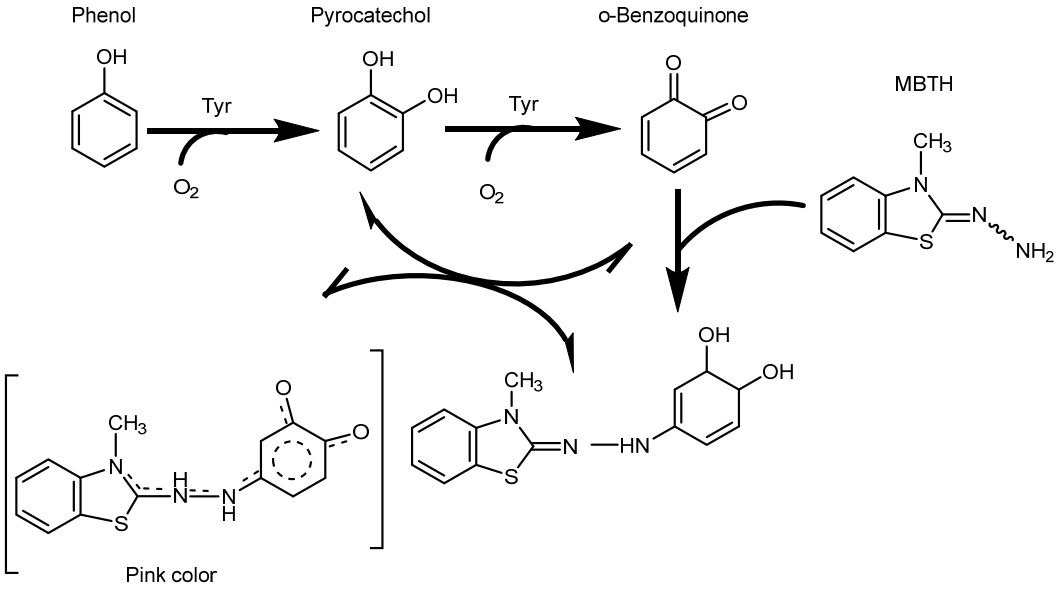

Fig 2. Mechanisms of the tyrosinase reaction

multilayer films on the chitosan surface [49]. This assembly technique uses a combination of enzymatic oxidation of phenolic compounds by chitosan and tyrosinase that is stable, effective, and very flexible. The technique allows the incorporation of various materials such as polymers, biomolecules, clays, metal oxides, colloidal nanoparticles, and even biologically active compounds into various multilayer systems. The most crucial part of constructing paper biosensors is biocomponent immobilization [50]. Tyrosinase-based paper biosensor technology is based on quantitative measurements of the formation of color intensities, which are then expressed digitally, such as red, green, and blue, and then quantified as analyte concentrations [51]. Therefore, the paper biosensor method is very appropriate for monitoring environmental quality and food and beverage products without the need for sophisticated techniques as these techniques continue to be developed.

■ PAPER BIOSENSOR APPLICATIONS FOR PHENOLICS MEASUREMENT

Advances in paper biosensor techniques over time are used in many activities such as in the food industry and the health and environmental fields because they can provide stability and sensitivity that is more effective

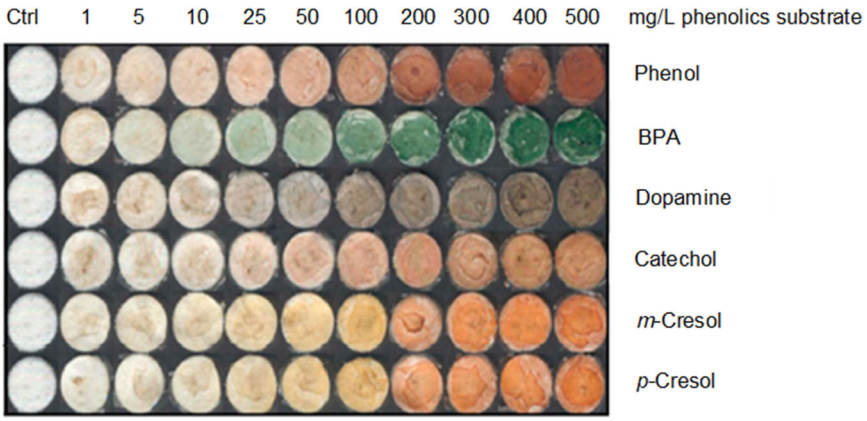

Fig 3. The information of colors for each compound [49]

than conventional methods, including utilizing effective chemicals in the measurement process. For example, paper colorimetric bioassays for detecting phenol have been successfully carried out to determine river water quality and, for the first time, without using chromophore reagents, were able to measure phenolic concentrations as low as 5 g/L with good results at the time of color formation (Fig. 3).

The paper biosensor in this study uses 11 cm diameter cellulose paper with medium porosity, which can remove 1 μm particles and has a particle retention time of 5–10 min. Thus, it is very suitable for clinical trials and phenolic detection. Tyrosinase was immobilized using chitosan and alginate to maintain enzyme performance for a long time, as reported in various storage processes. The tyrosinase-based paper bioassay was also evaluated using five layers of tyrosinase (200 units/μL) concentration, 1.25% chitosan 9 μL per layer. It was considered sufficient to form o-quinone enzymatically, 2% alginate from 6 μL per layer. Measurement of the color formed is then viewed using colorimetry for various compounds. Phenol gave a reddish-brown color, bisphenol A (BPA) gave a bluish-green color, dopamine exhibited a brown color, and orange for catechol, *m*-cresol, and *p*-cresol in the concentration range of 1–500 g/L. The work obtained by Alkasir et al. [49] offered very satisfactory results because it detected very low concentrations up to the range of 5 ppb and without adding chromophores in clean water and river water samples. This experiment is referred to by other researchers in developing paper biosensor technology to detect phenolic compounds in various media (Fig 3).

An experiment was then conducted by Arciuli et al. [52] to determine the quality of food products by testing the polyphenol content in five types of grapes (Franciacorta (Fc), Pinot (P), Sauvignon (S), Table wine (T), Frascati (F). The experiment used *o*-diphenolic L-DOPA as a compound that generally cannot give stoichiometric results since it has unstable properties and can inhibit the final separation of the products. Hence, a nucleophilic compound was added, namely the chromophore 3-methyl-2-benzothiazolinone hydrazone (MBTH) reagent, giving the bioactive paper a pink (stable) color. After adding the L-DOPA sample concentrations ranging from 0.01 to 10 mM, the *o*-quinone formed (enzymatic oxidation) reacted with MBTH giving a pink color to the bioactive paper after 5 min of exposure. There was no color change after that. The intensity of the resulting color depends on the concentration value (Fig. 4).

The results of wine samples (n = 3) were then compared with the Winder-Harris method (enzyme test, and it turns out that there is a difference in the *t*-test in the two wine samples, while the Folin-Ciocalteu method (phenolic test) shows higher yields (except for the Sauvignon wine sample) due to the presence of sulfite interfering which reduces the formation of antioxidants and non-phenolic sugars in the wine samples [53] (Table 3).

The two paper biosensor preparations qualitatively

Table 3. Phenol index is L-DOPA equivalent (mM) by performing three replications [52]

Wine type	Bioactive filter-paper	Winder-Harris assay	Folin-Ciocalteu assay
Frascati	0.19 ± 0.06	0.20 ± 0.05	1.70 ± 0.18
Franciacorta (sparkling wine)	0.28 ± 0.04	0.23 ± 0.04	1.06 ± 0.18
Pinot	0.26 ± 0.04	0.20 ± 0.08	0.73 ± 0.07
Sauvignon	0.28 ± 0.09	0.15 ± 0.10	0.22 ± 0.05
Table wine (sold in tetrapak)	0.26 ± 0.04	0.21 ± 0.09	1.0 ± 0.12

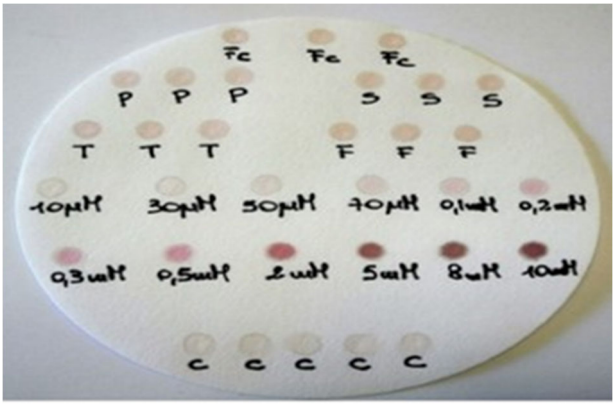

Fig 4. Image of filter paper with bioactive spots [52]

measure the phenolic content in wine samples using MBTH chromophore reagent, which reacts enzymatically with quinone to form a stable pink color. The subsequent work was then carried out by Hidayat et al. [54-55] in testing the total polyphenol content in green tea drinks from various local markets in Jember, Indonesia, using MBTH chromophore reagent. The results are not better than the Arciuli et al. [52] work in terms of the stable color formation time obtained up to 13 min despite using sodium periodate samples [55], which exhibits a faster ability as an oxidizing compound on polyphenols versus tyrosinase [53]. Water-soluble BPA is a hazardous chemical that can interfere with thyroid function, decrease the immune system and nervous system, and reduce sperm quality in humans. This compound is widely used in the manufacturing of polycarbonate plastics, epoxy resins, polyacrylate, and polysulphone resins used as packaging for food and drinks such as food cans and beverage bottles or plastic waste bins [56]. BPA concentrations were determined by biosensors using modified walled carbon nanotubes (MWCNTs), polycationic polymer poly(diallyl dimethylammonium chloride/PDDA), and Nafion. A mixture of TYR/TiO_2/MWCNTs/PDDA/Nafion was observed using scanning electron microscopy (SEM) (Fig. 5). Three-dimensional pore-shaped structure for the TYR/TiO_2/MWCNTs/Nafion and visible carbon nanotubes was found in the cracks. PDDA addition produces a matrix film with larger porosity allowing diffusion to occur. The results of this biosensor have a response of 0.28 and 45.05 mM, which can determine the BPA contained in the sample.

Paper biosensor technology for detecting BPA dust samples at the household scale was introduced [57]. The equipment was constructed into two parts, namely an air sampler and tyrosinase-based paper biosensors in the form of a 0.6 cm diameter plate. Plastic tapes are used as biosensor paper collection tools to measure the BPA based on its color changes. BPA dust sampling locations are around 100 cm^2 using portable biosensor equipment rate of 2.5 L/min through a long rubber tube of 25 cm. The BPA dust biosensor technique uses the LbL method, immobilized between chitosan and alginate. The formation of greenish color appears the first 60 s after

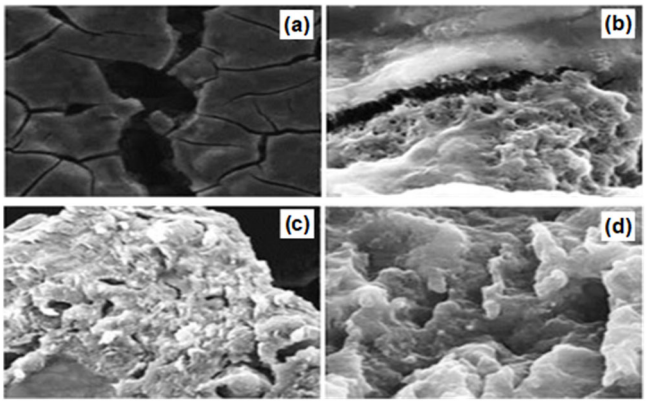

Fig 5. SEM results on graphite electrode surfaces for (a) TYR/TiO_2, (b) TYR/TiO_2/MWCNTs/Nafion and (c) TYR/TiO_2/MWCNTs/PDDA/Nafion (d) Higher magnification for 5(c) [56]

the addition of buffer (PBS pH 6.5). The stable color after 30 min confirms the presence of quinone-imine compounds as seen from the absorption band formed at the wavelength of 610 nm, indicating the presence of quinone carbonyl and nucleophilic amino groups for consisting of an aerosol sampling section, with a 6.0 cm hose size directly connected to the aerosol pump, a flow BPA compounds (Fig. 6). Phenol waste from wine, paper, and plastic industries was detected by paper biosensors [58] using chitosan and Tyr-AuNps bioconjugates from immobilized *Streptomyces* nanomaterials on different filter papers. The filter papers were ordinary filter paper, Whatman 1 paper, and NaTPP treated Whatman 2 paper.

The use of several types of filter paper aims to compare the detection time intervals and obtain poor detection results at 4 min with ordinary filter paper. The detection was quite good at 4 min for Whatman 1, and good detection results at 3 min for Whatman 2 of various types of industrial waste. Observation of detection color showed reddish-brown color for wine waste indicating the presence of phenol, dark brown color for paper waste indicating the presence of dopamine, and an orange color for plastic waste samples for catechol. The results were then compared with two standard methods, namely the 4-aminoantipyrine (AAP) and the fluorochromatic (FCR) test, showing fairly balanced results with the efficiency value of the inner paper biosensor in the wine industry waste (0.991 mM), paper industry waste (0.78 mM), and plastic waste (0.487 mM) using phenol standard [58].

■ BIOSENSOR PERFORMANCE TEST

The application of enzymes in industrial biosensors has limited functions, such as biosensor stability due to the loss of enzyme activity. Thus, over time, modifications were made in the biosensor assembly, which was able to increase enzyme activity and biosensor stability. Three critical aspects regarding the quality of biosensor performance are sensitivity, stability, and reproducibility. The shelf life after the biosensor fabrication can influence the containment of enzyme activity. The enzyme can generally be stabilized by avoiding the degradation process or minimizing degradation using a practical immobilization approach. Immobilization is also helpful in increasing sensitivity, response time, stability, and reproducibility. Reproducibility testing aims to see the uniformity of measurements of equivalent samples, carried out by different analysts and different equipment in one or more laboratories at the same time or different measurements using the same sample, performed by other analysts and other equipment in one or more laboratories at the same time or at different times. Immobilization between chitosan and alginate in the manufacture of paper biosensors can make the enzymes

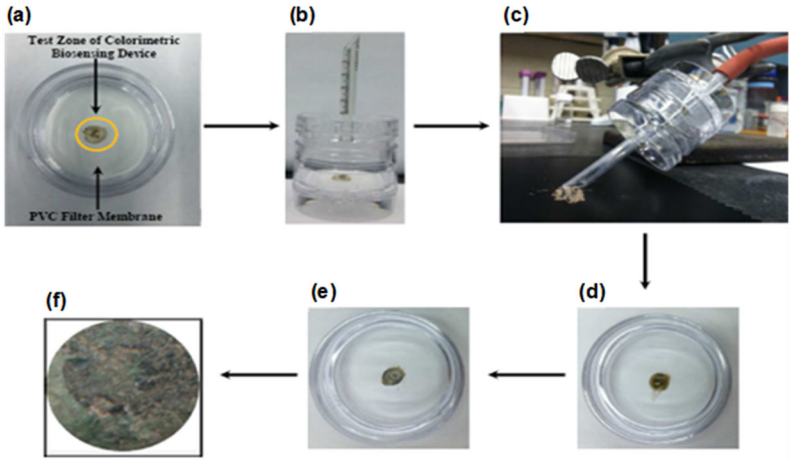

Fig 6. The shape of BPA dust-based paper biosensor (a) Air sampling cassette (b) Equipment put together with hose (c) Equipment connected to a pump with a rubber tube (d) BPA dust particle buildup (e) The color change after interacting with BPA dust (f) Greenish color is visible during the image scanning process [57]

trapped and result in strong electrostatic intermolecular interactions [59]. Biosensor stability is also evaluated from the storage factors, both temperature and storage time. Tyrosinase-based paper biosensors that were immobilized using chitosan and alginate can be stored for 260 d at room temperature (25 °C), giving a good stability rate of 92%; meanwhile, storing in a refrigerator (2–8 °C) within 54 d increased the stability up to 99%. In the freezer condition (−20 °C) for 260 d, the stability was 97%, which is the best achievement for enzyme-based paper biosensors without adding chromophore reagents. The reproducibility test was carried out using a concentration of phenol and BPA 100 μg/L, triplicates (n = 3). For phenol, the average color intensity was 139.6 (± 3.7) with a relative standard deviation (% RSD) value of 2.7%, and for BPA was 108.0 (± 3.4) with an RSD of 3.2%. Regarding the sensitivity, the paper biosensor was capable of measuring phenolics as low as 5 μg/L concentrations with a detection limit of 0.86 (± 0.1) μg/L [49].

The total polyphenols in green tea drinks from traditional markets in Indonesia were determined using tyrosinase, and MBTH was immobilized on a filter paper [55]. This enzymatic oxidation reaction can occur when 0.52 mM catechins as much as 5 μL are used as the substrates in detecting the total polyphenol content (TPC) (Table 4). The oxidized catechin by tyrosinase followed by a reaction of its corresponding quinone by adding MBTH chromophore changes the color from colorless to stable pink at 13 min at a pH ranging from 7.0–7.5 [60-61]. This paper biosensor can be stable for 8 days if stored at a temperature (−4 °C) and will drop 15% a day if stored at room temperature (25 °C) and in the refrigerator (4 °C). This method is then compared using ultraviolet-visible spectrophotometry.

It can be concluded that these two methods do not give a significant difference (using the t-test), as seen from the df = 4 and α = 0.05, with a value of R^2 = 0.9788. Therefore, biosensors can be used for testing the total levels of polyphenols in green tea drinks and would be appropriate to be applied in tea plantations. Meanwhile, sodium meta-periodate ($NaIO_4$) reagent and MBTH chromophore give a faster and more stable color response at 9 min. The device is stable for 20 d at 4 °C; activity decreases slowly. At room temperature, the activity continued to decrease due to the instability of the MBTH as the temperature increased. The reproducibility was evaluated six times (n = 6), giving good results with RSD values of 0.628%, less than 2%, with a measurement range of 25–300 ppm [48].

A paper biosensor for detecting BPA in a living room carpet has a sensitivity of 0.38 to 1.25 μg/g BPA [49] with a detection limit of 0.28 g/g. The concentration of 1.28 to 3.78 μg/g BPA resulted from sampling at the childcare center that holds about 60 children. The household-scale BPA results are known to come from floor cleaning resins and furniture, plastic beverage containers, plastic toys, clothes racks, mattresses, rubber shoes, and boots. The BPA dust biosensor reproducibility was evaluated using BPA concentrations of 100 μg/g carried out at three locations (n = 3), resulting in average color intensity of 112.6 (± 2.082) with a calculated RSD value of 0.018. This biosensor method is validated by GC. The comparative BPA analysis of the three samples of new carpet dust gives a BPA concentration of 0.8 ± (0.18) for the colorimetric method and 0.83 ± (0.15) for the GC method. The performance of both methods can be seen from the linear regression curve: y = 0.9484x + 0.2508; R^2 = 0.9743

Table 4. Results of various types of green tea drinks for total polyphenol by biosensor and UV-Vis spectrophotometry (n = 3 m, α = 0.05)

Sample	Polyphenol biosensor	Spectrophotometer	t_{cal}
K	0.747 ± 0.017	0.802 ± 0.005	0.174
L	0.403 ± 0.006	0.376 ± 0.022	2.000
M	0.557 ± 0.015	0.537 ± 0.003	2.194
N	0.448 ± 0.007	0.447 ± 0.003	0.170

*Results were obtained by independent t-test, with t value (t_{tab}) of 2.776 (df = 4 and α = 0.05) [55]

with a slope of 0.95, and this bias is considered to be within acceptable tolerance limits because it is seen from the error factor at the time the sampling process and the analysis process. The validation results show that the output of the two methods is in good agreement. The characterization was then continued by using GC/MS to confirm the presence of BPA in household dust. The mass spectrometer shows an abundance of 228 *m/z* for the BPA standard (MW = 228 g/mol).

■ CONCLUSION

Paper biosensors have been widely used in various applications to improve the quality of life and the environment in long-term monitoring because of their stability, low cost, and ease to use in the process of making biosensor equipment, environmentally friendly, quick and accurate measurement. Tyrosinase-based paper biosensors can test very low concentrations (ppm, ppb, and ppt) in analytes based on color changes when solid support media such as chitosan are enzymatically immobilized in quinones (tyrosinases) in the presence of cellulose additives in the filter paper. The performance of this enzyme-based paper biosensor can be evaluated based on its sensitivity, stability, and reproducibility properties. However, stability is the most crucial performance test on paper biosensors because they are very sensitive to temperature changes that occur during the storage period. Tyrosinase-based paper biosensors have enormous potential in recent decades and will continue to be developed. This review provides an overview of the efforts to develop paper tyrosinase-based biosensors based on their advantages: simple, inexpensive, fast, and reliable. The design of paper biosensors that can be connected directly through other portable and automated devices such as cellphones or smart watches through the programs used can make this technique more complete, popular, and a mainstay in the future. So that not only from certain aspects but can be used in various scientific fields.

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■ AUTHOR CONTRIBUTIONS

F. Yurike conducted the experiment; F. Yurike prepared the draft; D. Iswantini, S.S. Achmadi, and H. Purwaningsih revised the manuscript. All authors agreed to the final version of this manuscript.

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