JURNAL KIMIA VALENSI
p-ISSN: 2460-6065; e-ISSN: 2548-3013
Journal homepage: https://journal.uinjkt.ac.id/index.php/valensi
Research Article

Phenol Biosensor Development Using *Bacillus megaterium* And *Pseudomonas fluorescens* Microbes Consortium

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Article Info
Received: Nov 12, 2024
Revised: Dec18, 2024
Accepted: Oct 7, 2025
Online: Dec 22, 2025

Citation:
Mulyawan, R., Pradono, D. I., Nurhidayat, N., Saprudin, D., & Purwaningsih, H. (2025). Phenol Biosensor Development Using *Bacillus megaterium* And *Pseudomonas fluorescens* Microbes Consortium. *Jurnal Kimia Valensi*, 11(2), 272-283.

Doi: 10.15408/jkv.v11i2.42356

Abstract

Phenol is a toxic industrial pollutant that must be monitored at a low cost and in real-time. Conventional amperometric biosensors based on a single enzyme or a single microorganism often suffer from limited sensitivity and operational drift. Here, we report a microbial consortium biosensor that couples *Bacillus megaterium*, which supplies phenol-degrading enzymes and structural stability, with *Pseudomonas fluorescens*, whose electroactive biofilm enhances electron transfer to a screen-printed carbon electrode (SPCE). The electrode potential was swept at 100 mV s^{-1} between –1 V and +1 V (i.e., a 2 V window traversed in 20 s) to capture the full redox range of phenol by Cyclic Voltammetry (CV). The oxidation peak current grew linearly with phenol concentration from 27 to 137 mg/L (R^2 = 0.98), giving a sensitivity of 1.30 µA mg/L and a limit of detection of 13.6 mg/L. Compared with our previously tested single-microbe sensors, the consortium lowered the LOD threefold while maintaining long-term signal stability (>30 days). These results demonstrate that complementary metabolic pathways and the conductive biofilm of *P. fluorescens* synergistically enhance the electrochemical response provided by *B. megaterium*. The consortium-based SPCE platform, therefore, offers a robust, inexpensive tool for on-site phenol monitoring in environmental and industrial settings.

Keywords: *Bacillus megaterium*, biosensor, cyclic voltammetry (cv), phenol, *Pseudomonas fluorescens*

1. INTRODUCTION

An organic compound called phenol is widely used in various sectors of the economy, including the petrochemical, pharmaceutical, and plastics industries. However, because phenol is poisonous and difficult to break down, trash containing it can have a severe effect on the environment, particularly in aquatic habitats. Therefore, in order to lessen the harmful effects of phenol, it is crucial to detect and monitor its quantities in the environment[1–6].

Numerous analytical techniques have been established and are commonly employed in industry and research to ensure the precise identification of phenol in the environment. Some of the research was conducted to develop methods for analyzing phenol. The following are methods of phenol detection: a highly sensitive phenol biosensor utilizing selected Bacillus biofilm through an electrochemical method[7], evaluation of bacterial biofilm as a biosensor for detecting phenol, catechol, and 1, 2-dihydroxynaphthalene[8], and a development method of tyrosinase-based paper biosensor for phenolics measurement[9]. However, as described in APHA Method 5530 D, one of the most used techniques is UV-Vis spectrophotometry, which involves reacting phenol with 4-aminoantipyrine to create a colored molecule whose absorbance can be measured at particular wavelengths. Furthermore, in compliance

with EPA Method 604 and EPA Method 8270D guidelines, high-performance liquid chromatography (HPLC) and gas chromatography (GC) are also widely utilized for phenol analysis due to their strong separation and detection capabilities. These techniques provide a range of methods for phenol detection and quantification, offering flexibility in selecting the most suitable method based on sample conditions and the required sensitivity. However, the drawbacks of these methods include the need for relatively long preparation and analysis times, as well as high costs for materials and equipment.

Analytical instruments known as biosensors hold great promise for identifying chemical substances in the environment, like phenol. Biosensors have advantages over the previously mentioned techniques, namely high sensitivity, quick reaction times, and cheaper operating costs, when compared to chromatography or spectroscopy[10,11]. Since microorganisms naturally break down or transform phenol into less harmful molecules, the creation of a microbial-based phenol biosensor is a creative approach.

Pseudomonas fluorescens and *Bacillus megaterium* are two known microbial species with a strong capacity for phenol chemical metabolism. The Gram-positive bacterium *Bacillus megaterium* is widely employed in industrial biotechnology due to its diverse capacity to produce a range of enzymes and metabolite products. In the meantime, *Pseudomonas fluorescens*, a Gram-negative bacterium, is well-known for its capacity to break down complex organic substances in a variety of settings, including phenols[12–14].

Communities of microbes known as biofilms are affixed to certain surfaces and encased in a matrix made of extracellular polymeric materials that the bacteria themselves generate. Biofilms can develop on a variety of surfaces, including food, teeth, water, pipelines, medical equipment, and implant tissue. Because bacteria tend to build their own microenvironment and niche, biofilm formation occurs. This intricate and dynamic biofilm structure enables microorganisms to withstand changes in environmental conditions, absorb nutrients, and offer defense against antimicrobial agents. An application of biofilm for detecting phenol was developed for low-concentration phenol detection using a Pseudomonas fluorescens biofilm immobilized on SPCE. The lack of a single microbe was sensitive and specific in detecting phenol.

In this study, we utilized a microbial consortium comprising *Pseudomonas fluorescens* and *Bacillus megaterium* to develop a phenol biosensor. This microbial consortium is expected to provide a more environmentally friendly method for monitoring hazardous substances in the environment, while also enhancing the sensitivity and efficacy of biosensors in detecting phenol.

2. RESEARCH METHODS

Bacillus and *Pseudomonas* cultures were grown and revived in a heterotrophic medium and incubated in a Sanyo MIR-162 incubator (Osaka, Japan) at 37 °C for 24 hours using isolates from the Health Microbiology Laboratory at the Research Center for Biology (BRIN), Cibinong, Indonesia. The heterotrophic medium was composed of 200 mL of distilled water, mixed with 3.0 g of agar, 3.6 g of tryptone, 1.0 g of NaCl, and 0.5 g of K_2HPO_4. The medium was first sterilized for 15 minutes at 121 °C in an autoclave (HVE 50 Hirayama, Japan). The cultures were then kept in the medium for around 48 hours at 37 °C.

This study used SPCE refs. C110 from Metrohm Drop Sens, Sigma Aldrich's phenol standard, and the pH 7 phosphate buffer solution. Retrained in SPCE, there are two treatment options available. The first was with the single Pseudomonas and Bacillus, and the second was with their consortium. With a 1:1 ratio, all were given 48 hours to stand for biofilm development. They were then evaluated using current, maximum, linearity, detection limit, and precision methods in this study, employing CV with an EDAQ potentiostat over a range of -1 to 1 V and a scan rate of 100 mV/s.

Microbial Cultures

Bacillus megaterium and *Pseudomonas fluorescens* were cultured and revived in a heterotrophic medium. The bacterial strains were obtained from the Health Microbiology Laboratory at the Research Center for Biology (BRIN), Cibinong, Indonesia. To prepare the medium, 200 mL of distilled water was mixed with 3.0 g of agar, 3.6 g of tryptone, 1.0 g of NaCl, and 0.5 g of K_2HPO_4. The medium was sterilized by autoclaving at 121 °C for 15 minutes using an HVE 50 Hirayama autoclave (Japan). The bacterial cultures were incubated in the medium at 37 °C for 24 hours in a Sanyo MIR-162 incubator (Osaka, Japan) to ensure optimal growth.

Development of Biofilms

Following the incubation period, two treatment groups were prepared:

- *Bacillus megaterium* alone
- *Pseudomonas fluorescens* alone
- A consortium of both bacteria in a 1:1 ratio

Each treatment group was incubated for 48 hours at 37 °C to allow biofilm formation on the bacterial cultures. After this period, the biofilms were ready for electrochemical analysis.

Electrode and Electrochemical Setup

SPCE, Ref. C110 (Metrohm DropSens) was used as the working electrode. The electrode was pre-conditioned according to the manufacturer's guidelines. For phenol detection, a standard phenol solution was prepared using Sigma-Aldrich's phenol standard. A pH 7 phosphate buffer solution was used to maintain optimal conditions for bacterial activity and electrochemical measurements.

Cyclic Voltammetry (CV)

CV was conducted using an Edaq potentiostat to evaluate the electrochemical response of the biofilm-coated SPCEs. The CV measurements were performed within a potential range of -1 to 1 V with a scan rate of 100 mV/s. The following parameters were assessed:

- Current Evaluation: The maximum current response at different phenol concentrations.
- Linearity: The linear relationship between current response and phenol concentration.
- Detection Limit (LOD): The lowest detectable concentration of phenol based on the response.
- Precision: The reproducibility of the measurements across different trials.

Each experimental condition was replicated to ensure consistency and accuracy of the results.

3. RESULTS AND DISCUSSION

Characteristics of Consortium Biofilms

Scanning Electron Microscopy (SEM) observations revealed the distinctive surface of certain Bacillus biofilms on SPCE, which formed in 9 days and had more colonies than at 0 days into the biofilm formation process. After the bacterial suspension was dripped (0 days), **Figure 1a** depicts a group of consortium cells adhered to the surface; at this point, the consortium cells are rod-shaped and have not yet developed a biofilm. This is consistent with the findings, which showed that it has a rod-shaped cell morphology[15]. This is referred to as the attachment or colonization stage, occurring on day 0, when the SPCE working electrode surface is exposed.

Planktonic bacteria can naturally produce biofilms by adhering to both biotic and abiotic surfaces. As shown in **Figure 1**, the consortium biofilm thickened and formed an extracellular polymeric substance (EPS) matrix after 9 days. Polysaccharide-based EPS exhibits hydrophilicity due to its ability to bind water, forming a hydrogel complex that can bind bacterial colonies and construct them in three dimensions. The bacterial cultures were immobilized on the SPCE for electrochemical measurements. After the biofilm formation, the SPCE was used to assess the electrochemical response of the bacterial consortium. The bacterial colonies within the matrix are shielded by the matrix, which enhances their stability and resistance to external factors[16].

The formation and morphology of microbial biofilms play a pivotal role in the performance of biosensors, particularly when utilizing microbial consortia. Scanning Electron Microscopy (SEM) observations in this study (**Figure 1**) revealed clear distinctions between the early attachment stage (day 0) and the mature biofilm phase (day 9) formed by the Bacillus megaterium and Pseudomonas fluorescens consortium on the SPCE surface. On day 0, the rod-shaped bacterial cells were scattered and loosely attached to the carbon surface, indicating the initiation of the colonization phase. By day 9, the bacterial population significantly increased, and the surface was densely covered with cells embedded within an extracellular polymeric substance (EPS) matrix, confirming the progression to a mature biofilm structure.

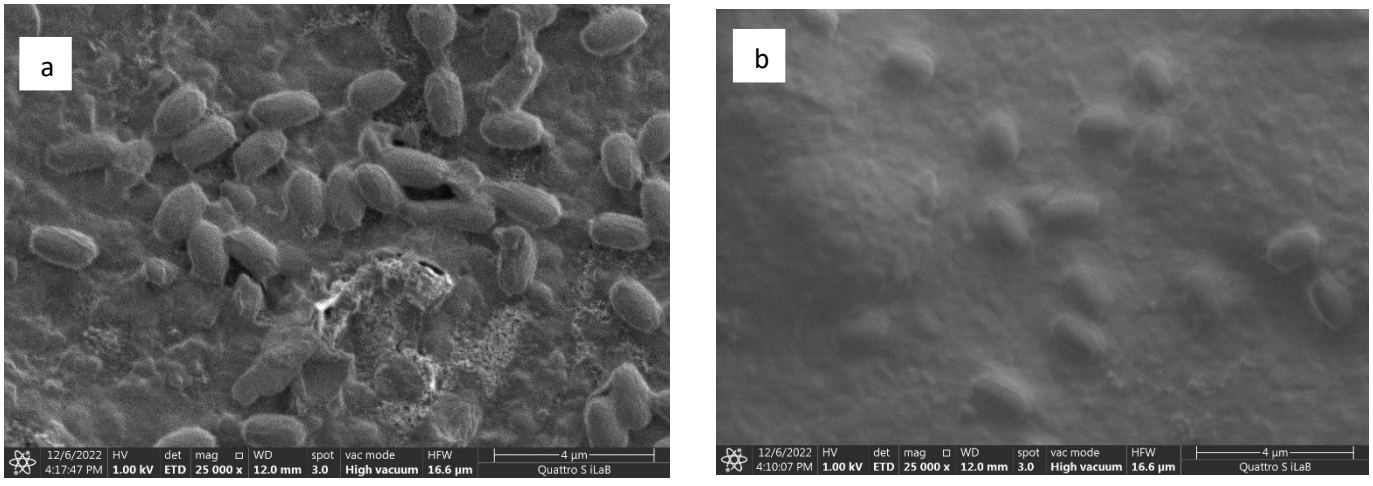

Figure 1. SEM morphology of the *Bacillus* biosensor (a) day 0 biofilm formation, (b) day 9 biofilm formation

This observation is consistent with previous reports that describe the biofilm life cycle, beginning with reversible attachment, followed by EPS production and microcolony formation, which leads to a structured and stable biofilm layer[17]. The EPS matrix, primarily composed of polysaccharides, proteins, and extracellular DNA, serves as a protective barrier and provides mechanical stability to the microbial community. Its hydrophilic nature enables the matrix to form a hydrated, gel-like network, facilitating nutrient retention, waste diffusion, and cell-to-cell communication, thereby enhancing bacterial viability and bioelectrocatalytic function.

Biofilm formation on the SPCE surface has substantial implications for biosensor design. The immobilization of bacterial consortia within the EPS matrix contributes to the biosensor's operational stability by shielding the cells from environmental fluctuations and potential toxicants. Furthermore, EPS facilitates improved electrical connectivity between the microbial redox-active components (e.g., outer membrane cytochromes or nanowires) and the electrode, ultimately increasing the efficiency of electron transfer during phenol oxidation. The role of EPS in improving biosensor performance through structural support and electron mediation has been previously demonstrated in electroactive biofilm systems[18,19].

Thus, the morphological transformation observed through SEM not only confirms successful biofilm establishment but also explains the increased electrochemical response observed in the CV measurements. The biofilm's ability to serve both as a microbial habitat and a conductive medium underscores its dual role in enhancing both stability and sensitivity in biosensor applications.

Phenol oxidation ability on a SPCE Consortium electrode

In the cyclic voltammetry (CV) measurements, the current values observed were low, particularly at lower phenol concentrations. This could be due to the non-conductive nature of the biofilm, which can reduce electron transfer efficiency. The SPCE electrode itself may also contribute to the lower current values, as its conductivity is lower compared to other electrode types. However, despite the low current, the sensor still shows a clear and reproducible oxidation peak at approximately +0.78 mV. The intensity of this peak increases with higher phenol concentrations, confirming that the sensor effectively responds to phenol. The linear correlation between current and phenol concentration ($R^2 = 0.98$) and the low detection limit (13.62 mg/L) demonstrate that the sensor retains its sensitivity, even with the observed low current values. The combination of *Bacillus megaterium* and *Pseudomonas fluorescens* in the microbial consortium plays a crucial role in enhancing the biosensor's performance by both lowering the limit of detection (LOD) and increasing sensitivity to phenol. These improvements are primarily due to two factors: biofilm formation and the increased metabolic activity of the bacterial consortium.

Microbial consortium approaches have been increasingly favored over single-strain systems. A recent study using a *Bacillus* and *Pseudomonas* consortium on SPCE achieved an excellent R^2 of 0.9924 and LOD of 0.5 μM for antioxidant phenolics, attributed to synergistic enzyme activity and improved electron transfer[20]. This aligns with our findings that such consortia can enhance sensitivity even when the absolute current magnitude is modest.

Biofilm Formation

When the bacterial consortium is immobilized on the electrode surface, it forms a stable biofilm. The biofilm matrix, composed of extracellular polymeric substances (EPS), creates a protective and supportive environment for the bacteria. This structure not only stabilizes the bacteria but also enhances the electron transfer efficiency between the bacteria and the electrode surface.

The biofilm matrix itself can exhibit some degree of conductivity, facilitating better electron transfer. This is crucial for improving the electrochemical response of the biosensor. As a result, the sensor's sensitivity is significantly improved, allowing it to detect lower concentrations of phenol with higher accuracy and a lower detection limit.

Increased Metabolic Activity

The metabolic activity of the microbial consortium accelerates the degradation of phenol, breaking it down into non-toxic intermediates. This faster phenol degradation increases the rate of electron transfer in the oxidation reaction, leading to a stronger electrochemical signal.

Pseudomonas fluorescens, in particular, is known for its ability to metabolize complex organic compounds, including phenol. The increased metabolic activity of the bacteria helps facilitate the oxidation of phenol at a faster rate, thus enhancing the overall sensitivity of the biosensor. The quicker degradation results in a more pronounced oxidation peak and a better current response, which allows the sensor to detect phenol at much lower concentrations.

Synergistic Effect of the Consortium

The synergy between the two bacteria in the consortium further optimizes the phenol degradation process. *Bacillus megaterium* provides stability and enzymatic activity for phenol breakdown, while *Pseudomonas fluorescens* enhances biofilm formation

and electron transfer, improving the overall electrochemical response of the biosensor.
This combined action results in a lower LOD and increased sensitivity, enabling the biosensor to detect phenol in a broader concentration range with high precision.

Phosphate-buffered saline (PBS), pH 7, was used as the electrolyte solution for SPCE electrodes. By using CV on the SPCE for ten cycles to monitor the redox reactions occurring on the surface of the SPCE working electrode, the stability of the solvent and electrolyte was measured. CV was used to examine the oxidation capacity of phenol on the surface of the SPCE (**Figure 2**). Voltammograms produced on the SPCE electrode surface with and without varying phenol concentrations are displayed in the **Figure 2.** It is evident that phenol in the electrolyte solution results in the formation of a low intensity oxidation peak at around +0.78 mV, which rises with phenol concentration[21–23].

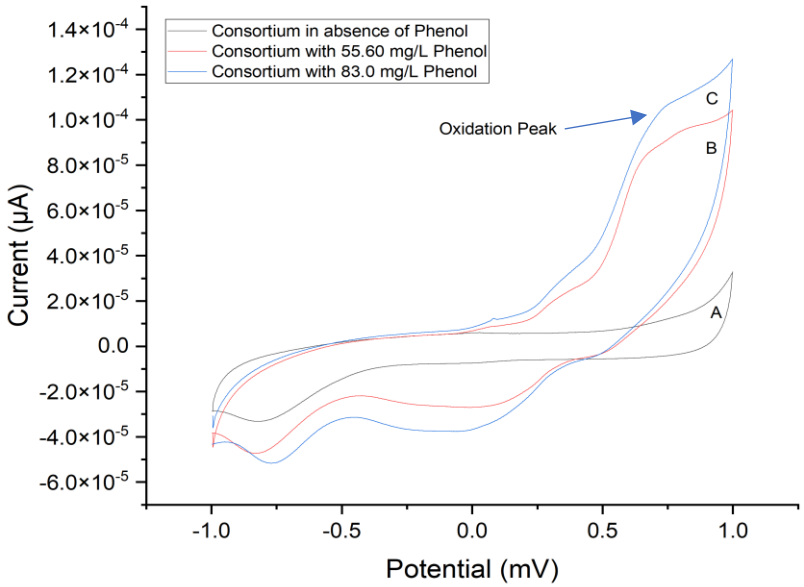

Figure 2. Cyclic voltamograms obtained by consortium immobilized at SPCE (A) in the absence (B) in the presence of 55.6 mg/L phenol, (C) 83.0 mg/L phenol in PBS (pH 7) at a scan rate of 100 mVs^{-1}.

In the CV measurements (**Figure 2**), we observed that the SPCE electrodes with the bacterial consortium, especially *Pseudomonas fluorescens*, exhibited enhanced electrochemical responses compared to bare SPCE electrodes. While biofilms are typically considered non-conductive, *Pseudomonas fluorescens* biofilms demonstrated improved electrochemical activity due to the bacteria's ability to facilitate electron transfer through conductive pili (nanowires) and outer membrane cytochromes. These specialized structures allow the bacteria to shuttle electrons between the biofilm and the electrode surface, enhancing the electrochemical response for phenol oxidation.

Furthermore, the biofilm matrix, composed of extracellular polymeric substances (EPS), supports this electron transfer by maintaining a conductive interface, despite the biofilm's general non-conductive nature. This biofilm-mediated electron transfer results in an increase in electrode conductivity and a stronger electrochemical signal for phenol oxidation, demonstrating the synergy between the biofilm and the electrode. The observed oxidation peak at +0.78 mV grows with phenol concentration, confirming the effectiveness of the biofilm-enhanced biosensor in phenol detection.

Consortium's impact on the electrode surface's phenol oxidation

In this study, we propose to use a consortium of bacteria, specifically *Bacillus megaterium* and *Pseudomonas fluorescens*, as biocatalysts to initiate the oxidation of phenol. In this system, *Bacillus megaterium* is involved in the enzymatic degradation of phenol through its metabolic pathways, which break down phenol into less toxic intermediates. *Pseudomonas fluorescens*, on the other hand, enhances the bioelectrochemical performance by forming a biofilm on the electrode surface. This biofilm not only provides a stable microenvironment for the bacteria but also facilitates electron transfer between the bacteria and the electrode through conductive structures such as cytochromes and pili (nanowires). These biofilm-associated electron transfer mechanisms enhance the efficiency of phenol oxidation by facilitating the transfer of electrons from phenol oxidation reactions to the electrode, thereby improving the electrochemical response. The combined action of both bacteria within the biofilm

matrix enhances the overall oxidation capacity and sensitivity of the biosensor. The cyclic voltammograms presented in **Figure 3** show the electrochemical behavior of the bare SPCE (a) without phenol, (b) with phenol present in PBS, and (c) with the bacterial consortium immobilized on the SPCE. When phenol is introduced into the electrolyte medium, the current density increases compared to the absence of phenol, indicating phenol oxidation on the SPCE surface. The presence of the bacterial consortium further enhances this response, suggesting that the bacteria play a beneficial role in improving the electrochemical reaction without altering the overall form of the voltammogram.

We propose using a consortium of bacteria as biocatalysts to initiate the oxidation of phenol. The cyclic voltammograms for the electrodes (a) without phenol and (b) with phenol present in PBS electrolyte medium at a sweep rate of 100 mVs−1 are shown in **Figure 3**, respectively.

In fact, a comparative investigation of the consortium's impact on the electrode surface in an electrolytic solution containing phenol is shown in **Figure 3**. The pure SPCE electrode exhibits a slight variation in. While phenol is present, the current density is higher than when the analyte is absent from the electrode, but after the consortium is immobilized, the current rises once more when phenol is present without damaging the form, hence these findings show that the existence of bacteria has a beneficial impact on the electrode's reaction[24,25].

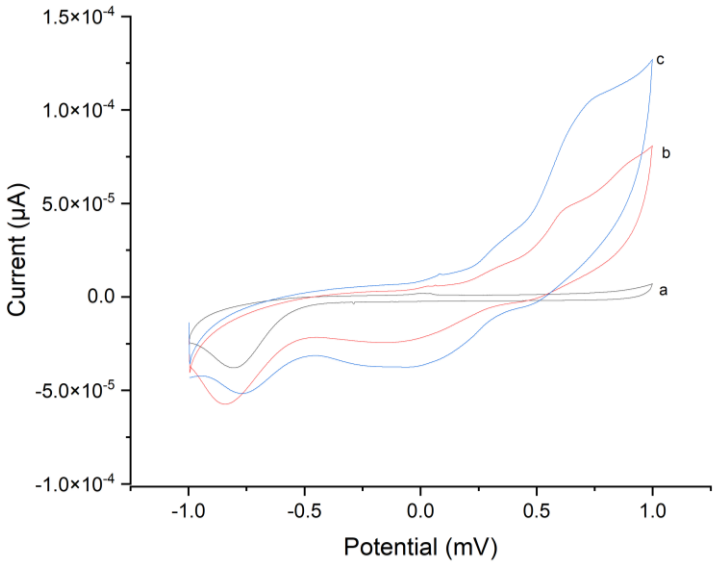

Figure 3. Cyclic voltamograms obtained by bare SPCE (a) in absence of phenol, bare SPCE (b) and consortium immobilized at SPCE (c) in the presence of 83.0 mg/L phenol in PBS (pH 7) at a scan rate of 100 mVs^{-1}.

Proposed reaction mechanism for consortium biosensor-based phenol detection

Catechol or hydroquinone are the products of phenol's initial electrochemical oxidation process (**Figure 4**). Phenol serves as the electron source when a positive voltage is applied to the working electrode, facilitating electron transfer. In order to create hydroquinone or catechol, phenol must lose electrons in this process. In the subsequent process, either hydroquinone or catechol undergoes oxidation to yield a quinone or a β-quinone, in that order. At the working electrode, electron transfer occurs again, but this time, quinone and a-benzoquinone are formed as a result of the hydroquinone or catechol losing electrons[26,27].

As shown in **Figure 4**, the cyclic voltammograms reveal a progressive increase in current from the bare SPCE (a–b), to electrodes modified with *Pseudomonas fluorescens* (c), *Bacillus megaterium* (d), and finally the consortium biofilm (e). While phenol can be weakly detected on bare SPCE, the oxidation current is significantly enhanced in the presence of microbial modifications, with the consortium showing the highest electrochemical response.

As compared to *Bacillus*-SPCE and *Pseudomonas*-SPCE (**Figure 5**), phenol can be detected by bare SPCE, but its oxidation current is much smaller. Phenol detection capability can be enhanced by combining these SPCEs into a consortium. Improvements in *Bacillus*, which produces tyrosinase to detect phenol, have been made. The addition of *Pseudomonas*, which produces peroxidase, oxidizes the enzyme, and then phenol reduces it once again. On the electrode surface, electrochemistry is used to reduce phenoxy radicals formed during the oxidation of phenolic compounds when hydrogen peroxide is present[27].

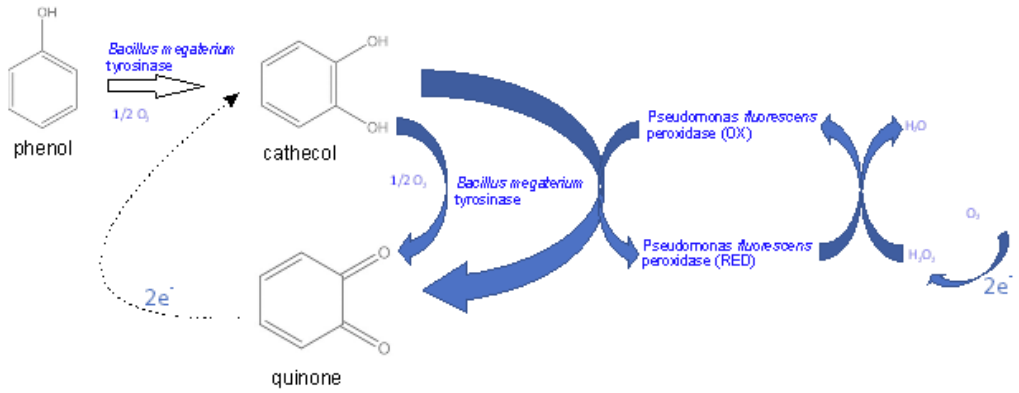

Figure 4. Proposed mechanism of reaction for consortium biosensor-based phenol detection

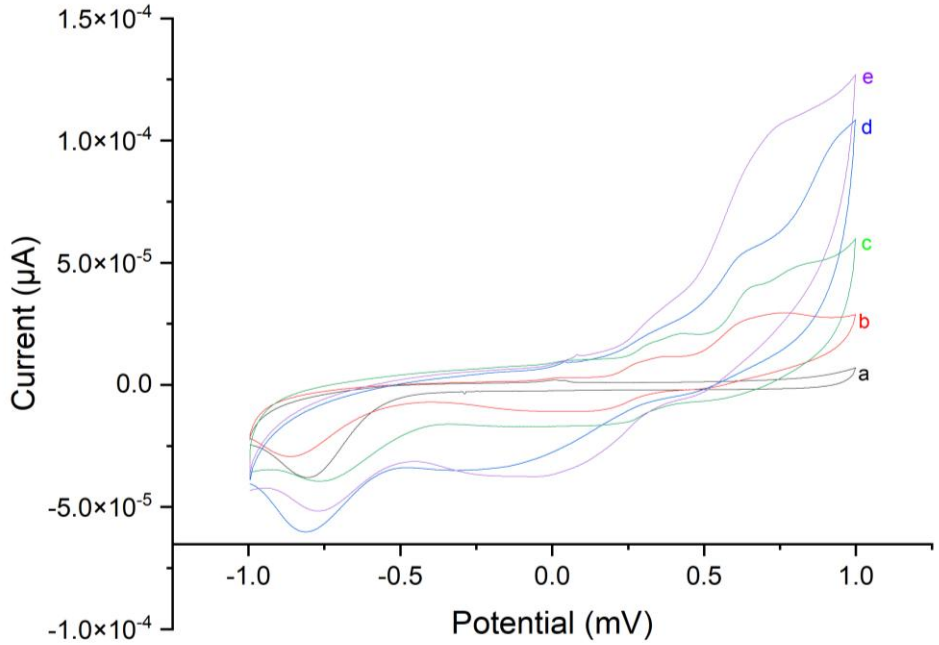

Figure 5. Cyclic voltamograms obtained by bare SPCE (a) in absence of phenol, bare SPCE (b), (c) *Pseudomonas*-SPCE, (d) *Bacillus*-SPCE and consortium immobilized at SPCE (e) in the presence of 83.0 mg/L phenol in PBS (pH 7) at a scan rate of 100 mVs^{-1}.

This improvement can be explained by the synergistic biocatalytic activities of both bacterial species, as illustrated in **Figure 5**. *Bacillus megaterium* expresses tyrosinase, which catalyzes the conversion of phenol to catechol and subsequently to o-quinone via oxidation. *Pseudomonas fluorescens*, on the other hand, contributes peroxidase enzymes, which cycle between oxidized and reduced states to support the electron transfer process and regenerate active tyrosinase. These complementary enzymatic functions result in the formation of phenoxy radicals, which are electrochemically reduced at the SPCE surface—yielding a higher oxidation peak. Additionally, Pseudomonas's ability to form conductive biofilms enhances electron transfer kinetics.

Together, **Figures 4** and **5** validate the hypothesis that a microbial consortium significantly enhances the biosensor's electrochemical performance, both by improving enzymatic degradation of phenol and by increasing electron transfer efficiency.

Analysis Capabilities of Phenol Biosensor

For concentrations ranging from 27 to 137 mg/L, phenol was gradually added to the electrolyte solution to establish the calibration curve (**Figure 6**). The oxidation current peaks increased linearly with the phenol concentration, as shown. The connection that follows can be used to represent this linearity.

The error bars in **Figure 4** were calculated by performing replicate cyclic voltammetry measurements for each phenol concentration. For each concentration, the current at the oxidation peak was measured in triplicate. The mean current value was determined from these replicate measurements, and the standard deviation (SD) was calculated to quantify the variability in the data. The standard error of the mean (SEM) was then computed by dividing the SD

by the square root of the number of replicates. The SEM values were used to generate the error bars in the concentration vs. current plot, which reflect the precision of the measurements for each phenol concentration. **Figure 6**, derived from the voltammetry data, shows the linear correlation between the current (μA) and the phenol concentration (mg/L). The strong correlation, with an R^2 value of 0.9855, demonstrates the biosensor's sensitivity to phenol. The sensor exhibits high sensitivity with a slope of 129.96 μA/mg/L, which is consistent with its ability to detect phenol across a broad concentration range. This highlights the potential of using the bacterial consortium-based biosensor for accurate phenol detection in environmental and industrial applications[28,29].

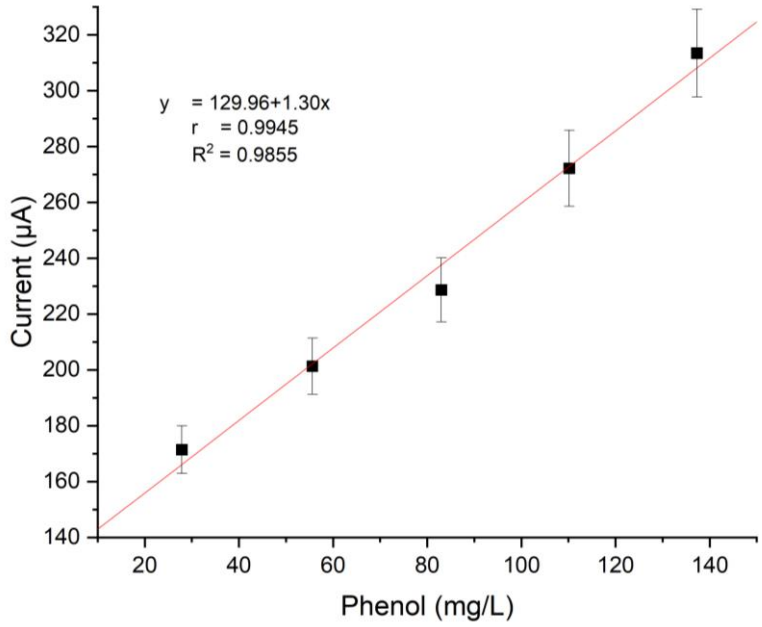

Figure 6. Phenol concentration's effect on the strength of oxidation peaks detected by CV at the Consortium Immobilized at SPCE.

Table 1. Phenol biosensors compared data

Bacteria	y = a + bx	R^2	Limit Of Detection (mg/L)
Bacillus	y = 0.99x-0.04	0.9733	23.72
Pseudomonas	y = 1.1577x+1.53	0.8463	38.69
Consortium	y = 1.30x+129.96	0.9828	13.62

The outcome clearly shows that a consortium outperformed *Bacillus* or *Pseudomonas* as a single microbe in terms of analytical performance (**Table 1**)[24]. The data in **Table 1** clearly demonstrate that the microbial consortium outperforms the individual strains in terms of overall analytical performance. Specifically, the consortium-based biosensor achieved the highest R^2 value (0.9828), indicating superior linearity between current response and phenol concentration compared to *Bacillus megaterium* (R^2 = 0.9733) and *Pseudomonas fluorescens* (R^2 = 0.8463) alone. This high linearity reflects the biosensor's consistent and predictable behavior across a wide range of phenol concentrations.

Furthermore, the consortium exhibited the lowest limit of detection (LOD) at 13.62 mg/L, significantly better than that of Bacillus (23.72 mg/L) and Pseudomonas (38.69 mg/L). A lower LOD means the biosensor can detect smaller amounts of phenol, which is crucial for environmental applications that require early detection of pollutants.

Although *Pseudomonas fluorescens* showed a higher slope (1.1577 μA/mg/L), its poor linearity (R^2 = 0.8463) suggests unstable signal responses. In contrast, the consortium combined the benefits of both bacteria: the enzymatic degradation ability of *Bacillus megaterium* and the electroactive biofilm formation of Pseudomonas fluorescens. This synergy improves not only signal strength but also electron transfer efficiency and biofilm stability on the SPCE surface.

These results confirm that combining both microbes leads to a more robust, sensitive, and reliable biosensor, offering clear advantages over single-strain systems for phenol detection. In reference to the earlier investigation of this study, which was conducted to determine an approach for enhancing the consortium's analytical performance, the data produced positive results. In contrast to earlier research, this study's outcome is a novel technique developed from an earlier investigation[28].

The matrix effect of Phenol Biosensor

Several significant factors that impact measurement accuracy and sensitivity are discussed in the context of the catechol matrix's impact on phenol analysis using potentiometric biosensors. As a phenolic component frequently present in environmental samples containing phenol, catechol can significantly impact the biosensor's response. Biosensors that employ ion-selective electrodes in potentiometry are typically calibrated to identify particular ion species, such as phenol. However, because catechol and phenol share similar chemical and electroactive characteristics, their presence in the sample matrix may create interference[30].

Catechol is found at 0.2–0.3 mV, while phenol is found at 0.7–0.8 mV. One way that catechols might cause interference is by changing the potential distribution at ion-selective electrodes, which are meant to react solely to phenol. This is known as catechol interference. The sensitivity and specificity of the biosensor to phenol can also be decreased by catechol by competing with phenol for binding sites on enzymes or other active components[31,32]. These influences may cause calibration shifts or erroneous readings, resulting in an inaccurate measurement of the phenol content. It can be verified that the biosensor approach with consortium can detect phenol even in the presence of catechol interference, given the data that the amount of phenol current at a concentration of 134 mg/L is not affected by catechol as seen on **Figure 7**.

In **Figure 7**, the CV profiles show that the oxidation peak typically associated with phenol, at approximately +0.78 V, is not distinctly detected in the presence of catechol. The overlapping redox signals of phenol and catechol can explain this phenomenon. Catechol, which is more electroactive and undergoes reversible redox transformation at lower potentials (typically between +0.2 and +0.4 V), exhibits a dominant signal in the CV curve. Due to its higher electrochemical reactivity and rapid electron transfer kinetics, catechol likely masks or suppresses the phenol oxidation signal, especially when present in concentrations comparable to or higher than those of phenol. Additionally, catechol's redox products, such as o-quinone, may adsorb onto the electrode surface, further hindering phenol oxidation by blocking active sites[33].

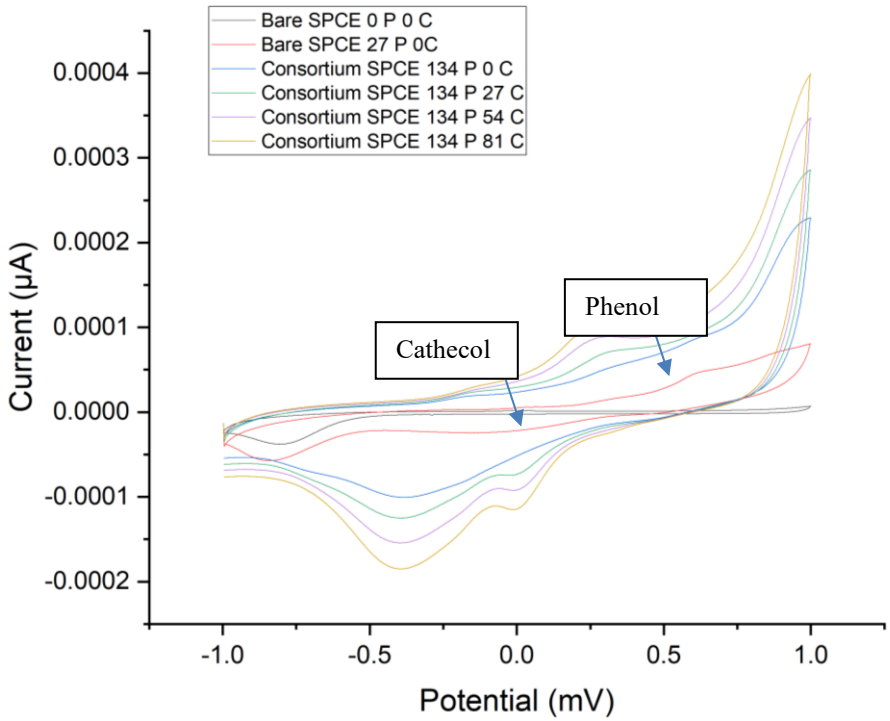

Figure 7. Phenol concentration's effect on the Cathecol oxidation peaks detected by CV

A distinct reduction peak observed around –0.1 to –0.2 V in the CV curve corresponds to the electrochemical reduction of o-benzoquinone back to catechol. This confirms the reversible redox behavior of catechol and the active participation of the microbial consortium in facilitating electron transfer during this redox cycling. Notably, despite the presence of catechol, the current response at high phenol concentrations (e.g., 134 mg/L) remains stable and elevated. This suggests that the biosensor retains its selectivity and can reliably detect phenol, even in the presence of matrix interference. The results affirm the robustness and electrochemical efficiency of the

microbial consortium immobilized on SPCE for phenol detection in complex mixtures[34].

4. CONCLUSIONS

The findings of this investigation benefited from earlier studies on the use of a single microbial biosensor for phenol detection. Using the consortium biofilm immobilized on the SPCE surface, a phenol biosensor has been successfully fabricated. The linearity ranges from 27 to 37 mg/L, with an R^2 value of 0.98. The detection limits are at 13.62 mg/L, and the average sensitivity is 1.30 μA/mg/L. Furthermore, the PBS had a pH of 7.0.

ACKNOWLEDGMENTS

We extend our gratitude to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for funding through the PDD Program 2022. In addition, appreciation is extended to the Department of Chemistry, IPB University, and the Central Research Department of Biology, BRIN, as well as Politeknik AKA Bogor, for the facilities provided.

REFERENCES

1. Kim JY, Park J, Hwang H, Kim JK, Song IK, Choi JW. Catalytic Depolymerization of Lignin Macromolecule to Alkylated Phenols over Various Metal Catalysts in Supercritical Tert-Butanol. *J Anal Appl Pyrolysis* 2015, *113*, 99–106. https://doi.org/10.1016/j.jaap.2014.11.011.
2. Emenike EC, Ogunniyi S, Ighalo JO, Iwuozor KO, Okoro HK, Adeniyi AG. Delonix Regia Biochar Potential in Removing Phenol from Industrial Wastewater. *Bioresour Technol Rep* 2022, *19*, 101195. https://doi.org/10.1016/J.BITEB.2022.101195.
3. Duan W, Meng F, Cui H, Lin Y, Wang G, Wu J. Ecotoxicity of Phenol and Cresols to Aquatic Organisms: A Review. *Ecotoxicol Environ Saf* 2018, *157*, 441–456. https://doi.org/10.1016/J.ECOENV.2018.03.089.
4. Trivellini A, Lucchesini M, Maggini R, Mosadegh H, Villamarin TSS, Vernieri P, Mensuali-Sodi A, Pardossi A. Lamiaceae Phenols as Multifaceted Compounds: Bioactivity, Industrial Prospects and Role of "Positive-Stress." *Ind Crops Prod* 2016, *83*, 241–254. https://doi.org/10.1016/J.INDCROP.2015.12.039.
5. Ahmaruzzaman M, Mishra SR, Gadore V, Yadav G, Roy S, Bhattacharjee B, Bhuyan A, Hazarika B, Darabdhara J, Kumari K. Phenolic Compounds in Water: From Toxicity and Source to Sustainable Solutions – An Integrated Review of Removal Methods, Advanced Technologies, Cost Analysis, and Future Prospects. *J Environ Chem Eng* 2024, *12* (3), 112964. https://doi.org/10.1016/J.JECE.2024.112964.
6. Xu Y, Guo L, Zhang H, Zhai H, Ren H. Research Status, Industrial Application Demand and Prospects of Phenolic Resin. *RSC Adv.* 2019, *9* (50), 28924–28935. https://doi.org/10.1039/C9RA06487G.
7. Ariyanti D, Iswantini D, Sugita P, Nurhidayat N, Effendi H, Ghozali AA, Kurniawan YS. Highly Sensitive Phenol Biosensor Utilizing Selected Bacillus Biofilm Through an Electrochemical Method. *Makara J Sci* 2020, *24* (1), 24–30. https://doi.org/10.7454/mss.v24i1.11726.
8. Iswantini D, Ghozali AA, Kusmana C, Nurhidayat N. Evaluation of Bacterial Biofilm as Biosensor for Detecting Phenol, Catechol, and 1,2-Dihydroxynaphthalene. *Hayati* 2021, *28* (4), 262–270. https://doi.org/10.4308/HJB.28.4.262-270.
9. Yurike F, Iswantini D, Purwaningsih H, Achmadi SS. Tyrosinase-Based Paper Biosensor for Phenolics Measurement. *Indonesian Journal of Chemistry* 2022, *22* (5), 1454. https://doi.org/10.22146/ijc.72607.
10. Kochana J, Wapiennik K, Kozak J, Knihnicki P, Pollap A, Woźniakiewicz M, Nowak J, Kościelniak P. Tyrosinase-Based Biosensor for Determination of Bisphenol A in a Flow-Batch System. *Talanta* 2015, *144*, 163–170. https://doi.org/10.1016/J.TALANTA.2015.05.078.
11. Han E, Yang Y, He Z, Cai J, Zhang X, Dong X. Development of Tyrosinase Biosensor Based on Quantum Dots/Chitosan Nanocomposite for Detection of Phenolic Compounds. *Anal Biochem* 2015, *486*, 102–106. https://doi.org/10.1016/J.AB.2015.07.001.
12. Min K, Park GW, Yoo YJ, Lee JS. A Perspective on the Biotechnological Applications of the Versatile Tyrosinase. *Bioresour Technol* 2019, *289* (May), 121730. https://doi.org/10.1016/j.biortech.2019.121730.
13. Zheng H, Yan Z, Wang M, Chen J, Zhang X. Biosensor Based on Polyaniline-Polyacrylonitrile-Graphene Hybrid Assemblies for the Determination of Phenolic Compounds in Water Samples. *J Hazard*

Mater 2019, *378* (January), 120714. https://doi.org/10.1016/j.jhazmat.2019.05.107.

14. Kolahchi N, Braiek M, Ebrahimipour G, Ranaei-Siadat SO, Lagarde F, Jaffrezic-Renault N. Direct Detection of Phenol Using a New Bacterial Strain-Based Conductometric Biosensor. *J Environ Chem Eng* 2018, *6* (1), 478–484. https://doi.org/10.1016/J.JECE.2017.12.023.
15. Balakrishna Pillai A, Jaya Kumar A, Thulasi K, Kumarapillai H. Evaluation of Short-Chain-Length Polyhydroxyalkanoate Accumulation in Bacillus Aryabhattai. *Brazilian Journal of Microbiology* 2017, *48* (3), 451–460. https://doi.org/10.1016/J.BJM.2017.01.005.
16. Kazmiruk V. *Scanning Electron Microscopy*; IntechOpen: London, 2012. https://doi.org/10.5772/1973.
17. Donlan, R. Biofilms: Microbial Life on Surfaces. *Emerging Infectious Disease journal* 2002, *8* (9), 881. https://doi.org/10.3201/eid0809.020063.
18. Frølund B, Palmgren R, Keiding K, Nielsen P. H. Extraction of Extracellular Polymers from Activated Sludge Using a Cation Exchange Resin. *Water Res* 1996, *30* (8), 1749–1758. https://doi.org/10.1016/0043-1354(95)00323-1.
19. Lovley D. Microbial Energizers: Fuel Cells That Keep on Going. *Microbe* 2006, *1*, 323–329. https://doi.org/10.1128/microbe.1.323.1.
20. Sukma RM, Iswantini D, Nurhidayat\ N, Rafi M. Bacterial Consortium Biofilm-Based Electrochemical Biosensor for Measurement of Antioxidant Polyphenolic Compounds. *Electrochem* 2024, *5* (4), 530–545. https://doi.org/10.3390/electrochem5040034.
21. Nurhidayat N, Iswantini D, Bestari P, Purwaningsih H, Sugiarti S. The Accuracy of Ethanol Biosensor Based with Acetobacter Aceti Biofilm in Certifying Halal Food Products. In *AIP Conference Proceedings*; American Institute of Physics Inc., 2020; Vol. 2243. https://doi.org/10.1063/5.0004769.
22. Galdino FE, Smith JP, Kwamou SI, Kampouris DK, Iniesta J, Smith GC, Bonacin JA, Banks CE. Graphite Screen-Printed Electrodes Applied for the Accurate and Reagentless Sensing of PH. *Anal Chem* 2015, *87* (23), 11666–11672. https://doi.org/10.1021/acs.analchem.5b01236.
23. Manan FAA, Hong WW, Abdullah J, Yusof NA, Ahmad I. Nanocrystalline Cellulose Decorated Quantum Dots Based Tyrosinase Biosensor for Phenol Determination. *Materials Science and Engineering C* 2019, *99* (January 2018), 37–46. https://doi.org/10.1016/j.msec.2019.01.082.
24. Mulyawan R, Iswantini D, Nurhidayat N, Saprudin D, Purwaningsih H. Biosensor Performance of Phenol Analysis Using Microbial Consortium of Bacillus Sp. and Pseudomonas Sp. *AIP Conf Proc* 2022, *2638* (1), 50009. https://doi.org/10.1063/5.0104743.
25. González-Sánchez MI, Gómez-Monedero B, Agrisuelas J, Iniesta J, Valero E. Electrochemical Performance of Activated Screen Printed Carbon Electrodes for Hydrogen Peroxide and Phenol Derivatives Sensing. *Journal of Electroanalytical Chemistry* 2019, *839*, 75–82. https://doi.org/10.1016/J.JELECHEM.2019.03.026.
26. Moutcine A, Laghlimi C, Ziat Y, Isaad J, El Bahraoui S, Chtaini A. Electroanalytical Analysis of Phenol Oxidation Using Bacteria Immobilized by a Polycaprolactone Coating on the Copper Electrode Surface. *Sci Rep* 2024, *14* (1), 13136. https://doi.org/10.1038/s41598-024-58281-7.
27. Cheol S, Rawson K, Mcneil CJ. Disposable Tyrosinase-Peroxidase Bi-Enzyme Sensor for Amperometric Detection of Phenols. 2002, *17*, 1015–1023.
28. Raymundo-Pereira PA, Silva TA, Caetano FR, Ribovski L, Zapp E, Brondani D, Bergamini MF, Marcolino LH, Banks CE, Oliveira ON, Janegitz BC, Fatibello-Filho O. Polyphenol Oxidase-Based Electrochemical Biosensors: A Review. *Anal Chim Acta* 2020, *1139* (xxxx), 198–221. https://doi.org/10.1016/j.aca.2020.07.055.
29. Forzato C, Vida V, Berti F. Biosensors and Sensing Systems for Rapid Analysis of Phenolic Compounds from Plants: A Comprehensive Review. *Biosensors (Basel)* 2020, *10* (9). https://doi.org/10.3390/bios10090105.
30. Congur G. Development of a Novel Methyl Germanane Modified Disposable Sensor and Its Application for Voltammetric Phenol Detection. *Surfaces and Interfaces* 2021, *25*, 101268. https://doi.org/10.1016/j.surfin.2021.101268.
31. Mossanha R, Erdmann CA, Santos CS, Wohnrath K, Fujiwara ST, Pessoa CA. Construction of a Biosensor Based on SAM of Thiolactic Acid on Gold Nanoparticles Stabilized by Silsesquioxane Polyelectrolyte for Cathecol Determination. *Sens Actuators B Chem* 2017, *252*, 747–756. https://doi.org/10.1016/j.snb.2017.06.001.

32. Cerrato-Alvarez M, Bernalte E, Bernalte-García MJ, Pinilla-Gil E. Fast and Direct Amperometric Analysis of Polyphenols in Beers Using Tyrosinase-Modified Screen-Printed Gold Nanoparticles Biosensors. *Talanta* 2019, *193*, 93–99. https://doi.org/10.1016/j.talanta.2018.09.093.

33 González-Costas JM, Caruncho-Pérez S, González-Romero E. Enhanced Catalytic Surfaces for Catechol Sensing: Combining Grafted Aryldiazonium Derivative with Cross-Linking Dopamine or Coupling Tyrosinase Immobilizations. *Applied Sciences* 2025, *15* (8), 4250. https://doi.org/10.3390/app15084250.

34 Bedendi G, De Moura Torquato LD, Webb S, Cadoux C, Kulkarni A, Sahin S, Maroni P, Milton RD, Grattieri M. Enzymatic and Microbial Electrochemistry: Approaches and Methods. *ACS Measurement Science Au* 2022, *2* (6), 517–541. https://doi.org/10.1021/acsmeasuresciau.2c00042.