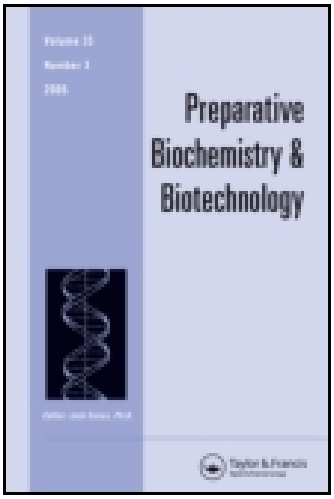

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A NOVEL BIOSENSOR BASED ON *Lactobacillus acidophilus* FOR DETERMINATION OF PHENOLIC COMPOUNDS IN MILK PRODUCTS AND WASTEWATER

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□ *Different branches of industry need to use phenolic compounds (PCs) in their production, so determination of PCs sensitively, accurately, rapidly, and economically is very important. For the sensitive determination of PCs, some biosensors based on pure polyphenol oxidase, plant tissu,e and microorganisms were developed before. But there has been no study to develop a microbial phenolic compounds biosensor based on* Lactobacillus *species, which contain polyphenol oxidase enzyme. In this study, we used different forms of* Lactobacillus *species as enzyme sources of biosensor and compared biosensor performances of these forms for determination of PCs. For this purpose, we used lyophilized* Lactobacillus *cells (containing* L. bulgaricus, L. acidophilus, Streptococcus thermophilus*), pure* L. acidophilus, *pure* L. bulgaricus, *and* L. acidophilus- *and* L. bulgaricus *adapted to catechol in Lactobacilli MRS Broth. The most suitable form was determined and optimization studies of the biosensor were carried out by using this form. For preparing the bioactive layer of the biosensor, the* Lactobacillus *cells were immobilized in gelatin by using glutaraldehyde. In the study, we used catechol as a substrate. Phenolic compound determination is based on the assay of the differences on the respiration activity of the cells on the oxygen meter in the absence and the presence of catechol. The microbial biosensor response depends directly on catechol concentration between 0.5 and 5.0 m*M *with 18 min response time. In the optimization studies of the microbial biosensor the most suitable microorganism amount was found to be 10 mg, and also phosphate buffer (pH 8.0; 50 m*M*) and 37.5° C were obtained as the optimum working conditions. In the characterization studies of the microbial biosensor some parameters such as substrate specificity on the biosensor response and operational and storage stability were examine. Furthermore, the determination of PC levels in synthetic wastewater, industrial wastewater, and milk products was investigated by using the developed biosensor under optimum conditions.*

Keywords biosensor, *Lactobacillius acidophilus*, oxygen electrode, phenolic compound, probiotics

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INTRODUCTION

Phenolic compounds (PCs) have been recognized as toxic substances and endocrine disruptors.[1,2] This interaction for humans and wildlife results in the induction of serious pathologies such as developmental abnormalities and carcinogenesis.[3,4] For these reason, the determination of PCs in environmental matrices, including tap and surface water, has become a matter of great concern and scientific interest. Determinations are usually carried out in centralized laboratories using high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS). Resent research activity has focused on the design and construction of biosensors that are capable of improving the efficiency of site monitoring and can be used for the necessary remediation activity.[5]

Amperometric determination of PCs is a simple available technique. Direct oxidation of PCs can be used, but another possibility is the use of polyphenol oxidase enzyme biosensors that oxidize the PCs into their corresponding quinones. However, very different problems have been found in using pure enzyme; their application in biosensor construction may be limited due to being time-consuming and due to the costly enzyme purification and need for cofactors/coenzymes.[6] Microorganisms provide an ideal alternative to these handicaps because they contain many enzymes and cofactors in the cells.[7]

For the determination of PCs some biosensors based on polyphenol oxidase[8] and plant tissue[9,10] have been developed. By using *Pseudomonas putida*[11] or *Moraxella* sp.[12] microorganisms, microbial phenolic compounds biosensors were also developed. Therefore, there is no study to develop a microbial phenolic compounds biosensor based on *Lactobacillus* species. These microorganisms exhibit a specific polyphenol oxidase activity,[13,14] and this enzyme oxidizes the PCs into their corresponding quinones.

$$\text{Catechol} \xrightarrow{\text{Enzyme in bioactive film} + O_2\,(\text{dissolved in medium})} o - \text{Quinone} + H_2O$$

Reduction of the resulting quinones accomplishes the amplification of the amperometric signal. Generally, catechol is used as a substrate model for determination of phenolic compounds biosensor.[5]

In this study, we used different forms of *Lactobacillus* species as enzyme sources of biosensor and compared biosensor performances of these forms for determination of PCs. For this purpose, we used lyophilized *Lactobacillus* cells (containing *L. bulgaricus, L. acidophilus, Streptococcus thermophilus*), pure *L. acidophilus*, pure *L. bulgaricus*, and *L. acidophilus* and *L. bulgaricus* adapted to catechol in Lactobacilli MRS Broth. The most suitable form was

determined and optimization studies of the biosensor were carried out using this form.

The oxygen electrode was used as a transducer to test the sensor responses of developed biosensor. Moreover, *Lactobacillus* species were immobilized in gelatin by using the cross-linking agent glutaraldehyde.

Measurements were carried out by standard curves, which were obtained by the determination of respiration activity of microorganisms or consumed oxygen level related to catechol concentration injected into the reaction medium. Developed biosensor was applied for determination of PCs in synthetic wastewater, industrial wastewater, and milk products.

EXPERIMENTAL

Materials

Lactobacillus cells (*L. bulgaricus, L. acidophilus, S. thermophilus* in lyophilized form), used for preparation of commercial yogurts, were obtained from DI-PROX (France) and stored at −18°C.

Lactobacilli MRS Broth was obtained from Acumedia Manufacturers (Lansing, MI).

PCs (catechol, phenol, resorcin, orcinol, *p*-cresol, pyrogallol, L-Dopa) were obtained from Merck (Germany), and stock solutions of PCs were prepared by dissolving the right amount of pure substance into phosphate buffer solution (50 m*M*, pH 8.0).

Potassium dihydrogen phosphate, sodium monohydrogen phosphate, and the cross-linking agent glutaraldehyde (25% v/v) were provided by Merck (Germany).

Gelatin used as a gel matrix during immobilization was obtained from Sigma Aldrich Chemical Co. (USA).

Synthetically concocted wastewater had the composition $(NH_4)_2SO_4$ (0.5 g), $MgSO_4$ (0.1 g), $MnSO_4$ (0.01 g), $FeSO_4$ (0.5 mg), and known amounts of catechol in tap water (100 mL, pH 7.2).[11]

An industrial wastewater sample was provided from the Ergene River at the Çerkezköy industrialized region in Trakya-Turkey, for comparing the results with synthetic wastewater.

Milk products (Activia, Yovita, Danone yogurts, Ülker Kefir and Pınar, Sütaş milk) were obtained from local markets.

Apparatus

Orion 3 star DO benchtop model dissolved oxygen meter and Orion 3 star 080113 series dissolved oxygen (DO) probes were used for the

determination of the consumed oxygen level by preparing the biosensor. A Rotina model 38 R centrifuge with refrigeration was used for collecting the bacteria. All the measurements were carried out at constant temperature with a Nüve model BM 302 water bath with external circulation. For preparing buffer solutions, a 213 microprocessor model pH meter was used.

Culture Medium of the Microorganism

In this study, we used different forms of *Lactobacillus* cells as enzyme sources of biosensor and compared biosensor performances of these forms for determination of PCs. For this purpose, lyophilized *Lactobacillus* cells (containing *L. bulgaricus, L. acidophilus, S. thermophilus*), pure *L. acidophilus* cells, pure *L. bulgaricus* cells, and *L. acidophilus* and *L. bulgaricus* cells adapted to catechol in Lactobacilli MRS Broth were prepared. Lyophilized *Lactobacillus* cells were used directly in the bioactive layer of biosensor. Pure *L. acidophilus* and pure *L. bulgaricus* cells were obtained by purification of lyophilized *Lactobacillus* cells in Lactobacilli MRS Broth. For this purpose, a quantity of lyophilized *Lactobacillus* cells was activated in a Lactobacilli MRS Broth for 24 hr at 35°C. After this activation process *S. thermophilus* in lyophilized *Lactobacillus* cells could not grow in Lactobacili MRS Broth medium, because the medium is not suitable for their growth.[14,15] Thus, only *L. acidophilus* and *L. bulgaricus* cells could be grown in Lactobacilli MRS Broth. The cells were removed from the culture medium by centrifugation at 4600 rpm for 25 min at 4°C. The pellet was washed with phosphate buffer (pH 8.0, 50 m*M*) and centrifuged again at 4600 rpm and 4°C for 25 min. The cellular pastes were separated into two portions. One portion was used for purification of *Lactobacillus* cells and the other portion was used for adaptation of *Lactobacillus* cells. For purification, one portion of the cellular paste that was spread on Petri dishes contained blood agar and was incubated at 37°C for 24 hr. The different bacterium colonies on petri dishes were investigated and identified by microbiologists in our group according to Baron et al.[15] Then the identified *L. acidophilus* and *L. bulgaricus* cells were grown in culture medium, as already described, at suitable conditions and time. Thus, a paste of pure *L. acidophilus* was obtained and used in the bioactive layer of the biosensor. In the same way, a paste of pure *L. bulgaricus* obtained was used for preparing a biosensor.

For adaptation of *Lactobacillus* cells, the other portion of the cellular paste was inoculated to the MRS broth medium containing gradually increasing catechol. It was inoculated daily until the medium reached 300 mg/L of catechol. After the adaptation of cells (20 hr) was completed, the cells were removed from the culture medium by centrifugation at 4600 rpm for 25 min at 4°C. The pellet was washed with phosphate buffer

(pH 8.0, 50 m*M*) and centrifuged again at 4600 rpm and 4°C for 25 min. Thus, a paste of *L. acidophilus* and *L. bulgaricus* cells adapted to catechol in Lactobacilli MRS Broth was obtained and used for preparing the biosensor.

Cell growth was followed spectrophotometrically by measuring optical density at 560 nm, and the relationship between optical density and the living cells was also investigated. In all experiments, log-phase bacterial cells ($OD_{560} = 0.450$) were used.

Procedures

Preparation of the Microbial Biosensor

In the microbial biosensor construction to prepare the bioactive layer of the biosensor the *Lactobacillus* cells (10 mg) and gelatin (10 mg) were mixed and dissolved in 400 μL phosphate buffer (pH 7.5, 50 m*M*) at 37.5°C. Then 200 μL of the solution was spread over the Teflon membrane on dissolved oxygen probe, and the bioactive layer was allowed to dry at 4°C for 30 min. After that, the bioactive layer was treated with a cross-linking agent, glutaraldehyde (0.625%, in phosphate buffer; pH 7.5, 50 m*M*) for 5 min to immobilize the cells on the surface of the dissolved oxygen probe membrane.

Measurement Procedure

There are two possibilities for the measurement by the microbial biosensor: endpoint measurement (steady-state mode, in which the differences in dissolved oxygen concentration [ΔDO] reflect the respiration rate of catechol), kinetic measurement (first derivative of dissolved oxygen concentration–time curve corresponding to the acceleration of respiration) (Figure 1). We carried out our measurements according to endpoint measurement.

In this study the biosensor based on lyophilized *Lactobacillus* cells was put into the thermostatic reaction cell containing working buffer (pH 8.0, 50 mM phosphate buffer) at constant temperature (37.5°C) and the magnetic stirrer was fixed at constant speed. A few minutes later (10 min), dissolved oxygen concentration was equilibrated because of the diffusion of dissolved oxygen between working buffer and dissolved oxygen probe. At this time catechol was injected into the thermostatic reaction cell. The dissolved oxygen concentration started to decrease, and 18 min later it reached a constant dissolved oxygen concentration due to enzymatic reaction equilibration. At this point, dissolved oxygen concentration was recorded; measurements were carried out by noting the decrease of dissolved oxygen concentration in relation to phenolic substrates concentration added to the reaction cell.[16] The reactions that occurred in the bioactive layer of the microbial biosensor are shown in Figure 2.

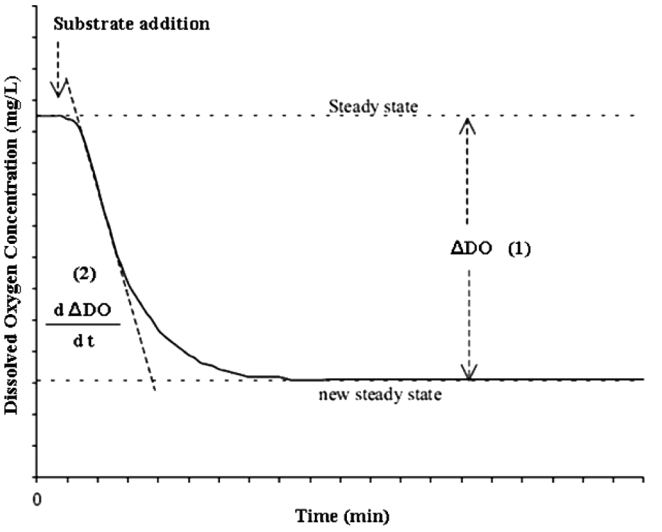

FIGURE 1 Measurement principle of the dissolved oxygen biosensor: (1) Endpoint measurement. (2) Kinetic measurement.[17]

Applications

The microbial biosensor was developed for the determination of PC concentrations in wastewaters and in milk products that contain various concentrations of PCs. For this purpose, the samples were necessarily diluted before the determination of PC concentration. Five measurements for every sample were made. Standard deviation (SD) and coefficient of variation (CV%) were calculated from the calibration curve.

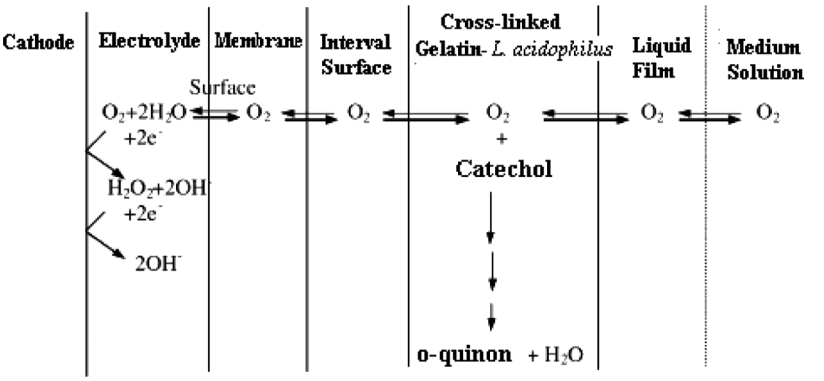

FIGURE 2 The reactions occurred in the bioactive layer of the microbial biosensor.

RESULTS AND DISCUSSION

Selecting of Suitable *Lactobacillus* Cells as the Enzyme Source

In this study, we used different forms of *Lactobacillus* species as enzyme sources of biosensor and compared biosensor performances of these forms for determination of PCs. For this purpose, lyophilized *Lactobacillus* cells (containing *L. bulgaricus, L. acidophilus, S. thermophilus*), pure *L. acidophilus* cells, pure *L. bulgaricus* cells, and *L. acidophilus* and *L. bulgaricus* cells adapted to catechol in Lactobacilli MRS Broth were used.

Biosensors were prepared by using the *Lactobacillus* cells described earlier. Biosensor responses of each *Lactobacillus* cells were measured in the same conditions. According to the results in Figure 3, the best biosensor responses were obtained by using the biosensor prepared with lyophilized *Lactobacillus* cells (contains *L. acidophilus, L. burglaricus, S. thermophilus*). The biosensor based on lyophilized *Lactobacillus* cells displayed a linear increase and reproducibility against the increase in substrate concentration. The biosensors prepared with pure or adapted *Lactobacillus* cells showed poor biosensor response. This circumstance may be explained by the synergetic effects. *Lactobacillus acidophilus, L. bulgaricus,* and *S. thermophilus* in lyophilized *Lactobacillus* cell mixture like living together. A symbiotic relationship between these three bacteria is known from the literatures.[13] But *S. thermophilus* can't live in Lactobacilli MRS Broth because the medium is not suitable for their growth.[14,15] Thus, pure *L. acidophilus* cells, pure *L. bulgaricus* cells, and adapted *Lactobacillus* cells grown in Lactobacilli

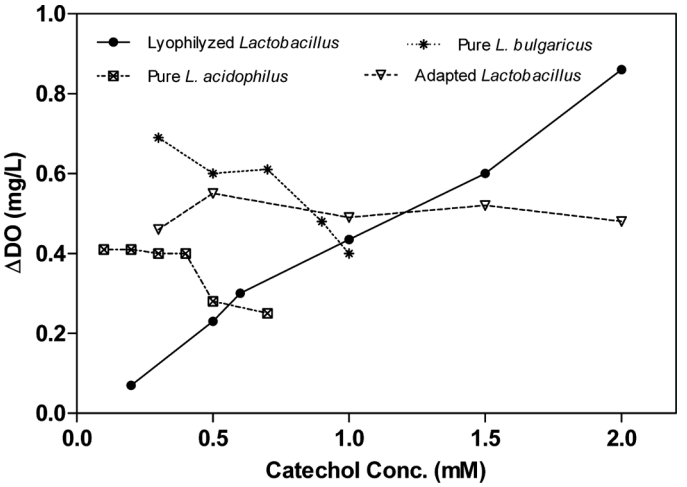

FIGURE 3 The effect of different *Lactobacillus* cells on the biosensor response. Phosphate buffer (pH 8.0; 50 m*M*), T 37.5°C; the amounts of microorganism and gelatin and percentage of glutaraldehyde were kept constant at 10 mg, 10 mg, and 0.625%, respectively.

MRS Broth do not contain *S. thermophilus* cells. Therefore, lyophilized *Lactobacillus* cells were chosen as the most suitable cells in further experiments.

Effects of Immobilization Material on Biosensor Response

After chosing the most suitable cells, effects of thepercentage of glutaraldehyde on the microbial biosensor response were investigated for the optimization and the determination of effects of immobilization materials such as amount of lyophilized *Lactobacillus* cells and gelatin.

Effects of the Amount of Lyophilized Lactobacillus *Cells on Biosensor Response*

In order to determine the effect of the amount of lyophilized *Lactobacillus* cells on the biosensor response, the amounts of gelatin (10 mg) and glutaraldehyde (0.625%) on the bioactive layer were kept constant; the amount of lyophilized *Lactobacillus* cells was changed to 5, 10, and 20 mg in the biosensor preparation. Figure 4 shows the results obtained from this study. According to the figure, the best relation between the microbial biosensor response and the amount of the lyophilized *Lactobacillus* cells and also the most suitable linear range were obtained when 10 mg lyophilized *Lactobacillus* cells was used.

Effects of Gelatin and Glutaraldehyde Amount on the Biosensor Response

After detection of the effects of lyophilized *Lactobacillus* cells amount on the biosensor response, other parameters such as effects of gelatin and glutaraldehyde amount on the biosensor response were investigated.

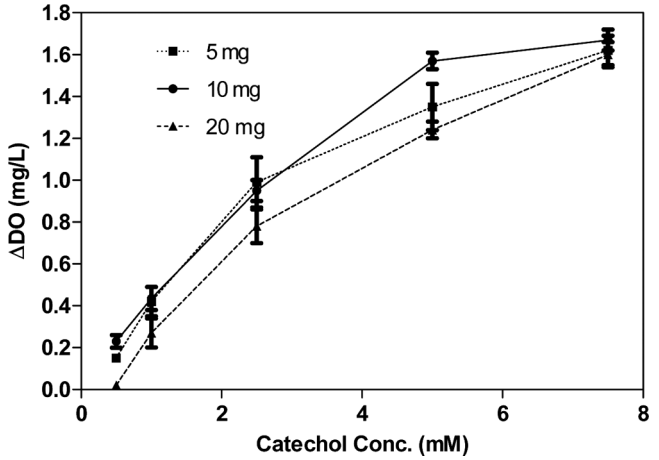

FIGURE 4 Effect of the amount of lyophilized *Lactobacillus* cells on biosensor response. Phosphate buffer (pH 8.0; 50 m*M*), T 37.5°C; gelatin amount and percentage of glutaraldehyde were kept constant at 10 mg and 0.625%, respectively.

For this purpose, the amounts of lyophilized *Lactobacillus* cells and glutaraldehyde were kept constant as 10 mg and 0.625%, respectively, and different amounts of gelatin (5, 10, and 20 mg) were used in the biosensor construction. According to the results obtained, the most suitable biosensor responses were obtained when 10 mg gelatin was used in the biosensor construction (Figure 5). When less than and more than this amount of gelatin was used in the biosensor construction, decreases in the biosensor response were observed. When 5 mg gelatin was used, formation of a porous surface on the bioactive layer formed, and after few measurements were realized, the bioactive layer was destroyed because of the lack of physical stability. When 20 mg gelatin was used, a steric structure on the biosensor layer of the biosensor formed. The steric structure did not give any opportunity for substrate diffusion from the reaction medium to the electrode and as a result the biosensor responses decreased.

After the best amounts of lyophilized *Lactobacillus* cells and gelatin were determined as 10 mg and 10 mg, respectively, for the investigation of the effect of the cross-linking agent, glutaraldehyde on the biosensor response the amounts of lyophilized *Lactobacillus* cells and gelatin were kept constant at 10 mg and each of the newly prepared microbial biosensors was treated with 0.25, 0.625, and 1% glutaraldehyde. Figure 6 shows the results obtained. The best biosensor response was obtained when 0.625% glutaraldehyde used. When 1% glutaraldehyde was used, a decrease was observed in the biosensor response due to more gelatin–gelatin cross-links that caused steric hindrance, which would lead to a diffusion problem of the substrate. Using 0.25% glutaraldehyde in the biosensor construction gave

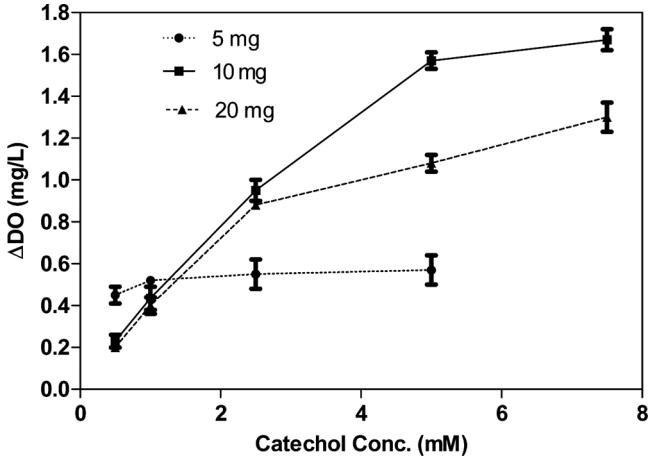

FIGURE 5 Effect of the gelatin amount on the biosensor response. Phosphate buffer (pH: 8.0; 50 m*M*), T 37.5°C; the amounts of microorganism and glutaraldehyde were kept constant at 10 mg and 0.625%, respectively.

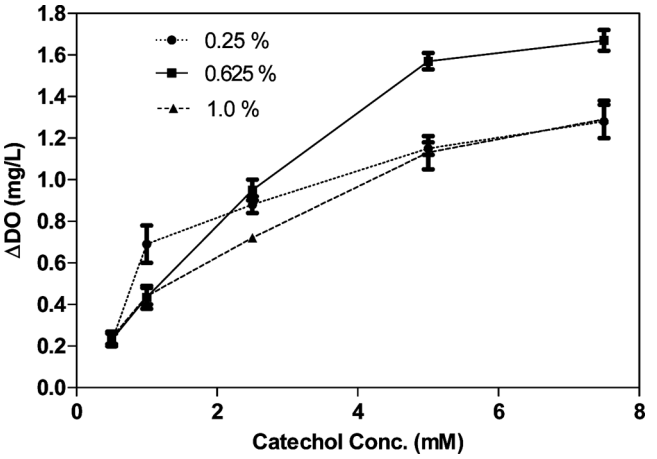

FIGURE 6 Effect of the glutaraldehyde percentage on the biosensor response. Phosphate buffer (pH 8.0; 50 m*M*), T 37.5°C; the amounts of microorganism and gelatin were kept constant at 10 mg and 10 mg, respectively.

a porous structure on the bioactive layer of the biosensor and also this structure resulted in low dissolved oxygen concentration change, which resulted from the respiration activity of lyophilized *Lactobacillus* cells in the presence of catechol.

Optimization Parameters for the Biosensor

Effect of pH

To determine the effect of pH value on the microbial biosensor response, different buffer systems were investigated. For this purpose, a 50 m*M* concentration of phosphate (pH 5.0, 6.0, 7.0, 7.5, 8.0) and glycine (pH 8.5, 9.0, 10.0) buffers were used in the experiments. From the experiments the optimum pH value was obtained as 8.0 (Figure 7). It was expected that the best working of the microorganism used in the biosensor construction could be at this pH value because the optimum pH value of free polyphenol oxidase is between 7.0 and 8.0. The biosensor was subjected to 2.5 m*M* catechol in buffer solution at various pHs. According to Figure 7, when pH was increased from 5.0 to 8.0, increases for the microbial biosensor response were observed; on the other hand, when pH increased from 8.0 to 10.0, decreases were recorded for the biosensor response. Consequently, pH 8.0 was selected as the most suitable and optimum pH value for the microbial biosensor.

Effect of Temperature

For the determination of temperature effect on the microbial biosensor response, the experiments were carried out between 21 and 50°C under the

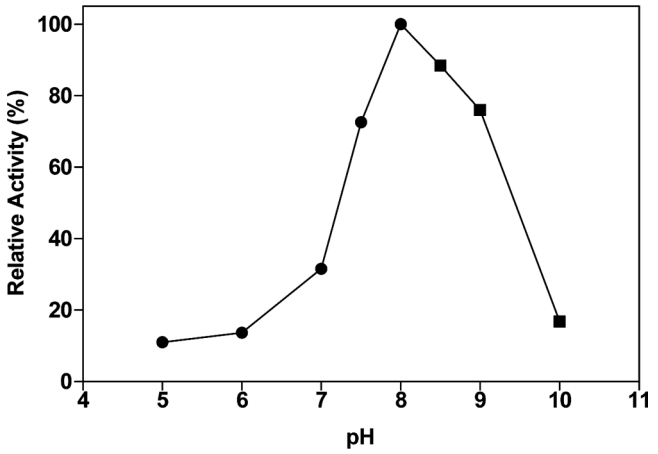

FIGURE 7 Optimum pH of the microbial biosensor: ●, 50 m*M* phosphate buffer; ■, 50 m*M* glycine buffer. T 37.5°C; the amounts of microorganism and gelatin and percentage of glutaraldehyde were kept constant at 10 mg, 10 mg, and 0.625%, respectively.

best working conditions obtained from the optimization studies. Results obtained are given in Figure 8. According to this figure, the highest biosensor response was observed at 37.5°C. Below and above 37.5°C, decreases in the biosensor responses were recorded. *Lactobacillus* species are thermophilic bacterium and they can resist and live at high temperatures[13]; for that reason, the reducing of activity of biosensor was not great above 37.5°C.

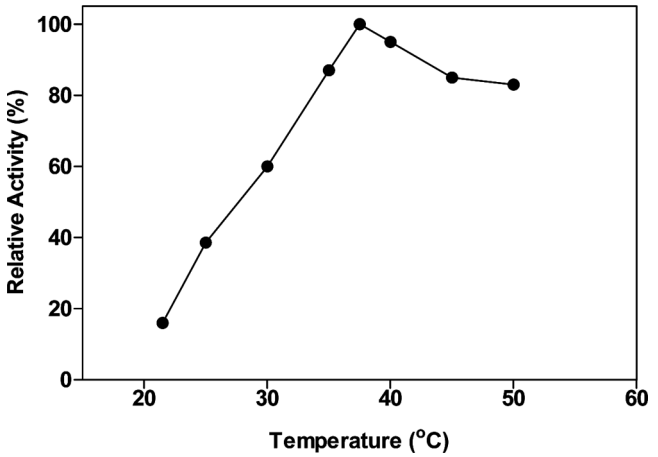

FIGURE 8 Optimum temperature of the microbial biosensor. Phosphate buffer (pH 8.0; 50 m*M*), T 37.5°C; the amounts of microorganism and gelatin and percentage of glutaraldehyde were kept constant at 10 mg, 10 mg, and 0.625%, respectively.

Characterization Studies of the Biosensor

Determination of the Detection Limits for Catechol

Figure 9 shows the calibration curve of the biosensor for catechol. A good linear relationship with a correlation coefficient of 0.9981 was obtained over the concentration range from 0.5 to 5.0 m*M* catechol.

Repeatability

In the repeatability experiments, by using 1.0 m*M* catechol, standard assays ($n = 7$) were made by applying the optimal conditions. From the assays, the average value (X), the standard deviation (SD), and coefficient of variation (CV) were calculated as 1.022 m*M*, ±0.045, and 4.39%, respectively.

Substrate Specificity

For determination of substrate specificity of the lyophilized *Lactobacillus* cells biosensor, 2.5 m*M* of standards of various compounds such as catechol, phenol, orcinol, resorcinol, *p*-cresol, pyrogallol, and L-Dopa were examined. The biosensor response obtained for catechol was accepted as 100% and compared to the biosensor responses obtained for the other substances (Table 1). From the results of the experiments, it can be said that alkaline pyrogallol naturally absorbs oxygen. Therefore, it is well expected to reduce DO level without any enzimatic reaction. Thus, we can say that the best and maximum responses were obtained for catechol.

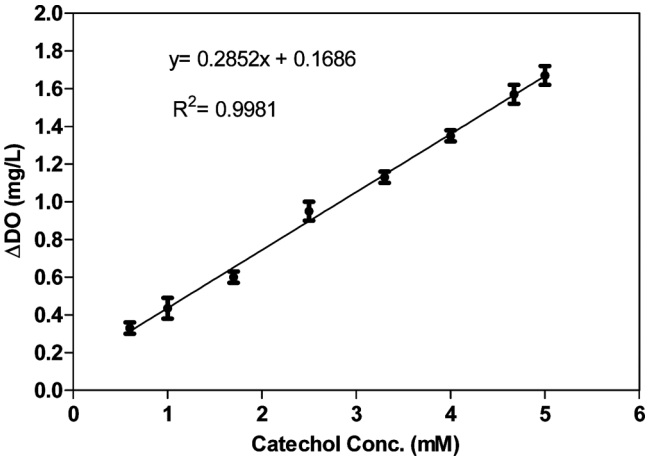

FIGURE 9 Standard curve of the microbial biosensor. Phosphate buffer (pH 8.0; 50 m*M*), T 37.5°C; the amounts of microorganism and gelatin and percentage of glutaraldehyde were kept constant at 10 mg, 10 mg, and 0.625%, respectively.

TABLE 1 Substrate Specificity

Substrate[a]	Activity (%)
L-Dopa	89.2
Phenol	37.11
Catechol	100.0
Pyrogallol	227.83
Resorcin	87.63
p-Cresol	5.15
Orcinol	22.77

[a]Concentration of the substrates is 2.5 m*M*.

Operational and Storage Stability

Consecutively, 11 measurements were made with biosensor prepared under the optimum conditions (37.5°C and pH 8.0, 50 m*M* phosphate buffer) determined in the optimization studies. After 7 injections the biosensor started to lose activity for 1 m*M* catechol.

For the determination of the storage stability of the biosensor developed, the experiments were carried out periodically each day for 18 days of storage by detecting the decreases in the biosensor response. The biosensor was used for only this purpose, and on the other days the biosensor was stored at 4°C in a flask containing phosphate buffer (pH 8.0, 50 m*M*). It was not in contact with the phosphate buffer. This condition provided a moisture medium for the biosensor. Thus, it is possible to prevent drying of the bioactive layer of the biosensor. During the storage period, the biosensor lost only 22% of its initial activity related to storage

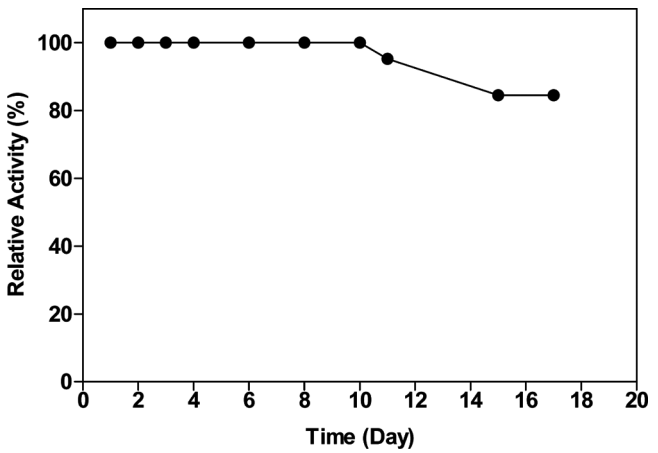

FIGURE 10 Storage stability of the microbial biosensor. Phosphate buffer (pH 8.0; 50 m*M*), T 37.5°C; the amounts of microorganism and gelatin and percentage of glutaraldehyde were kept constant at 10 mg, 10 mg and 0.625%, respectively.

TABLE 2 Catechol Measurements in Samples with Biosensor Based on Lyophilized *Lactobacillus* Cells

Sample	Catechol Concentration Reported (m*M*)	Detected Amount of Catechol Concentration (m*M*)
Wastewater (synthetic)	1.0	0.87 ± 0.04
	2.5	2.11 ± 0.07
Wastewater (industrial)	1.0	1.72 ± 0.12
	2.5	3.09 ± 0.44
Pasteurized milk (Pınar)	1.0	1.41 ± 0.03
	2.5	2.61 ± 0.08
Pasteurized milk (Sütaş)	1.0	1.09 ± 0.37
	2.5	2.56 ± 0.24
Kefir (Ülker-içim)	1.0	1.38 ± 0.22
	2.5	2.76 ± 0.31
Natural yogurt (Danone)	1.0	1.10 ± 0.12
	2.5	2.61 ± 0.24
Probiotic yogurt (Activia)	1.0	1.27 ± 0.19
	2.5	2.69 ± 0.20
Probiotic yogurt (Yovita)	1.0	1.25 ± 0.18
	2.5	2.56 ± 0.37

time (18 days). These results are considered in Figure 10. These results showed that storage stability of the biosensor is better than for some other biosensors.[16]

Application of Prepared Lyophilized Lactobacillus *Cells-Based Biosensor*

To determine PC levels in synthetic wastewater, industrial wastewater, and milk products that contain various concentrations of PCs, the lyophilized *Lactobacillus* cells-based biosensor developed was used. Before the measurements each sample was diluted in order to contain 1.0 m*M* and 2.5 m*M* of catechol in the reaction medium. The dilution procedure also minimized the interference effects. By using the biosensor, three measurements for every sample were made, and results obtained for the samples were compared with each other by calculating standard deviations (SD) (Table 2).

CONCLUSIONS

Biotechnological processes have a crucial role on several industrial productions, such as biomedical applications, food analysis, and environmental remediation. Interest was focused on biomediators as purified enzymes or metabolic pathways of cells, tissues, etc., with specific biochemical properties for analytical and either production or degradation applications.[18] Bacterial cells, with or without adaptation steps, enable us to get biosensor systems with different substrate specificities. Thus, it could be

possible to analyze different PCs individually in the mixed samples by using these sensors.

In this study, the lyophilized *Lactobacillus* cells-based biosensor was developed for determining the PCs in food samples as milk products and environmental wastewater. When we consider the characterization study results of the lyophilized *Lactobacillus* cells-based biosensor, obtained from parameters such as catechol detection limits (0.5–5.0 m*M*), substrate specificity, reproducibility, and operational and storage stability, it may be said that the lyophilized *Lactobacillus* cells-based biosensor is more suitable especially for routine catechol analysis as can be seen in milk products and environmental wastewater. In the previous studies, different types of microorganisms were used in biosensor systems.[19–21] In comparison to the others, our biosensors have higher sensitivity as a bacterial sensor. Furthermore, immobilization of microorganisms as bacteria like *Lactobacillus* cells, instead of pure enzymes on the thick film electrodes, provided economic and practical disposable biosensors without requiring pretreatment (growing, adaptation, and purification). The immobilization method was also useful for the protection of microbial activity. All our data showed that the obtained biosensor may be used as a simple, rapid, and direct method of determining PCs (based on catechol) in various media such as industrial wastewater samples and milk products as food samples.

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