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The Scientific and Technical Research Council of Turkey (TÜBITAK)

Consiglio Nazionale delle Ricerche (CNR)

JOINT RESEARCH PROJECT PROPOSAL

- Please provide the required information briefly. Condensed statements in the boxes provided are preferred. However, additional sheets may be used if necessary.
- Please attach the curricula vitae of all the participating scientists.
- Please submit 3 copies of the proposal to TÜBITAK.

Project title
Screen printed microbial sensors: Biodetection of phenolic compounds

Duration	**Starting date**
24 months	November 2002

Turkish Side			Italian Side		
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Date	**Signature**		**Date**	**Signature**	

Project Information

State of the art (Please present a critical and comparative summary of a literature survey justifying the work proposed)

The development of analytical systems and devices for the control of environmental contaminations is by now an inalienable requirement for the safeguard of the ecosystem. In the course of the years several analytical techniques, mainly chromatographic-spectrometric (HPLC/MS and GC/MS) have been developed to being employed for the determination of the environmental pollutants. These traditional techniques of analysis are extremely powerful in terms of sensitivity, selectivity and specificity, but apart from economical consideration, they present also some disadvantages, due to the need of qualified staff, and to the necessity to carry out an extensive pre-treatment processes on the sample to be assayed. More important, it is not possible to employ this technique **on the spot** for the monitoring in real time of the area under investigations. These reasons have given new impulse towards the development of alternative analytical devices and methods, to be applied in the screening of various contaminants in environmental matrices, minimizing the pre-treatment of sample, reducing cost and time of analysis and extending the number of sampling sites and/or of samples per site. Biosensors represent one of these alternatives, and their application to environmental monitoring has been continuously growing the last years [1].

The application of thick-film technology to the construction of sensors is well-documented [2]. Compared to other technologies that are available for manufacturing electrodes, such as thin-film, thick-film electrodes are relatively inexpensive, simple to fabricate and are congruent for mass production. For these reasons, the scientific and technical communities regard thick-film electrodes as disposable. In addition to these very attractive advantages, the technology enables biomolecules, such as enzymes, etc. and ion-selective membranes to be deposited onto the electrode surface in a straight-forward manner.

Over the past few years interest has been increasing in the application of simple, rapid, inexpensive and disposable biosensors in fields such as clinical, environmental or industrial analysis. The most common disposable biosensors are those produced by thick-film technology. A thick-film biosensor configuration is normally considered to be one which comprises layers of special inks or pastes deposited sequentially onto an insulating support or substrate. One of the key factors which distinguishes a thick-film technique is the method of film deposition, namely screen printing, which is possibly one of the oldest forms of graphic art reproduction.

Screen printing seems to be one of the most promising technologies allowing biosensors to be placed large-scale on the market in the near future because of advantages such as miniaturisation, versatility and low cost and particularly the possibility of mass production. The use of thick-film technology for the production of sensors and biosensors is an emerging field [3].

Problem statement (Please describe accurately the subject of the work proposed)

Phenolic compounds are a class of polluting chemicals, easily absorbed by animals and humans through the skin and mucous membranes. Their toxicity is direct toward a great variety of organs and tissues, primarily lungs, liver, kidneys and genitor-urinary systems. A great number of agricultural and industrial activities release phenolic compounds in the environment [4]. Concern over the pollution risk to environment from industrial manufacturing processes and intensive agriculture has highlighted the need for rapid, easy to operate, low-cost screening procedures which can operate in the field [5]. Thick film technology was chosen as one of the most promising technology, allowing biosensors to be largely on the market especially with disposable, easy-to use, inexpensive devices [6]. On the other hand, instead of enzymes, use of microorganisms as a biological sensing material in the fabrication of biosensors presents some advantages: They are present ubiquitously and are able to metobolize a wide range of chemical compounds. Furthermore microorganisms have a great capacity to adapt to adverse conditions and to develop the ability to degrade new molecules with time. It is also known that microbial biosensors have been investigated a variety of environmental applications[7].

To use of thick film electrodes based on induced/adapted microorganisms will provide reduced cost, practical biosensors with increased stability.

Object and scope (Please list the objectives clearly and specify the scope accurately)

In this work; preparation of thick film electrodes by screen printing technology based on adapted/induced microorganisms which are metabolize phenolic compounds as a disposible sensor strips for the monitoring of phenol derivatives is aimed. Following steps will be investigated:

- Preparation of thick film electrodes in different configurations
- Adaptation of microorganisms into the phenolic medium
- Biosensor preparation, characterization
- Application

Method (Please itemise the parameters to be studied and specify the method to be applied)

Tramates versicolor, Pseudomonas putida and P*hanerochate chrysosporium* which have a metabolic pathway for the phenol degredation are chosen as the biocatalytic materials. The planned steps is given below:

- Microbial cells will be adapted/induced in the presence of different phenolic compounds that are detected.
- Thick Film Sensors will be prepared: screen printed graphite electrodes with different configurations based different substrates like PVC, acetate, ceramic etc.
- Chemical characterization of thin film electrodes
- Cells will be immobilized by using different techniques on the electrode surfaces
- Characterization of biosensors
- Application in environmental monitoring

International co-operation (Please explain in detail the work items to be realised through international co-operation)

Thin/thick film Electrodes will be provided from ENEA, Technological Dept., Biotechnology and Agriculture sector, Electrochemical Biosensor Laboratory (Rome/Italy). The other steps (microorganism production, biosensor preparation and measurements) will be performed at Biochemistry department of Ege University (Faculty of Science).

Results

Expected results (Please list the expected results of the work proposed)

This work will allow to combine thick/thin film technology which is a long established technique for the fabrication of miniature, rugged, low-cost electronic subsystems and biotechnological advances as an easy to use and economical biosensor systems.

Implementation possibilities (Please discuss the expected modes of implementation of the results obtained)

- The immobilization of microorganisms on the thick/thin film surfaces opens the way for such online, real-time monitoring,
- Adapted/induced cells provide more specific and sensitive analysis with good operating lifetimes,
- On the other hand, intake protection in water industry requires sensors with rapid response for use on-line. Microbial sensor strips could be easily used for this aim.

Expected benefits (Please explain the expected scientific, technological, economical benefits to the partner countries)

A great number of agricultural and industrial activities release phenolic compounds in the environment. In Italy waste waters from crushers in production process of olive oil (green waters) are one of the main source of polyphenol contaminants. Concentration of phenol derivatives in green waters is 3000-24000 ppm, while acceptability limits in waste are 0.5-1 ppm according to Italian regulation [8]. It is also well known that phenol is one of the major pollutants of agricultural and industrial waste waters in Turkey. Generely, the detection of phenolic compounds is usually accomplished by means of extremely sensitive and reliable techniques (chromatography and/or spectrophotometry), which unfortunately do not allow continous in situ monitoring in real matrices [9]. Biosensors based on phenol degrading microorganisms will be useful tools to solve the problems related to phenolic compounds in waste waters like green waters and wastes from textiles and paint industry especially for low cost and maintenance for the partner countries.

Execution Matters

Time table (Please indicate each major step in project evolution referring to the intended time schedule)

- To Obtain the consumbles and set up the systems for the analyses……. 6 months
- Production of microorganisms (adaptation/induction) and preparation of screen printed electrodes(chemical characterization) …… …… 6 months
- Preparation of microbial electrodes……. 9 months
  (Immobilization, characterization)
- Application of the proposed systems in real matrices…….. 3 months

Request justification (Please give detailed justification for each item requested)

Potentiostat: To set up an equipment to make chronoamperometric measurements with thin film electrodes.

Computer: To combine with the potentiostat to monitore the signals and also for the evaluation of the results.

Consumbles: Chemicals that will be used during the experiments. And also other consumbles such as printer ink, paper etc.

Additional notes (Please provide additional appropriate information, if any)

Attachment-1: References