



REVIEW ARTICLE

Microbial Biosensor for Marine Environments

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ABSTRACT

A biosensor is an analytical device which integrates a biological recognition element with a physical transducer to generate a measurable signal proportional to the concentration of the analyses. A microbial biosensor consists of a transducer in conjunction with immobilized viable or non-viable microbial cells. Non-viable cells obtained after permeable or whole cells containing periplasmic enzymes have mostly been used as an economical substitute for enzymes. Based on the sensing technique, recent reported microbial biosensors can be classified into two major groups: electrochemical microbial biosensors and optical microbial biosensors. Microbial biosensors based on light emission from luminescent bacteria are being applied as a sensitive, rapid and non-invasive assay in several biological systems. Bioluminescent bacteria are found in nature, their habitat ranging from marine (*Vibrio fischeri*) to terrestrial (*Photobacterium luminescens*) environments. Bioluminescent whole cell biosensors have also been developed using genetically engineered microorganisms (GEM) for the monitoring of organic, pesticide and heavy metal contamination. The major application of microbial biosensors is in the environmental field. Currently, different microorganisms have been genetically engineered to respond to particular stresses or toxicity and are described in the literatures as biosensors for environmental stresses though for them to be biosensors in these of this publication these organisms would have to be coupled to a suitable transducer. Based on the respiration of cells, *Pseudomonas* sp. entrapped polycarbonate membrane modified oxygen electrode was also applied to detect PNP which serves as the sole carbon and nitrogen source for the bacteria. By application of these biosensor in the marine environment the pollution in these ecosystems can be better manage.

Key words: Bacteria, Biosensor, Contamination, Environment, Ecology, Detection.

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INTRODUCTION

Biosensors have been under development for over 35 years and research in this field has become very popular for 15 years. Electrochemical biosensors are the oldest of the breed, yet sensors for only one analyze (glucose) have achieved widespread commercial success at the retail level (1). What is a biosensor? In the early days (the 1960s and 1970s), a sensor seemed to always be a probe of some sort, perhaps due to a vision inextricably linked to pH, ion selective or oxygen electrodes. If you follow the old literature, you will find biosensors that were called bio electrodes or enzyme electrodes or bio catalytic membrane electrodes (2). More recently, we have seen the definition broadened to include sensors buried within large automate instruments (3). There are some who see mass spectrometries, chromatography or electrophoresis as a viable sensor component (4). Many sensors used for biological purposes are therefore not biosensors, including those for temperature, pressure, electrocardiograms, pH, Ca²⁺, catecholamines and the like. By contrast, it is fair to consider surface Plasmon resonance (SPR) devices as utilizing biosensors (3, 5). Even labeled nanoparticles imbedded in the cytosol of individual cells that report optically are viable sensors.

Main description of biosensors

A biosensor is an analytical device which integrates a biological recognition element with a physical transducer to generate a measurable signal proportional to the concentration of the analyses (6, 7, 8, 9, 10, 11, 12). In the general scheme of a biosensor, the biological recognition element responds to the target compound and the transducer converts the biological response to a detectable signal, which can be

measured electrochemically, optically, acoustically, mechanically, calorimetrically, or electronically, and then correlated with the analyze concentration (7, 8, 12, 13). Since Clark and Lyon developed the first biosensor for glucose detection in 1962, biosensors have been intensively studied and extensively utilized in various applications, ranging from public health and environmental monitoring to homeland security and food safety (1, 11, 14, 15, 16). Various biological recognition elements, including cofactors, enzymes, antibodies, microorganisms, organelles, tissues, and cells from higher organisms, have been used in the fabrication of biosensors (9). Among these biological elements, enzymes are the most widely used recognition element due to their unique specificity and sensitivity (17). However, the purification of enzyme is costly and time-consuming. In addition, the *in vitro* operating environment could result in a decrease of the enzyme activity (13). Microbes (e.g., algae, bacteria, and yeast) offer an alternative in the fabrication of biosensors because they can be massively produced through cell culturing. Also, compared to other cells from higher organism's such as plants, animals, and human beings, microbial cells are easier to be manipulated and have better viability and stability *in vitro* (13), which can greatly simplify the fabrication process and enhance the performance of biosensors.

Microalgae usually proposed as bio receptors in sensors for water toxicity testing are not simple to handle and need to be appropriately sampled, diluted and immobilized on Alteration membranes before use. The species employed in this study, being a colonial microalga forming macroscopic mats, does not require any immobilization procedure, thus making easier and faster the preparation of each analysis. The proposed biosensor was quite sensitive to atrazine and showed intermediate sensitivity to carbonyl, while the detection of toxicity in the case of heavy metals was slow. This could be due either to biological factors (related to the adsorption characteristics of the cell walls as well as to specific pathways for sequestration, metabolization and release of the algal species used) or to chemical reasons such as the presence of natural competing agents and the high pH of the experimental medium. Another circumstance that may have resulted in decreased metal toxicity is the production of extra-cellular ligands by the alga, a pattern displayed by many cyanobacteria in response to metal stress (18, 19). Figure (1) shows the schematic representation of a biosensor.

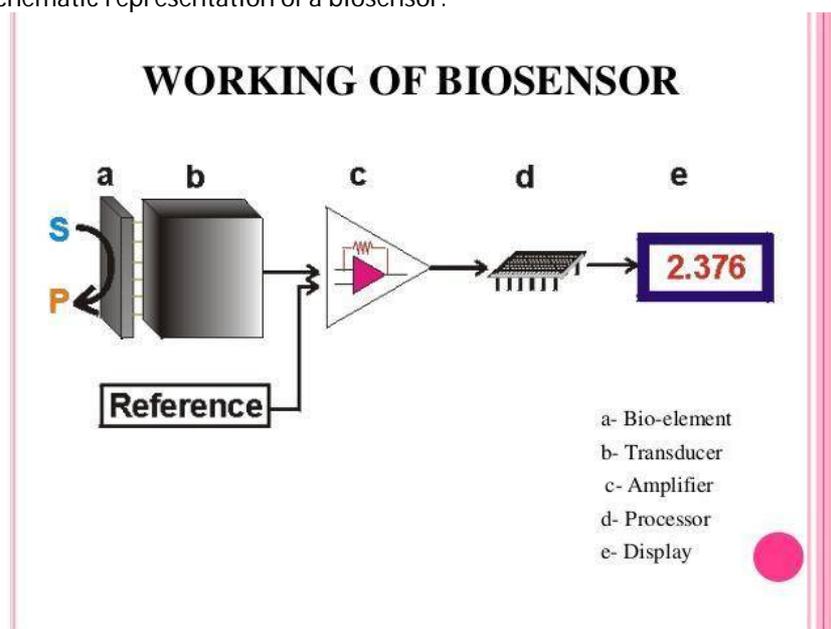


Figure 1. The schematic representation of a biosensor

What is microbial biosensor?

A microbial biosensor consists of a transducer in conjunction with immobilized viable or non-viable microbial cells. Non-viable cells obtained after Permeable or whole cells containing periplasmic enzymes have mostly been used as an economical substitute for enzymes. Viable cells make use of the respiratory and metabolic functions of the cell, the analyze to be monitored being either a substrate or an inhibitor of these processes. Bioluminescence-based microbial biosensors have also been developed using genetically engineered microorganisms constructed by fusing the *lux* gene with an inducible gene promoter for toxicity and bioavailability testing. Figure (2) show the schematic representation of a microbial biosensor (20).

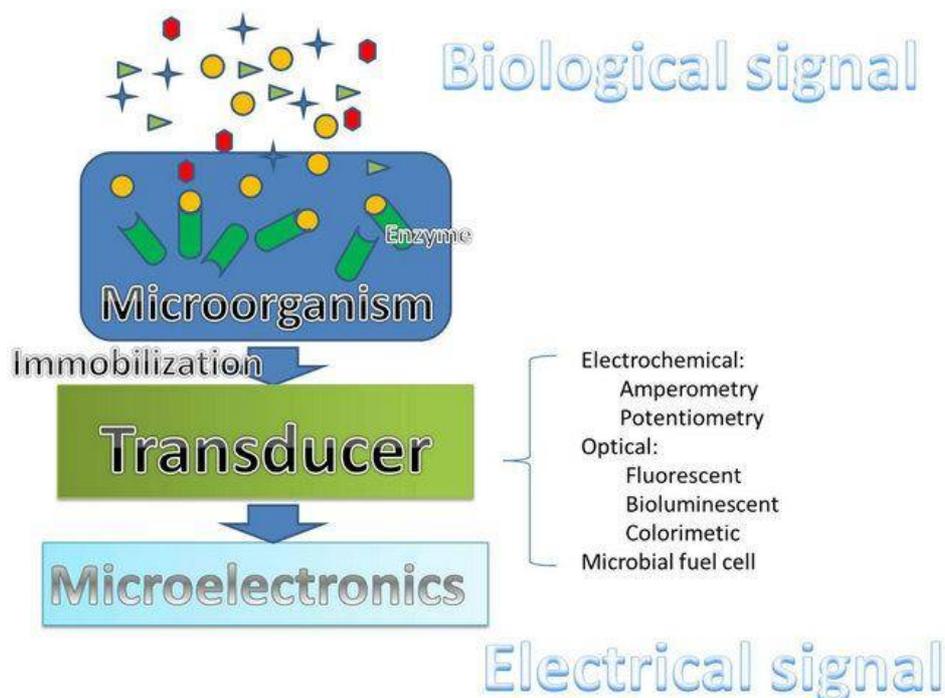


Figure 2: The schematic representation of a microbial biosensor

Classification of biosensors:

Based on the sensing technique, recent reported microbial biosensors can be classified into two major groups: electrochemical microbial biosensors and optical microbial biosensors. Amperometry is the most widely used technique in electrochemical microbial biosensors. Amperometric microbial biosensors have been extensively exploited for environmental applications (17, 21, 22). However, this method is time consuming and not suitable for on-line monitoring (23). Other electrochemical microbial biosensors: The other recently published electrochemical microbial biosensors based on conductometric, potentiometric, voltammetric transducers as well as MFC-based biosensors.

The conductometric microbial biosensor is attractive and appealing owing to its fast and sensitive response to the analyses. In this regard, a conductometric biosensor using *C. vulgaris* microalgae as the bioreceptor was constructed to detect heavy metal ions and pesticides in water sample (24). Potentiometric microbial biosensors detect the amount of analyses by measuring the potential difference between the working electrode and the reference electrode separated by a selective membrane. Recently, a potentiometric biosensor based on the pH electrode modified by permeable *P. aeruginosa* was developed for selective and rapid detection of cephalosporin group of antibiotics (25). The hydrolysis of cephalosporin, due to the enzyme activity of the microbial layer, was accompanied by the production of protons near the pH electrode. The response came from the change of electric potential difference between the working electrode and the reference electrode. Another potentiometric biosensor for the identification of Beta-lactam residues in milk was also reported (26). These biosensors classified as follow:

A) Fluorescent microbial biosensors:

Based on the detection mode, fluorescent microbial biosensors can be divided into two categories: in vivo and in vitro. In vivo fluorescent microbial biosensor makes use of genetically engineered microorganisms with transcriptional fusion between an inducible promoter and a reporter gene encoding fluorescent protein. Green fluorescent protein (GFP), encoded by *gfp* gene, is among the most popular tools due to its attractive stability and sensitivity, and the fluorescence emitted by GFP can be conveniently detected by modern optical equipments with little or no damage to the host system(27).

B) Bioluminescent microbial biosensors:

A bioluminescent microbial biosensor measures the luminescence change emitted by living microorganisms. The luminescence change is, in fact, caused by lux gene-coded luciferase responding to the target analyze in a dose-dependent manner. The expression of lux gene in microorganisms can be controlled in either a constitutive or inducible manner. In the constitutive manner, the lux gene exists constitutively in the sensing microbe and the bioluminescence will change directly with the addition of chemicals of interest. Based on the act that the light intensity produced by the bacteria could be reduced

in the presence of toxic compounds, a *Vibrio fischeri* based bioluminescent microbial biosensor was developed for the rapid determination of the toxicity of some common environmental pollutants in a continuous flow system (28). A tetracycline (TC) luminescent whole-cell biosensor was developed for the rapid and specific TC residue assay in poultry muscle tissue with membrane permeabilizing agent polymyxin B and sensitizing agent EDTA (29).

C) Sodium channel-based biosensors:

The effect of PSP toxins (STX, gonyautoxin and tetrodotoxin) as sodium channel blockers has been exploited for the development of a tissue biosensor (30, 31). The authors covered a Na electrode with a frog bladder membrane, rich in sodium channels, and integrated into a flow cell. Investigating the transport of Na⁺ ions, they could detect the toxin presence. The toxicity levels of the toxins correlated with those determined with the mouse bioassay and, in the tetrodotoxin case, the biosensor was able to detect concentrations more than one order of magnitude below the limit of the detection of the bioassay. A particular case is the neuronal network biosensor developed by Kulagina et al. (32) which exploits the effect of STX and PbTx-3 on the extracellular action potentials. The biosensor was constructed by growing cultured mammalian neurons from spinal cord tissue of embryonic mice over a 64-site microelectrode array. Despite the distinct actions of these two toxins on the nervous tissue (STX inhibits propagation of action potentials and PbTx-3 enhances activation of the sodium channels), both inhibited mean spike rate of spinal cord neuronal networks. The detection limits for STX and PbTx-3 were, respectively, 12 and 296 pgmL⁻¹ in buffer and 28 and 430 pgmL⁻¹ in 25-fold-diluted seawater. These extremely low values (approximately 30 000 times below the mouse bioassay detection limit for STX and 300 times below the regulatory limit for PbTx-3) are due to the extremely high sensitivity of the spinal cord networks. Additionally, the array responded to the presence of toxin-producing algae but not to the presence of non-toxin isolates of the same algal genera. Although this generic approach cannot fully identify or quantify individual toxins, its application as screening tool is clearly justified.

D) Enzyme inhibition-based biosensors:

Hamada-Sato et al have recently developed a biosensor that combines the PP2A inhibition with the phosphate ion consumption by pyruvate oxidase (PyOx) into a flow injection analysis (FIA) system (33). However, the inhibition step is performed in a microtube and only the second enzyme is immobilized. Nevertheless, they measured OA with a detection limit of 0.1 ngmL⁻¹, the biosensor being 50 times more sensitive than ELISA.

Our group is currently working on the development of an electrochemical PP2A inhibition-based biosensor for the determination of OA. Colorimetric experiments with the enzyme immobilized by entrapment with poly (vinyl alcohol) azide-unit pendant water-soluble photopolymer (PVA-AWP) on screen printed carbon electrodes have demonstrated the viability of the approach and its applicability to the detection of the toxin in mussels. Our strategy is much simpler than the one mentioned above, since the enzyme inhibition is detected directly using appropriate PP2A substrates, electrochemically active only after the dephosphorylation by the enzyme (33).

USE OF MICROBIAL CELLS AS BIOSENSING ELEMENTS

Microbes have a number of advantages as biological sensing materials in the fabrication of biosensors. They are present ubiquitously and are able to metabolize a wide range of chemical compounds. Microorganisms have a great capacity to adapt to adverse conditions and to develop the ability to degrade new molecules with time. Microbes are also amenable for genetic modifications through mutation or through recombinant DNA technology and serve as an economical source of intracellular enzymes. (34, 35)

Selection of an appropriate culture is essential as the specific microbial species used in biosensors have characteristic substrate spectra which may or may not correspond well with the spectrum of compounds present in the sample. Adaptation of a microbe for induction of desirable metabolic pathways and uptake systems by cultivation in medium containing appropriate substrates may often be desirable (36, 37, 38).

Microbial biosensors based on light emission from luminescent bacteria are being applied as a sensitive, rapid and non-invasive assay in several biological systems (39) (40). Bioluminescent bacteria are found in nature, their habitat ranging from marine (*Vibrio fischeri*) to terrestrial (*Photobacterium luminescens*) environments. Bioluminescent whole cell biosensors have also been developed using genetically engineered microorganisms (GEM) for the monitoring of organic, pesticide and heavy metal contamination. The microorganisms used in these biosensors are typically produced with a constructed plasmid in which genes that code for luciferase are placed under the control of a promoter that recognizes the analyze of interest. When such microbes metabolize the organic pollutants, the genetic control mechanism also turns on the synthesis of luciferase, which produces light that can be detected by luminometers. (41)

Microbial biosensors for environmental applications

The major application of microbial biosensors is in the environmental field (42, 43, 44, 45, 46, 47, 48, 49, 50, 51). Microbial biosensors have been developed for assaying BOD, a value related to total content of organic materials in wastewater. BOD sensors take advantage of the high reaction rates of microorganisms interfaced to electrodes to measure the oxygen depletion rates.

A microbial biosensor consisting of an oxygen microelectrode with microbial cells immobilized in polyvinyl alcohol has been fabricated for the measurement of bio available organic carbon in toxic sediments. The biosensor allows the estimation of available dissolved organic carbon in sediment profiles on a micro scale (52). Optical fiber (53) and calorimetric (54) based transducers have been used in BOD biosensors.

Halogenated hydrocarbons used as pesticides, foaming agents, flame-retardants, pharmaceuticals and intermediates in the polymer production are one of the largest group of environmental pollutants. Microbial bio assays using immobilized cells of *Rhodococcus* strain containing alkyl-halide hydrolase has been described by Hutteret al. (55). The enzyme presents in the cell liberate shalogen ions from halogenated hydrocarbons. These studies were extended in the fabrication of a microbial sensor (56). The sensor can be stored in the dry form at 277 K for 1 week. More recently a gram-positive actinomycete like organism, exhibiting a broad spectrum for the dehalogenation of halogenated hydrocarbons, has shown better promise and may have potential in the fabrication of a broad specificity biosensor for halogenated hydrocarbons (57).

Applications of microbial biosensors in food, fermentation and allied fields

In recent years, the demand for quick and specific analytical tools for food and fermentation analysis has increased and is still expanding. Both industry and government health agencies require a wide array of different analytical methods in the quality assurance of food materials. Analysis is needed for monitoring nutritional parameters, food additives, food contaminants, microbial counts, shelf life assessment and other factory characteristics like smell and odor. A variety of sensors based on enzymes and antibodies (58, 59, 60, 61) as wells electronic noses (62, 63) have been reported. Microbial biosensors have also shown potential in food analysis (60). A number of reports are available on microbial biosensors for amino acids such as tyrosine (*Aeromonasphenologenes*), tryptophan (*Ps. fluorescens*) and glutamic acid (*B. subtilis*). (64) (65). Determination of phenylalanine is needed not only forthe process control of the phenylalanine fermentation but also for the neonatal diagnosis and dietary management of hyper phenylalaninaemia. A microbial biosensor based on *Proteus vulgaris* cells immobilized in Ca-alginate on an amperometric oxygen electrode has been reported (66). Phenylalanine deaminase present in the cell oxidises phenylalanine to phenylacetic acid.

The importance of microbial biosensors in the marine environments:

Recently appeared around shellfish (diarrheic, paralytic, amnesic, neurologic and azaspiracid) and fish (ciguatera and puffer) poisonings produced by different types of phycotoxins, making evident the urgent necessity of counting on appropriate detection technologies. With this purpose, several analysis methods (bioassays, chromatographic techniques, immune assays and enzyme inhibition-based assays) have been developed. However, easy-to-use, fast and low-cost devices, able to deal with complicated matrices, are still required. Biosensors offer themselves as promising bio tools, alternative and/or complementary to conventional analysis techniques, for fast, simple, cheap and reliable toxicity screening.

A whole-cell sensor system that has been applied to the analysis of seawater utilizes the marine algae *Spirulina subsalsa* coupled to a Clark-type oxygen electrode in a flow through system to estimate pollution as indicated by variation in photosynthetic activity (67). Substances tested included chlorophenols, pesticides and surfactants. A similar system based on *C. vulgaris* and fiber optic signal detection was described for atrazine, simazine, isoproturon and diuron, with sub-ppb detection limits for photosystemII inhibitors (68). The advantage of whole cell sensors in this context is that they give information concerning bioavailability and potentially measure physiological responses, which are relevant to marine processes. The disadvantages that the obtained signals are generally less specific than those collected with enzymatic or affinity sensors and might have to be backed up by chemical analysis to resolve causative relationships between contaminants.

A topic specific to marine monitoring is the pollutants introduced to the marine environment through produced water and drilling fluids from oil exploration platforms (69, 70, 71). Produced water can contain a cocktail of ingredients from hydrocarbons to antifoam agents, biocides, surfactants, corrosion inhibitors and emulsifiers, which can have acute or chronic toxic effects. Given the large range of chemicals potentially implicated, it is likely that an estimate of toxicity/risk posed using a complex and

preferably relevant biological system would be a useful tool in impact assessment. Currently, different microorganisms have been genetically engineered to respond to particular stresses or toxicity and are described in the literatures "biosensors for environmental stresses" (72, 73, 74, 75), though for them to be biosensors in these of this publication these organisms would have to be coupled to a suitable transducer.

Another typically marine problem is the widespread contamination of water and sediments with antifouling agents, most notoriously in compounds such as tributyltin (TBT). Despite the fact that an outright ban agreed by the International Maritime Organization (IMO) is currently being implemented, TBT is still prevalent in many sediments and its quantification and removal is a problem that will face marine scientists and regulators for years to come (76). Among the best-documented effects of organotins on biota are direct toxicity, shell thickening in oysters, a decline in recruitment of their juvenile stages, and endocrine disruption. Given that TBT has been found to yield effects at concentrations in the low ng/l range (77), any detection methods would have to be extremely sensitive. A method based on a bacterial bioluminescence based bioassay for the specific detection of organotin compounds was reported (78). The detection limits were found to be 0.08 μ M for TBT (26_g/l) and 0.0001 μ M for DBT (0.03_g/l) with a linear range of one logarithm. The application of the bioassay to environmental samples is still under development and will depend on the contamination levels in relation to the detection limit of the bioassay for TBT and DBT. A flow-through sensor based on this assay with the bacteria immobilized within a chip and luminescent detection has recently been presented at the Eighth World Congress on Biosensors (79). Accumulation of the organotin within the immobilization matrixes assumed to be responsible for the improved detection limit of 1 nM TBT (325 ng/l).

Trace metals

Trace metals in the marine environment can have dual roles: in some cases they can act as essential limiting trace elements such as, for example, iron which has been found to limit algae growth in some parts of the ocean (80, 81, 82), in other cases they can present a pollution issue. A number of biosensors for metals have been described in the literature such as are combining luminescent bacterial sensors for the detection of zinc and chromate (83), a whole-cell sensor using the alkaline phosphates activity of the algae *C. vulgaris* and inter digitized conductometric electrodes for the detection of cadmium ions (84), an amperometric detection system based on urease inactivation for the screening of heavy metals such as mercury, copper, cadmium and tin in soil leachates (85), a catalytic cDNA sensor with fluorescent detection for lead (86) and many more. One factor of great importance in determining the fate and effect of metals in the environments their bioavailability and this is an issue that can, in certain instances, be addressed by the use of biosensors rather than chemical analysis in their detection. Two different sensor systems, one whole-cell and one protein-based, for metal bioavailability have been described (87) and some of the issues underlying the use of biosensors in assessing metal bioavailability have recently been discussed (88). Since the analysis and speciation of trace metals is a vast field in marine chemistry, the use of biosensors to obtain additional information appears a sensible route forward.

Food safety

Pollution of seafood through algal toxins is a concern that requires a comprehensive monitoring programme. Paralytic shell fish poisons (saxitoxin, gonyautoxin and tetrodotoxin) act as sodium channel blockers. A tissue biosensor based on frog-bladder membrane and a sodium electrode has been developed for the sensitive detection of these toxins (89, 90). An electrochemical immune sensor for saxitoxin and brevetoxin using glucose oxidase as enzyme label has been described (91), as well as one for okadaic acid, brevetoxin, domoic acid and tetrodotoxin, based on a screen-printed electrode (SPE) system and alkaline phosphatase as enzymatic label (92). Okadaic acid, a diarrhetic shellfish poison, was detected by chemi luminescent immune sensor in mussel homogenate (93) and also using a quartz crystal micro balances transducer for the immunoreaction (94).

The biosensors can have a range of applications in marine science, some of which will complement chemical methods in providing information about interactions with biological material; some will have advantages in terms of field-applicability, automation or cost.

What kinds of biosensors have been used for water material?

When the Nernstian biosensor response was used for calibration, up to 20,000 mgL⁻¹ glucose standard was measured without sample dilution. BOD calibrations were accomplished using the two more commonly used standard artificial wastewaters, GGA and OECD solutions. The results showed that the potentiometric CO₂ electrode was a useful transducer, allowing us to build, calibrate and characterize a BOD-like biosensor. Moreover, limitations present at oxygen amperometric electrode (customarily used

as BOD biosensor-based transducer) such as oxygen low solubility and its reduction at the cathode were avoided.

Fluorescence can be detected at a longer wavelength after the excitation of the fluorescent substance at a shorter wavelength. Fluorescent biosensors have been widely applied in analytical chemistry due to their easy construction using standard molecular biology techniques (95). Fluorescence-based microbial biosensors can be divided into in vivo and in vitro type. For the in vivo type fluorescent microbial biosensor, the microorganisms are able to produce the fluorescent substance (e.g., green fluorescent protein) without the addition of an exogenous fluorescent element. For the in vitro type, the metabolic activity of microorganisms changes the environment surrounding them, resulting in the change of light emission due to the exogenous fluorescent element.

Based on the respiratory activity of the bacteria, target analyze adapted *P. putida* has been used as the sensing element in the detection of other pollutants, such as BTE (benzene, toluene, ethylbenzene) (96) and 2,4-dichloro phenoxy acetic acid (97).

Candida tropicalis (98) have been applied to the construction of ethanol whole-cell biosensors. Moreover, a G. oxidants-based amperometric biosensor with ferric cyanide serving as the mediator for the measurement of ethanol in FIA system was constructed (99).

Material	Kind of bacteria	Substrate	Reference
Naphthalene	<i>Sphingomonas Sp.</i> Or <i>P.Fluorescens</i>	Hydrogel	(135, 136)
Acrylamide/Acrylic Acid/Acrylonitrile	<i>Brevibacterium Sp.</i>	Waste Waters	(137)
Acrylonitrile	<i>P. Pseudoalcaligenes</i>	Waste Waters	(137, 138)
Cyanide	<i>S. cerevisiae</i>	Cyanide	(139)
Nitrification	A Microbial Sensor	Waste Waters	(140)
Determination Of Phytotoxicity	<i>Synechococcus Sp.</i>	Isolated Chloroplasts Or Photosynthetic Membranes	(141)
Detecting Pollutant- Induced Effects	<i>Cyanobacterium</i>	Photoelectrochemical Cell	(141)
Sulphite	<i>Thiobacillus thiooxidans</i>	Foods	(142, 143)
Pharmaceutical	<i>B. Subtilits</i>	Enalaprilmaleate (Ema)	(38)
Highly Toxin Materials, Including Heavy Metals And Organic Chemicals	<i>Photobacterium phosphoreum</i>	Based On Heat Shock Gene- Bioluminescence	(144) (145) (146) (147)
Biocides	<i>E. coli</i>	Biocides	(148)
GEM	Biolumine Scene (<i>E. coli</i>)	Environment	(149)
Cytotoxicity And Lysis	<i>S. cerevisiae</i>	Sense Chemicals	(150)
For The Measurement Of Sugars	<i>Dienococcus</i>	Food And Allied Industies	(151)
Treatment Of Mixed Radioactive Wastes	<i>D. radiodurans</i>	Radioactive Wastes	(152)

Marine microalgae, especially phycotoxin producers such as several species of dinoflagellates and diatoms, are one of the main problems in the exploitation of marine resources around the world. Phycotoxins are toxic compounds that enter into the food chain as components of the phytoplankton. Shellfish ingest these toxins and act as vectors, transmitting them to humans; and not only shellfish: several marine carnivores, such as some fish species and crabs, may also act as vectors. Phycotoxins accumulate in the digestive glands of shell fish without causing any toxic effect on it. However, when

humans consume a sufficient amount of contaminated seafood (phyco toxins are odorless and tasteless), intoxication occurs. Table (1) shows the recently used biosensor.

Table (1): Recently used biosensor

Examples of Field application of biosensor

Several genes encoding the OP-degrading enzyme have been identified and, with the benefit of molecular biology technology, applied in whole cell biosensors as the recognition element (9) (100). For example, genetically engineered p-nitrophenol (PNP)-degrader *Pseudomonas putida* JS444 and *Moraxella* sp., displaying organophosphorus hydrolase (OPH) activity, were constructed to selectively and sensitively detect paraoxon, methyl parathion, parathion, fenitrothion, and ethyl p-nitrophenol thiobenzene phosphonate (EPN) (101, 102, 103, 104, 105).

A microbial BOD sensor equipped with an oxygen electrode and activated sludge was developed and successfully applied to the real-time monitoring of anaerobic reactors (106). Table (2) illustrates some biosensor that used in field.

Table (2): some biosensor that used in the field

Field application	Bacteria	Description	Reference
Determination of choline	<i>Arthrobacter globiformis</i>	Modified oxygen electrodes	(122)
Determination of caffeine	<i>Pseudomonas alcaligenes</i>	Modified oxygen electrodes	(123)
Analysis of genotoxicity	<i>Salmonella typhimurium</i> TA1535 psk1002	With a screen- printed	(125)
NA (nalidixic acid) and (IQ)	<i>E. coli</i> RFMuu3/PBR2TTs and <i>S.typhimurium</i> ta1535psk1002	Micro chip	(126)
Detect Cd ²⁺	<i>C.valgaris</i>	Detection limit was a slow as 1ppbcd ²⁺	(127)
Detection urea	<i>Brevibacterium ammoniagenes</i>	In the ph sensitive polyaniline on apt	(128)
Detection of cephalosporin group of antibiotics	<i>P.aeruginosa</i>	Phelectord modified	(129)
Determination Cu ²⁺	<i>Circinella</i> sp.	Modified carbon paste electrode	(131, 130)
Detection of genotoxin mitomycin	<i>E. coli</i> DH5a	A miniature flow-through optical cell-based disposable fluorescent microbial	(132)

Based on the respiration of cells, *Pseudomonas* sp. entrapped polycarbonate membrane modified oxygen electrode was also applied to detect PNP which serves as the sole carbon and nitrogen source for the bacteria (107). In addition, *Moraxella* and *P. putida* modified glassy carbon electrodes (GCE) were utilized in the detection of *E. coli* in water based on the highly sensitive amperometric detection of PNP, a metabolic product of *E. coli*, with the addition of p-nitrophenyl- β -d-glucuronide (108).

The decrease of oxygen consumption rate due to the inhibition of bacterial respiration can also be utilized to detect highly toxic chemicals. According to this mechanism, photosynthetic microalgae *Chlorella vulgaris* modified screen-printed carbon electrode was reported for the continuous evaluation of the toxicity of atrazine and DCMU (109).

Generally, the bacteria, which can uptake glucose, also possess the enzyme activity to metabolize other carbohydrates, such as galactose (110) (111), catechol (112), mannose, and xylose (110). Selective biosensors can still be developed for different sugars as long as the bacteria are adapted to the specific analyze in advance through the selective cultivation. The qualitative and quantitative detection of alcohols with high sensitivity, selectivity, and accuracy is required in many fields (113). Several microorganisms which can metabolize ethanol with the consumption of oxygen, such as *G. oxydans* (114, 115), *Pichia angusta* (116), and *Candida tropicalis* (117) have been applied to the construction of ethanol whole-cell biosensors. Moreover, a *G. oxydans* based amperometric biosensor with ferricyanide serving as the mediator for the measurement of ethanol in FIA system was constructed (118). The experimental results showed that the response was very stable during 72 h of continuous monitoring and the detection limit was as low as 3.3M for ethanol. Another microbial biosensor based on *G. oxydans* for the selective determination of 1,3-propanediol (1,3-d) in the presence of glycerol (Gly) in the flow system was also reported (119). With the help of either ferricyanide or Osmium redox polymers, a good operational stability was demonstrated by 140 h of continuous operation with a selectivity ratio (1,3-d/Gly) of 118 or 145, respectively.

Microbial biosensors are prospective in the determination of some compounds, which is costly and time-consuming by using traditionally analytical method. *S. cerevisiae* coupled with an amperometric oxygen electrode was shown to be sensitive in the detection of thiamine (120) and L-lysine (121). *Arthrobacter globiformis* (122) and *Pseudomonas alcaligenes* (123) modified oxygen electrodes were reported for the determination of choline and caffeine, respectively. A L-lactate selective biosensor was developed using permeabilized, genetically engineered *Hansenula polymorpha* immobilized on a graphite electrode with phenazine methosulphate as the free-diffusing redox mediator (124). Recently, a whole-cell biosensor on the basis of recombinant *Salmonella typhimurium* TA1535 pSK1002 coupled with a screen-printed gold electrode was designed for the amperometric analysis of genotoxicity, and the result obtained therein was consistent with that obtained by standard SOS-umu-test (125). In another recent study, *E. coli* RFM443/pBR2TTS and *S. typhimurium* TA1535 pSK1002 were integrated onto a microchip to fabricate novel microfluidic whole-cell biosensors for the detection of two genotoxic compounds, nalidixic acid (NA) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), respectively (126). The detection limits of 10 µg/mL for NA and 0.31 µM for IQ were achieved.

Demonstrate that OP compounds can inhibit the activity of acetylcholinesterase. A similar conductometric biosensor using *C. vulgaris* as the sensing element was fabricated to detect Cd²⁺ and the detection limit was as low as 1 ppb Cd²⁺ (127). Another conductometric biosensor was constructed by entrapping lyophilized *Brevibacterium ammoniagenes* in the pH sensitive polyaniline on a Pt twin wire electrode to detect urea (128). The catabolic activity of bacteria produced ammonia from urea, thereby causing an increase of the local pH. The variation in pH resulted in the change in the resistivity of the CP, which was detected by the working electrode.

Recently, a potentiometric biosensor based on the pH electrode modified by permeabilized *P. aeruginosa* was developed for selective and rapid detection of cephalosporin group of antibiotics (129). The hydrolysis of cephalosporin, due to the enzyme activity of the microbial layer, was accompanied by the production of protons near the pH electrode. The response came from the change of electric potential difference between the working electrode and the reference electrode. Another potentiometric biosensor for the identification of Beta-lactam residues in milk was also reported (26).

Recently, a voltammetric microbial biosensor for the determination of Cu²⁺ was reported (130). The biosensor was based on *Circinella* sp. modified carbon paste electrode. Cu²⁺ was pre-concentrated on the electrode and measured by CV, which was interpreted as peak current to determine the concentration of the target analyte. The detection limit of this biosensor could be as low as 54 nM Cu²⁺. Furthermore, a modified stripping voltammetry was proposed by taking advantage of both bacterial adsorption on a mercury surface and metal fixation capacity on *A. ferrooxidans* (131).

Martineau et al. reported a miniature flow-through optical cell-based disposable fluorescent microbial biosensor for the detection of genotoxin mitomycin C (132).

Another bioluminescent microbial biosensor based on luxCDABE marked *Acinetobacter* sp. bacterium was used to assay the toxicity of wastewater contaminated by heavy metals (133). In addition, a bioluminescent biosensor with *P. fluorescens* HK44 pUTK21 recognition element was designed to reflect the available fraction of naphthalene in soil since there was a linear relationship between the luminescence from microbes and the naphthalene concentration (134).

CONCLUSION

All literature indicated that biosensor can be applied in the marine environment especially for detection of pollutant such as: Crude oil, Heavy metal, insecticide and etc. Between all described biosensors the biosensor that work based on bioluminescence better applied in the marine environments.

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