



Biosensors technology and analytical instrumentation for the automated detection and quantification of biological makers and chemical agents of interest

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1. BRIEF PRESENTATION

BIOSENSORES S.L. is an **R&D company developing biosensor technology and analytical instrumentation for the automated detection and quantification of biological markers and chemical agents of interest.** It can be applied and be beneficial to several fields: **chemical and biological research, biomedicine, agriculture, food, water quality, environmental pollution,** etc.

Two major lines of investigation are now pursued:

- Molecular affinity biosensors.
- Microbial biosensors for the analysis of water quality

BRIEF DESCRIPTION OF THE OFFERED TECHNOLOGY

The **principal activity** of the company is to **develop automated systems based on specific biosensors for real-time detection and quantification of chemical or biological molecules.** The company develops analytical instrumentation for the monitoring of these molecules in an automotive way.

The analytical technology used gives the possibility to **analyze very small quantities of molecules,** quantities which are too small to be detected with most common techniques. The instrumentation developed is engineered to drastically **reduce the time per each analysis and to automate the analytical procedure.**

This kind of technology can be useful in **several different fields:** chemical and biological research, biomedicine (e.g. disease markers for, tumors, degenerative disease, autoimmune disease etc.) agriculture, food science, water quality, environmental pollution, security, etc. The company has previous experience on these topics, having taken part in six European projects related to this aim.

POSSIBILITY OF COLLABORATION AND SERVICES

If you think that our technology or expertise can be useful or can solve what you need, or if you like to collaborate in a research project with BIOSENSORES S.L., through autonomous, national or European Programmes, please contact us.

The following booklet intends to give an overview of our company, to help you to know what we can offer and do.

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2. GENERAL DESCRIPTION OF THE ACTIVITY OF THE COMPANY.

2.1. BACKGROUND OF THE COMPANY.

BIOSENSORES S.L. is a pioneering company in Europe in the field of the biosensor technology (report on biosensor technology, 1992, Prof. M. Mascini.). **BIOSENSORES S.L.** has a **broad experience in European Union (EU) projects** through the participation in **six projects of previous Framework Programmes (FPs)**

The company was **constituted in 1990**, and firstly located on the Technological Park of Valencia. At present, its **R&D and production facilities are in Moncofa (Castellón)** and it has a **sales office in the Scientific Park of Valencia.**

The fundamental activity of **BIOSENSORES S.L.** is the **accomplishment of R+D projects applying among others the technology of biosensors**, to develop analytical instrumentation for the **monitoring "on line" of molecules and chemical agents of interest.**

The **technology, the know-how and the instrumentation** that the company develops, **contribute to the advances and improvements that characterize to biosensor technology.** These improvements have been recognized by different concerted actions of the **EU: BIOSET, BIOSENSORS STABILITY; etc.**

2.2. INFRASTRUCTURES.

The company has own facilities to develop its work, such as, cell cultures installation, dark camera, cold camera and differentiated areas of work as: Electronics, transducers, automatism, molecular engineering, molecular biology and immunology. The company has reserved a wide space for the constructions of prototypes and occasional repair of equipments. This space is equipped with the infrastructure and needed tools. Other facilities of the company are: library, seminar room, administration area, etc.

The company has its own web page (www.biosensores.com) and a service of electronic mail (biosensores@biosensores.com), suitable for an agile management of consultation and exchange of information with other laboratories and groups of investigation.

2.3. THE TEAM.

The scientific staff of the company is formed by **engineers, biologists, doctors, and a group of economists**, all of them with a specific knowledge in its area. Moreover, **BIOSENSORES S.L. takes part in many National and European Projects in collaboration with Universities and the Spanish Research Council (CSIC) as well as with private Companies.**

As an integrated manner, we manage to get the knowledge and experience in this multidisciplinary environment to reach the ambitious objectives and planned technological advances in the R+D projects. Thus, different, but convergent, technological areas are covered with this philosophy of the company, such as microbiology, molecular biology, cell culture, bioelectronic prototypes and development of control software.

2.4. SCIENTIFIC ACTIVITY.

The principal activity of our company, **BIOSENSORES S.L.**, is to develop automated systems based on specific biosensors for real-time detection and quantification of chemical or biological molecules in different kind of samples.

Moreover, the company develops analytical instrumentation for the monitoring of these molecules in an automotive way. The analytical technology used gives the possibility to analyze very small concentration of molecules, concentrations which are too small to be detected with most common techniques. The instrumentation the company has developed is engineered to drastically reduce the time per each analysis and to automate the analytical procedure. This kind of technology can be useful in several different fields: chemical and biological research, biomedicine (e.g. disease markers for, tumors, degenerative disease, autoimmune disease etc.) agriculture, food science, water quality, environmental pollution, security, etc.

The two present lines of investigation of the company are centered in:

- R&D on equipment for the purification and analysis of the quality of the water.
- R&D on molecular affinity biosensors.

2.5. NATIONAL SCIENTIFIC AND TECHNICAL ACTIVITY

“Biosensors in different formats for the evaluation “in situ” from Policiclic Aromatic hydrocarbons and nitrates” (2006).

Application for a “Project for the Stimulation of Result Transference of Investigation” (PETRI), for the development of microbial biosensors genetically modified for the detection of Policiclic Aromatic hydrocarbons and nitrates. The general purpose of the Project is to exploit control systems of regulation of two components, which respond to aromatic hydrocarbons and nitrate to design biosensors that allow monitoring the bioavailability of these polluting agents, which are considered toxic by diverse environmental agencies.

This project has been participated by, along with **BIOSENSORES S.L.**, the **Research Group of the Superior Council of Scientific Research** led by the *Prof. Juan Luis Ramos* (Experimental Station of the river Zaidin. Granada, Spain)



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"Detection of traces of pathogen microorganisms and toxic substances using antibodies and immobilized probes of DNA nano particles integrated in Microsystem" (2006).

Application for a "Project for the Stimulation of Result Transference of Investigation" (PETRI), the general purpose of the Project is the design, construction and industrial validation of a hybrid optic/electrochemical biological Microsystems of analysis that allows to determine the presence of diverse types of micro organisms. This project has been participated by, along with **BIOSENSORES S.L.**, Research Groups of the Superior Council of Scientific Research (*Dr Muñoz Alpaca*. CNM. Barcelona; *Dr. JM Guisan*. ICP. Madrid)

"Intelligent Biosensor with transducer of fluorescence for the detection of Legionella" (2006).

Development and execution of a project of R+D, within the Plan of Aid for Promotion of the scientific research and the Technological Development of the Valencia Region (DOGV nº5063 of 3 of August of 2005). In this project they participate, with **BIOSENSORES S.L.**, different groups from investigation of the Universitat of Valencia (UVEG).

"Microbiosensor-DBO" (1995-2005)

BIOSENSORES S.L. has developed in 4 years a **microbiosensor to continuously measure the Biochemical Demand of Oxygen (DBO) and the toxicity in liquids**. Its innovating design allows maintaining the measurement procedure, even after the entrance of a toxic without interruption of the measurement, maintaining the metabolic activity of the sensorial component.

The product is adapted for its use in open surroundings and for the remote capture of data. **The response time is under 15 minutes**, allowing the strategic control of the water treatment plant and the **optimization of the power consumption**. It also allows the **monitoring of water quality in rivers and dams**; the **control of treatment of organic waste processing and biological cleaning contaminated land**.

At the moment, **the company has four equipments**, one of them placed permanently in a treatment plant for test purposes. The other three equipments are located in different water treatment plants of the Valencia Region, in conditions of real operation with satisfactory results.

At the present time, the equipments are being engineered to reduce their volumes and costs, that will make possible their use internationally, among others, in the following sectors: **potable water, aquaculture, spill control of polluting companies, monitoring of beaches for BLUE FLAG qualification, control and optimization of the processes in Waste Water Treatment Plants**, continuous monitoring of waste water quality for later applications, etc.



WATER ON - LINE ANALYSIS EUROPE

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"Automated Immunobiosensor with magnetic particles as a renewable active surface, for the detection and quantification in gaseous phase of molecules and micro organisms for its application in Human Security and Bio-terrorism" (2003-2006)

This project has been presented jointly with the **Ministry of Science and Technology** (PROFIT) and the **Ministry of Defense** (File 3140101), obtaining subsidies by both ministries. The primary target is to develop new technologies that allow the union of controlled bio molecules (DNA probes; antibodies, fluorophores...) to magnetic non-reactive particles, providing a renewable active surface.

The generic process of the immune-sensor system (Ab) or DNA probes is based on a first union step of magnetic particles. The union to these particles is made in an ordered and specific way, which is crucial to make the Ab-Ag/DNA-DNA union possible. On the other hand, the magnetic particles have the particularity of non-absorbing, in a nonspecific way, the present analytes in the environment, which produces a specific interaction between the analyte and the biosensor, increasing its reliability and reproductivity. Finally, the system can be connected to a second Ab/DNA marked with fluorophore, that allows the detection of the signal through a transducer (optical sensor, ISFET, etc.) that transform the light signal into an electrical signal, that can as well be integrated and analyzed with a suitable software.

"Development of on-line measuring equipment for the quantification of the microbial water contamination" (BIOCOUNTER) (1998)

This project develops specific measuring equipment that, valuing the microbial contamination on-line, allows the control of systems of liquid sterilization (ozone, UV, etc.). It is a new technology in the field of the control of the microbiological water contamination. **The analysis is made less than 20 minutes, within a cycle of only 1.5 hours, compared with the present technology that requires 24 hours or more.**

This equipment takes advantage of a part of the technology patented of microbiosensor- BOD that has developed BIOSENSORES S.L. Consequence of these new markets, to which the original technology developed by BIOSENSORES S.L. can be applied, the company has decided **to constitute a new company to the shelter of the Neotech Programme, Water On-line Analysis S.A (WOLA S.A).**, for the exploitation and future developments in microbial biosensors for water analysis: **BOD; Polytox-Res and Microbial counter.**

"ISFET-Immunobiosensor" (1995-1996)

Micro mechanized System made of an ISFET transducer (Ion Selective Field Effect Transistor), with later contacts and cell of flow of 6 microliters of capacity, for the quantitative detection of analytes whose specific interaction with the sensorial component (antibody conjugated with an enzyme), determines a detectable change of pH by the ISFET. A basic prototype has been developed in collaboration with the Department of Analytical Chemistry of the University of



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Barcelona and the National Centre of Microelectronics, within the framework of a project GAME (Group Activator of Spanish Microelectronics).

"Study of toxic factors and its incidence in citric cultures" (1996)

Pre-study made by BIOSENSORES S.L. on the incidence of the atmospheric contamination on citric cultures, including historical registry of the evolution of the tree area by means of aerial photography.

2.6. EUROPEAN SCIENTIFIC AND TECHNICAL ACTIVITY

BIOSENSORES S.L. in the European space centers its activity in developing, in collaboration with advance technological companies and Public Centres of Research of the UE, new products and technologies, with a high added value, within the analytical and biotechnological field.

The results of these union projects improved the analytical technology currently in use and will allow opening to new applications and markets in the midterm. **BIOSENSORES S.L.** at the moment participates in the following projects and activities.

"Enzyme technology for Lean and Green food processing" (LeanGreenFood) (2009-2011)
"Biosensors for in situ evaluation of bio availability of pollutants based on transcriptional regulators a la carte" (BIOCARTE) (2003-2006).

"Controlled Environment Bio piling for Contaminated Land Treatment" (TERRANOVA) (2000-2002).

"Ultra-sensitive Multianalyte Immunoassay based on DNA-sensor" (ULISA) (1998-2000).

"Basic Research on the use of magnetic fluids in microsystems" (MICROMAG) (1998-2000).

"Molecularly Imprinted materials for Integrated Chemical Sensors" (MIMICS) (1996-1999).

Development of multiparametric detection of Avian Influenza Virus infections of humans and animals based on bio-molecule conjugated magnetic beads system. Viability Study for the VII Framework Programme.

"Concerted Action on Biosensor stability" (1996-1999).

"Concerted Action BIOSET" (1997-2000).

Another six applications has been presented to the VII European Framework Programme. Resolutions are pending.

Note: A list of other main projects developed by Biosensores S.L. since 1990 can be found in the annexes.



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For the development of the previously mentioned projects, collaborations with different public research centres have been established, like: **Institute of Petrochemical Catalysis** (ICP.CSIC.Madrid) and **National Centre of Microelectronics** (CNM.CSIC.Barcelona), **Experimental Station of the Zaidín** (CSIC. Granada), **Optical fiber Group**. Applied physics (UVEG), **Laboratory of Industrial Electronic** (UVEG), **Laboratory of Organic Chemistry of metallic complexes** (UVEG), **Laboratory of Biochemistry and Molecular Biology** (Univ. Cardinal Herrera CEU).



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3. TECHNOLOGY DEVELOPED BY BIOSENSORES S.L

3.1 MICROBIAL BIOSENSORS.

BIOSENSORES S.L. has developed a new technology for the real time analysis of biodegradable organic matter and toxicity using Biochemical Oxygen Demand (BOD) and microbial respirometry. This technology, the **BOD-Microbiosensor**, provides updated information on water quality in real time.

The BOD–Microbiosensor has been recognized as:

A “novel technology for implementation in the routine monitoring of BOD.” Fulfilling the suitable specifications required by the Confederación Hidrográfica del Tajo (The Hydrographic Confederation of the River Tajo, Madrid) for the real time monitoring of BOD

Prof. Damilá Barceló (Professor, Spanish Research Council -CSIC- and head of the Department of Environmental Chemistry).

A major interest to water authorities in Spain, permitting the continuous monitoring of BOD for the control of water quality. Furthermore, it is recommended that water quality entities are informed about the availability and industrial performance of this device.

D.José Díez Lazaro, Head of Water Quality.

Particularly useful for the early warning of increasing BOD levels as it is operational day and night and has an alarm generating capacity allowing for the control of Waste Water Treatment Processes.

Javier Ruiz-Jarabo Ferrán, Head of Water Management, Madrid.

Furthermore, the BOD – Microbiosensor has been objectively evaluated and acknowledged as fulfilling the desired criteria for an ‘ideal biosensor’ as drawn up by academic experts in the field of biomonitoring, government officials and the WWT industry (BIOSET Concerted Action Meeting, Paris, 2000).

Dr. Susan Alcock (Cranfield University and BIOSET EU Concerted Action Coordinator).

The BOD-Microbiosensor **fulfills the major criteria recommended for a Biological Early Warning System (BEWS)** in providing, as proposed by Cairns and van der Schalie (1980), biological information on which sound decisions can be made with respect to the hazard posed to human health or the environment of a particular chemical or waste discharge.



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Figure 1. Microbiosensor for BOD determination developed by **BIOSENSORES S.L.**

The accuracy, reliability and efficiency of the **BOD-Microbiosensor** device have been demonstrated through the many years that **it has been successfully applied to the monitoring of wastewater treatment plants and water courses in both Spain and on a European scale.**

The device is characterized by its **automation, ease of use and consequent low maintenance requirements.** The **specialized software for the generation of alarm signals also permits the storage of toxic samples in a refrigerated unit for posterior analysis in the laboratory.**

This BOD-Microbiosensor is the **culmination of work with the Valencia Community Authority for Residual Water Quality Management and, particularly, with the Water Sanitation Entity.**

In addition, **BIOSENSORES S.L.** has also developed two other useful technologies for the biomonitoring of water quality:

- **POLYTOX-RES** – for the online determination of integral toxicity.
- **MICROBIAL COUNTER** – for the online determination of microbial concentrations in water samples.

PRACTICAL APPLICATION

The measurement of on-line BOD, toxicity, and multi-parametric testing using our apparatus facilitates:

- Monitoring of critical points (key lines) in WWTP and in Waters of Ecological Value.
- Remote capture and centralized data control.
- Toxic assessment of water samples.
- More information for the optimization of WWTP (Waste Water Treatment Plant) parameters and maximal use of the assimilative capacity of the environment.
- Microbiological reactor use – providing information on the organic matter available for microbial respiration.

- Increased efficiency of WWTP energy consumption.
- BOD assessment in different critical points of the WWTP.
- Accurate waste water assessment without functional toxic interference.
- A Reliable analytical cycle using two measurements of BOD-Microbiosensor reference standard for double-calibration.
- Low cost sampling.
- Low maintenance.
- Industrial utility. Real time evidence of the accuracy, reliability and efficiency of the equipment. The MBO-Microbiosensor is currently being used to monitor waste water treatment plants in Spain.
- Other advantages may include increased quantitative sensitivity to toxic events (especially mixture toxicity) than chemical indicators.

3.2 BIOSENSOR TECHNOLOGY MOLECULAR AFFINITY USED IN BIOSENSORES S.L. TECHNOLOGY

DEVELOPMENT OF BIOSENSORS FOR THE DETERMINATION OF ANTIBODIES BIOLOGICAL SAMPLES

Immunobiosensors are based in antigen-antibody interaction and allow quantification, in a heterogeneous liquid sample, of specific molecules with high sensitivity, reducing human intervention during sample preparation and analytic process, thus minimizing cost and saving time.

Our strategy is based in immunoassay sandwich technique, using two different antibodies for two distinct recognition sites in a single antigen. Molecular affinity immunobiosensors are based in specific antibodies against the target products. Then, the specific antigen/antibody recognition can be revealed and subsequently converted into an electrical signal and the whole analytical process can be automated and controlled by means of suitable software (**see Figures 2 to 7 below**).



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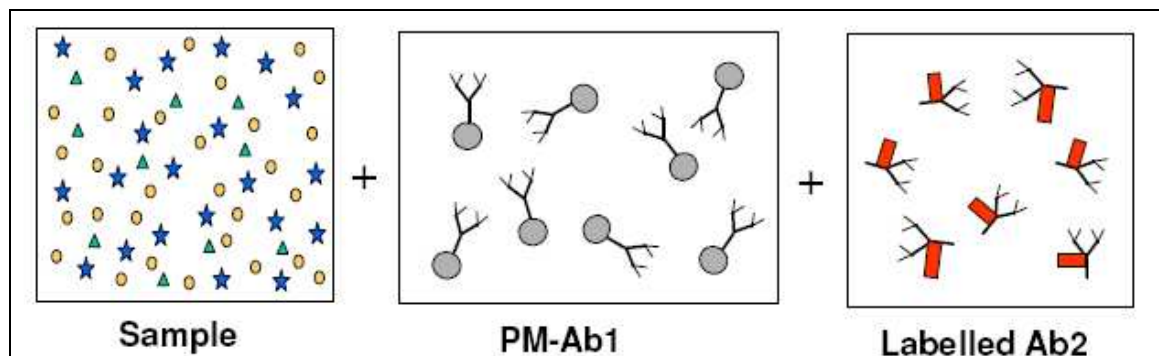


Figure 2. Main components in an affinity immunobiosensor, based in immunoassay sandwich technique with two different antibodies for a single antigen, allowing quantification of specific molecules in a heterogeneous liquid sample. Ab1 is a specific antibody against the target molecule and attached to magnetic particles (PM). Ab2 is a specific labeled antibody linked to a marker molecule (enzyme or fluorophore) for enzymatic or fluorescent detection of the target molecule in a later step.

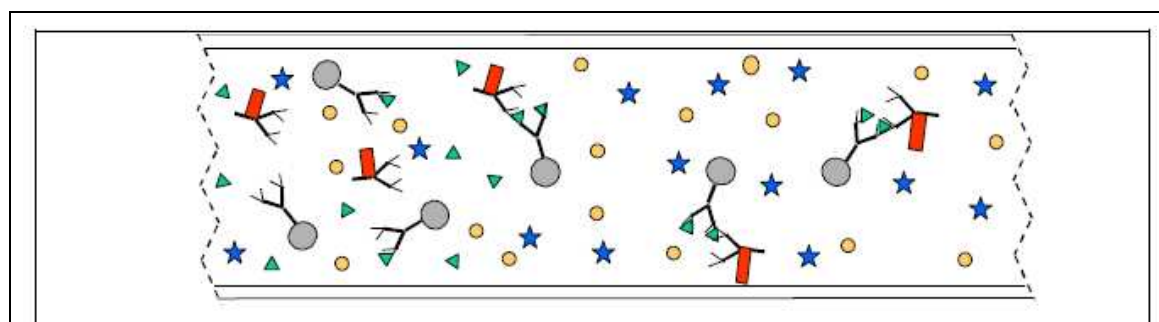


Figure 3. Specific interaction and PM-Ab1-A-Ab2 complex formation, allowing immobilization of the antigen (A) and thus fixing both the capture and labeled antibodies and the antigen in a highly stable way.

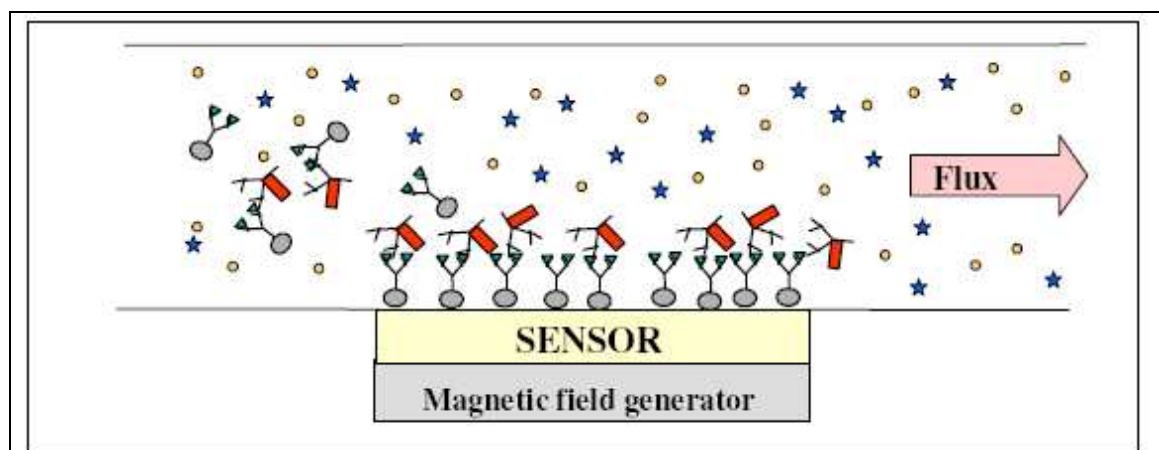


Figure 4. Magnetic particles retain and concentrate the target molecule using a magnetic field.

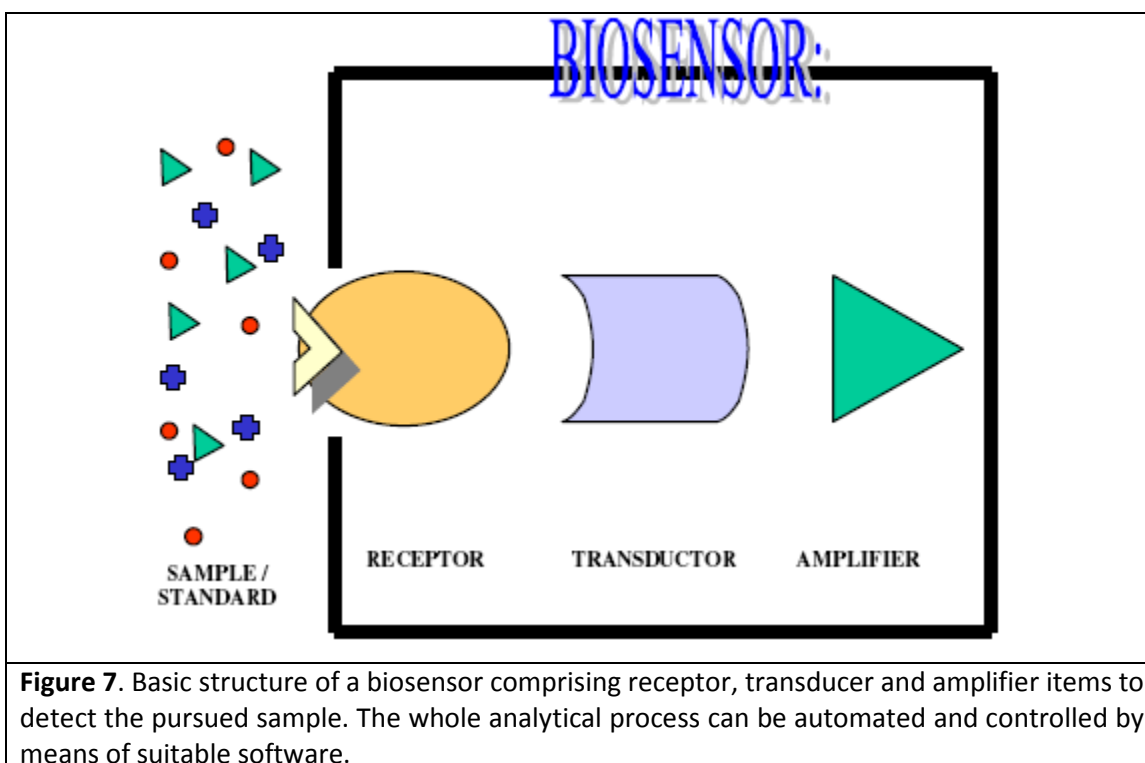
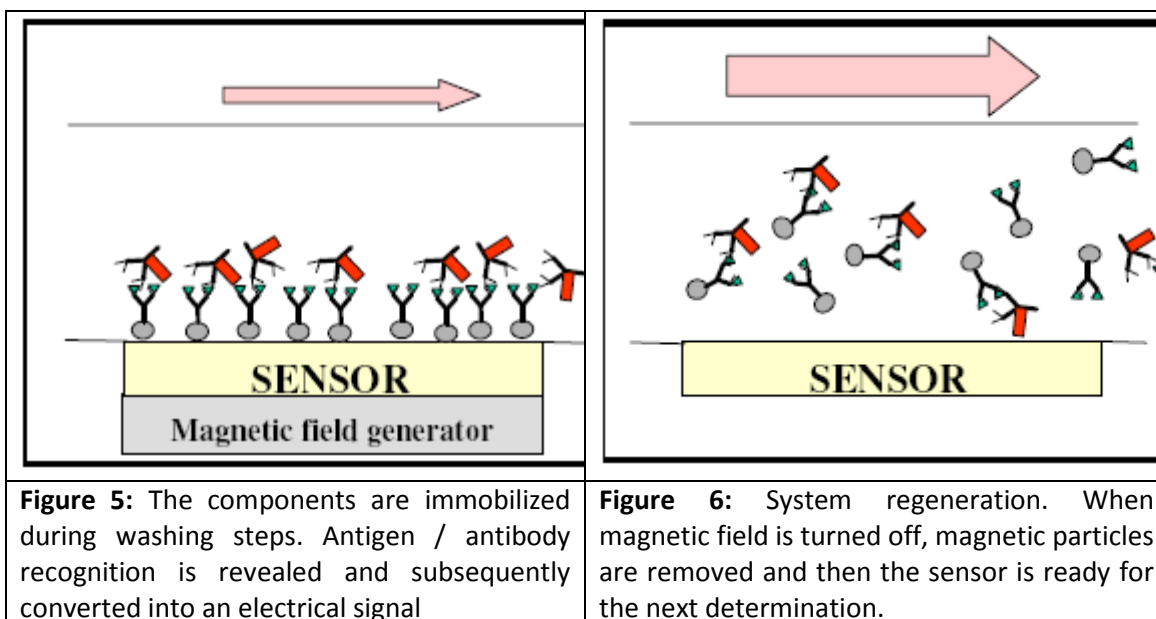


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DEVELOPMENT OF BIOSENSORS FOR THE CONTINUOUS DETERMINATION OF DNA IN BIOLOGICAL SAMPLES

Molecular affinity biosensors are based in nucleic acid interaction and allow quantification, in a heterogeneous liquid sample, of specific molecules with high sensitivity, reducing human intervention during sample preparation and analytic process, thus minimizing cost and saving time (Sole et al., 1998).

Our strategy is based in two nucleic acid probes, one of them for capture and immobilization of the analyte and the second one for detection. Molecular affinity nucleic acid-based biosensors are based in specific probes for the target sequences. Then, the specific probe interaction can be revealed and subsequently converted into an electrical signal and the whole analytical process can be automated and controlled by means of suitable software (See figures below).

Development of specific probes

Specific nucleic acid probes for the target molecules (miRNA sequences) will be obtained, characterized and selected.

* Specific capture probe (P1)

The first probe (P1) is specifically synthesized for the target sequence and is a capture probe that specifically recognizes the target nucleic acid (A) (Figure 8). Biotinilated P1 molecules will be attached to magnetic particles (PM) that are covalently linked to streptavidin. This way, complex PM-P1-A is formed, allowing immobilization of the target and thus fixing both the capture probe and the target in a highly stable way by means of the strong biotin/streptavidin interaction. This strategy is aimed to concentrate the nucleic acid target molecule in a later step using a magnetic field.

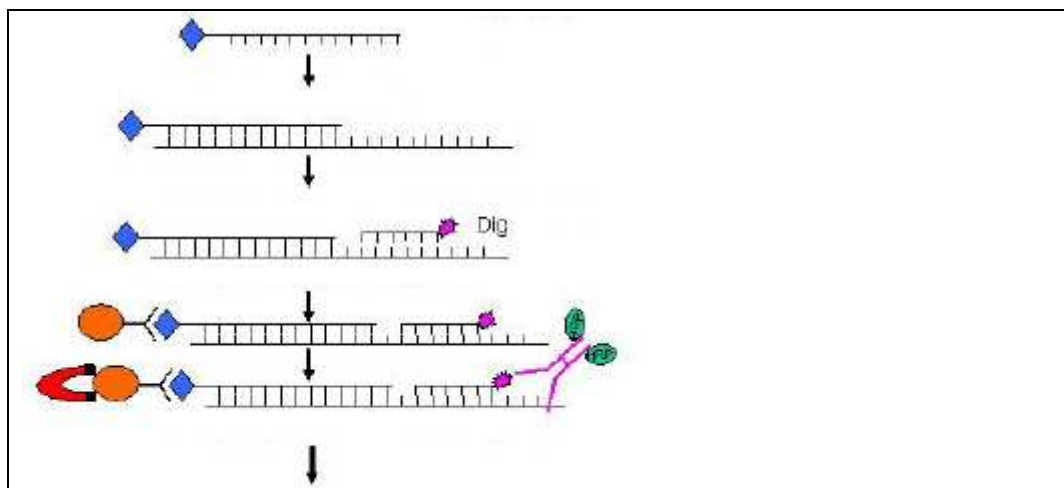


Figure 8. Specific recognition of the target nucleic acid; first with capture biotinilated probe and then with labeled detection probe, followed by magnetic concentration and



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immunoenzymatic detection.

*** Specific detection probe (P2)**

The second probe (P2) is designed for a different region of the same target molecule and is also specific to avoid any non-desired interaction. P2 is subsequently labeled to a marker molecule (enzyme or fluorophore) to allow enzymatic or fluorescent detection of the target molecule (Figure 8). P2 will recognize complex PM-P1-A, thus forming PM-P1-A-P2 that will be later retained and concentrated by a magnetic field. So, a layer of the sandwich is covering the magnetic particles, constituting a so-called renewable membrane allowing immobilization of all the components for washing steps. When magnetic field is turned off, the renewable membrane can be regenerated, preparing magnetic particles for the next determination.



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4. WOLA: WATER ON-LINE ANALYSIS EUROPE S.L.



Currently, **BIOSENSORES S.L.** has developed automated instrumentation based on biosensor technology that is on a final prototype phase and ready for production. These instruments are designed to measure different aspects of water quality. Three main aspects of water quality received our attention. Firstly, **the contamination of water that is biologically reactive** i.e. biochemical oxygen demand (or BOD for short). Secondly, **the integral toxicity levels of water** (Polytox) and thirdly the quantification **of micro organisms in the water** (Microbial counter). In addition, other more conventional water quality measurements such as **pH level, clarity, conductivity and dissolved oxygen** can be added to the instruments to give a complete picture of the quality of water being measured.

The instrument **MB-BOD** is a good example of our technology. Using this instrument, it takes **only 30 minutes to measure BOD automatically with a cost of 0.85€/ sample**, while conventional methods are manual and require five days with a cost of approximately 48€ / sample. This “real time” analysis allows the practitioner to get feed-back results to improve industrial / environmental processes. These could be for example to monitor accidents that may occur in the cleaning process of water and thus avoiding the use of contaminated water for various applications.

The company **Water On-Line Analysis Europe S.L.** (WOLA Europe S.L.) is a spin-off company specifically created to exploit and develop, at a national and international level, the technologies mentioned above (namely MB-BOD, Polytox, and Microbial counter), developed by **BIOSENSORES S.L.**

WOLA Europe S.L. will be owned by **BIOSENSORES S.L.** and external investors. At the same time, **WOLA Europe S.L.** will develop and exploit new equipments and technology products derived from its own Research and Development, in the area of water quality. This market has a very high growth rate.

The goal of **WOLA Europe S.L.** is to become a leading contender in the market of water control, and to penetrate significantly all major markets with its products that are already

developed. The technological advancements and know-how in **BOD, Toxicity and Microorganisms quantification** will allow this goal to be achieved within four to five years.

The instruments now ready for production can be summarized as follows:

- **MB-BOD:** Analysis of water biodegradable contamination.
- **POLYTOX-RES:** Analysis of integral toxicity.
- **MICROBIAL COUNTER:** Microbial quantification.

If the **WOLA Europe S.L.** venture could be of some interest to your company, we can send you more information and a brief business plan of the company to study the possibility of partnership or business collaboration






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5. ANNEXES

A. COPY OF THE EUROPEAN PATENT OF THE TECHNOLOGY OF BIOSENSORES S.L.

	European Patent Office 80056 MUNICH GERMANY Tel: +49 89 2339-0 Fax: +49 89 2339-4433	Europäische Patentamt	European Patent Office	Office européen des brevets
Sanz-Hermel Martínez, Alejandro C/Alétva, 4 46004 Valencia ESPAÑA		 Formal/Ess Officer Name: TN		
		Date: 11.10.07		
Reference: A.23154 MSD/Gr	Application/Officed No. 05917133.5 - 0401 / 05917133			
Applicant(s): Biosensores, S.L., et al				
Decision to grant a European patent pursuant to article 97(2) EPC Following examination of European patent application No. 05917133.5 a European patent with the title and the supporting documents indicated in the communication pursuant to Rule 51(4) EPC dated 05.04.07 is hereby granted in respect of the designated Contracting States.				
Patent No. Date of filing Priority claimed	05917133 07.06.98 19.06.97/ESA 9701075			
Designated Contracting States and Prosecutor(s)	AT BE CH DE DK FI FR GB GR IE IT LI LU NL PT SE Biosensores, S.L. Calle Ausias March, 1 12395 Moncofar/ES AT BE CH DE DK FI FR GB GR IE IT LI LU NL PT SE Dez-Caballero Ansu, Teofilo Calle Conde Salvatierra, 35 46004 Valencia/ES			
This decision will take effect on the date on which the European Patent Bulletin mentions the grant (Art. 97(4) and (5) EPC).				
The mention of the grant will be published in European Patent Bulletin 0746 of 07.11.07.				
Examining Division Stokowski O Maul n V Hilbrand G				
				
Registered letter EPO Form 5006A 07.02 05.10.07			to EPO postal service: 05.10.07	

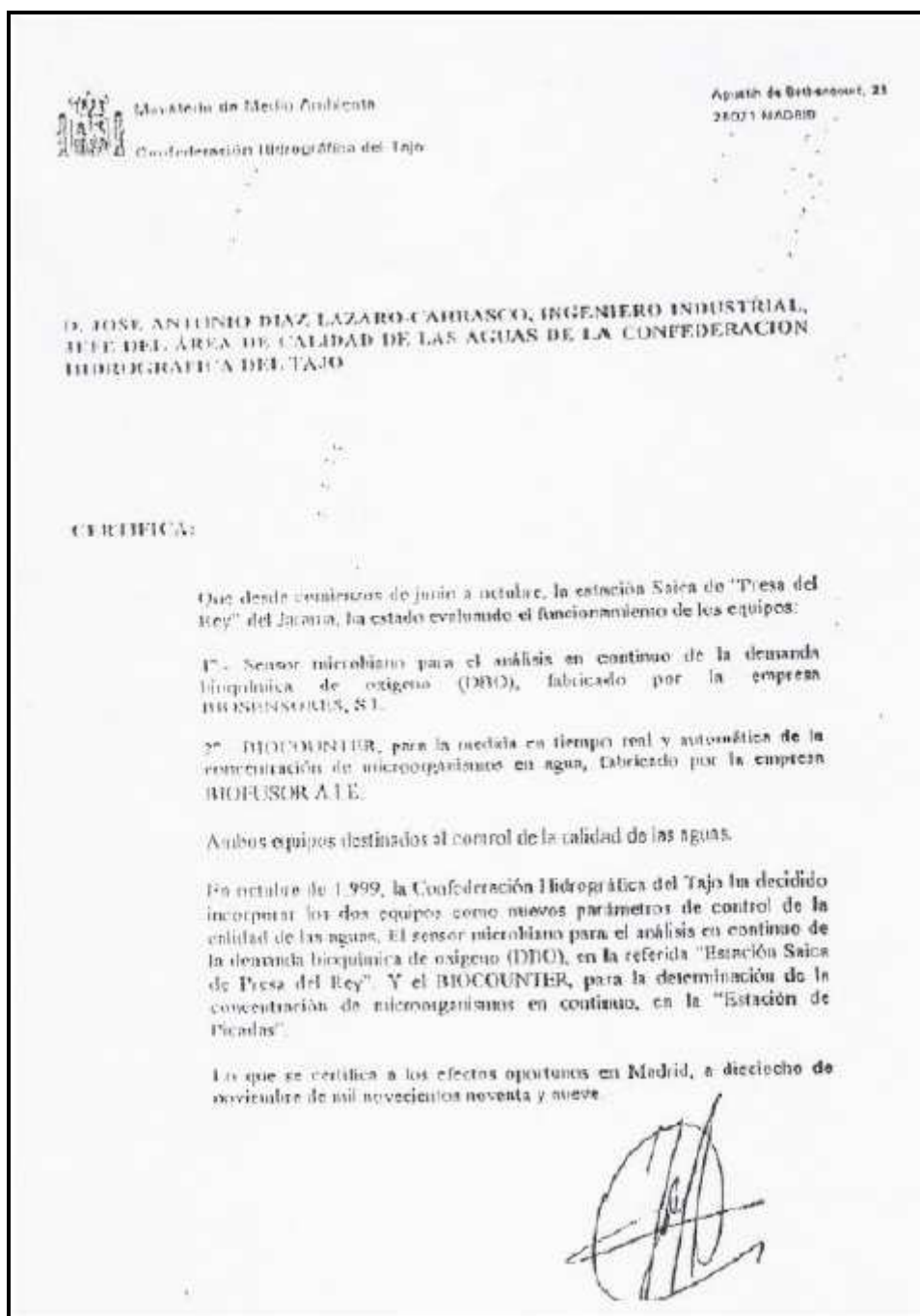


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B. .LETTER OF THE RIVER TAJO BASIN CONFEDERATION.



WATER ON - LINE ANALYSIS EUROPE

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C. LETTER OF THE DEPARTMENT OF ENVIRONMENTAL CHEMISTRY OF THE CSIC.

De: Damià Barceló <dbcqam@cid.csic.es>
Para: <biosensores@ctv.es>
Enviado: jueves, 19 de abril de 2001 12:41
Asunto: carta

A QUIEN CORRESPONDA

Me complace en informar favorablemente de los diferentes desarrollos analíticos para la medida de la calidad de las aguas residuales de la empresa BIOSENSORES. Tmnego que hacer especial referencia del sistema de medida a tiempo real de la Demanda Biológica de Oxígeno (DBO) que esta empresa ha desarrollado y que se utilizo con exito en la Tercera Reunion de Experimentos de Campo de la Union Europea a traves de la Accion Concertada BIOSET en la Depuradora de Aguas Residuales (EDAR) de la llagosta, en Barcelona. Este sistema moderno permite medir la DBO a tiempo real y por tanto actua como sistema preventivo de las descargas industriales en una estacion de depuracion de aguas. De esta manera se preserva el tratamiento secundario de depuracion de las aguas y se consigue un efecto preventivo excelente.

Por todo ello me complace en recomendar a la empresa BIOSENSORES para la puesta a punto y desarrollo de sistemas de vigilancia de la calidad de las aguas a nivel de España

Prof.Dr. D. Barceló
 Profesor de Investigacion CSIC
 Jefe del Departamento de Quimica Ambiental
 IQAB-CSIC
 Barcelona

Dr. Damià Barceló
 Environmental Chemistry
 IQAB-CSIC,
 Jordi Girona, 18
 08034-Barcelona

Tel.: 34- 93 400 61 18
 Fax: 34- 93 204 59 04

<http://www.cid.csic.es/departaments/ambiental>



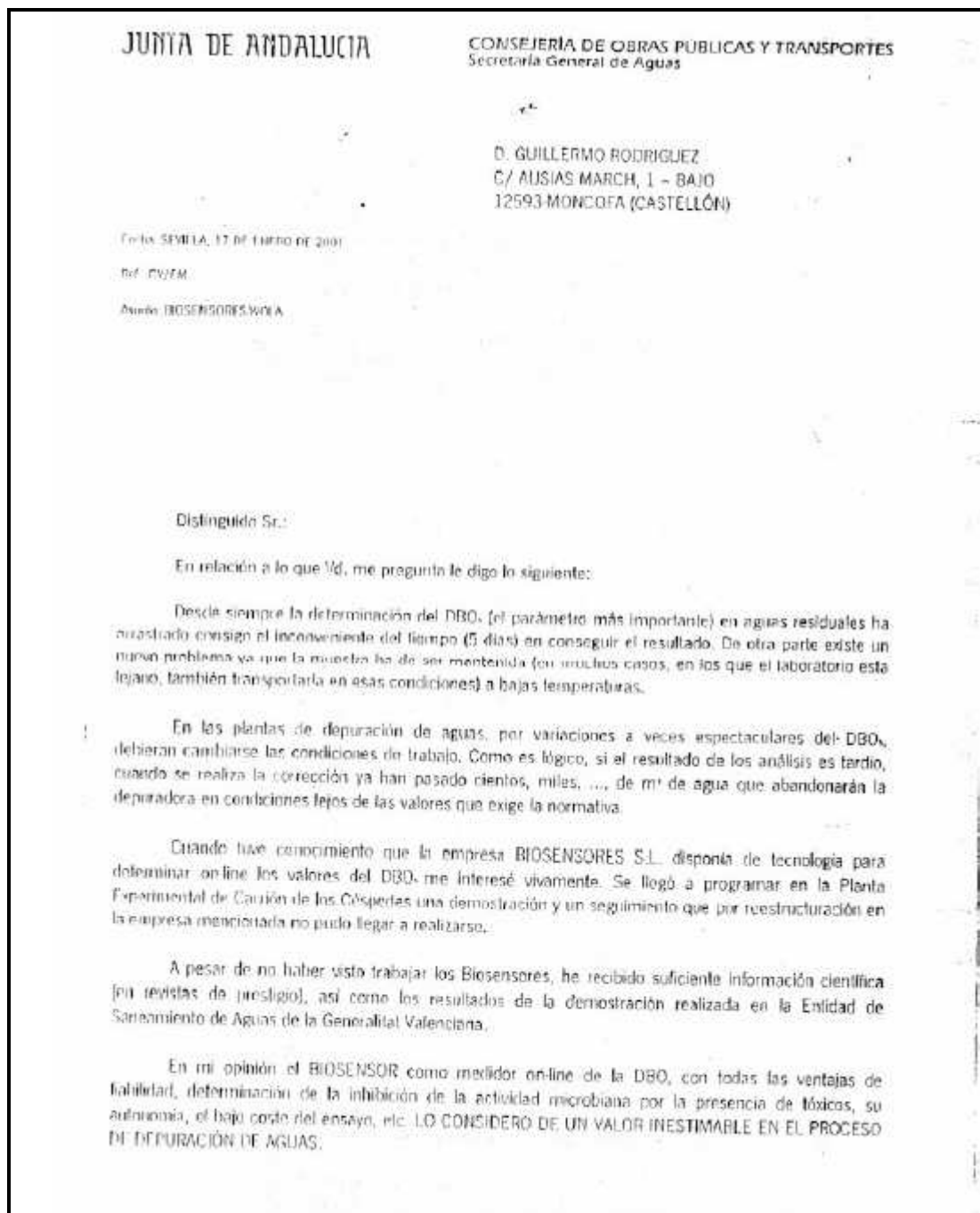
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D. LETTER OF THE AUTONOMOUS GOVERNMENT OF ANDALUCÍA.



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E. LETTER OF THE CENTER OF STUDIES AND EXPERIMENTATION OF PUBLIC WORKS.



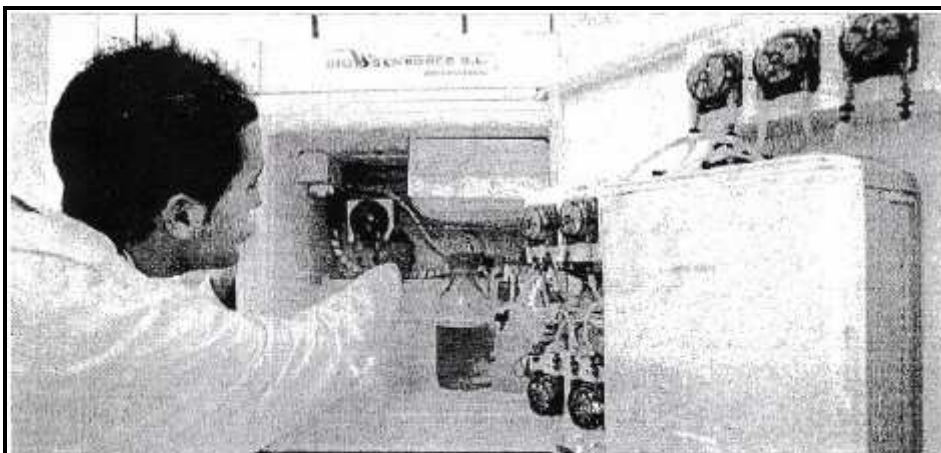
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F. INTERVIEW FOR THE NEWSPAPER "LAS PROVINCIAS".



En el laboratorio de la empresa Biosensores, en la localidad de Moncofa, muestran el prototipo para el análisis de aguas. /R06 11645

Biosensores de Moncofa, única empresa de la Comunitat en la feria biotecnológica de Chicago

El ICEX coordinó la presencia de 12 firmas españolas en la mayor muestra de empresas de biotecnología que se celebra en EE. UU.

La empresa castellanense Biosensores, SL, con sede en Moncofa, es la única representante de la Comunitat Valenciana en la feria de biotecnología BIO 2006, que se celebró hasta ayer en Chicago (EE. UU.). Al evento, la mayor cita de expositores de esta industria en la historia, acudieron otras 11 firmas españolas.

REDACCIÓN ■ CASTELLÓN
La feria BIO 2006, que este año se celebra en Chicago, cuenta en esta edición con la participación de una empresa de Moncofa que trabaja en el campo de la biotecnología. El Instituto Español de Comercio

Exterior (ICEX) invitó a la empresa Biosensores de Moncofa, así como a otras 12 firmas del resto del Estado, a formar parte de esta feria para analizar las posibilidades de esta tecnología en el mercado de EE. UU. De ellas, cinco empresas proceden

de Madrid, dos de Barcelona y una de Sevilla, Guipúzcoa, Vizcaya, Zaragoza y Castellón.

Es la tercera vez consecutiva que el ICEX acude a la feria BIO, aunque en esta ocasión lo hace con un pabellón de 16 expositores (12 firmas y tres instituciones), que integrarán el recinto oficial que compartirá con la Oficina Económica y Comercial de la Embajada de España en Chicago.

BIO 2006 es la mayor cita de expositores de la industria biotecnológica

BIOSENSORES, SL

Actividades. La empresa realiza proyectos de Investigación y Desarrollo (I+D) aplicando entre otras tecnologías las de biosensores. Además también desarrolla instrumentación analítica en aplicación en la monitorización "on line" de molibdeno y productos químicos.

Investigación. La empresa con sede central en la localidad de Moncofa realiza estudios en el área de investigación. Forma parte en equipos de estudio y análisis de la calidad del agua y participa en biosensores de actividad molecular.

gica en la historia, con más de entidades representadas, entre ellas, organizaciones e instituciones. Se estima que, hasta ayer, dieron lugar a 18.000 personas

Presencia de tres institutos. En el pabellón del ICEX participan además tres organismos: Genovisa España, 4 iThimoware i Desenvolupament Empresarial (CIDEM) y Prodesid. Esto certifica una oportunidad para adelantarse, con establecer alianzas en el mundo de EE. UU, el más importante en materia biotecnológica según el ICEX.

"Johnny Depp está simplemente impresionante" *The Observer*
"Una obra maestra... Johnny Depp se ha superado" *TM*



"Impresionante historia de Sexo y Pecado" *Mark Clute*
"Johnny Depp es una maravilla" *Publity Dome*

Mercado Mosen Soroll

Dombosera, próxima remodelación. Camicería-charcutería-polería, en pleno centro Valencia (Bº El Carmen). Junto a la boca del Niño (en construcción). 5 palcos-43 mesas, juntos o dividibles. Se trasporta por no poder alentar. 96 367 26 73



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G. INTERVIEW WITH "PANORAMA ACUÍCOLA".

Biosensores

un nuevo método de medición de DBO en tiempo real

El equipo permite un funcionamiento totalmente autónomo. Posibilidad de captura de datos en remoto. Frecuencia de análisis programable, habitualmente entre 12 y 15 análisis/día. Bajo costo por determinación. Buena correlación con el análisis clásico de laboratorio (DBO5).

Biosensores es una empresa pionera en Europa en el campo de la emergente tecnología de bio-medición, que comenzó a principios de la década de los 90. Después de una etapa inicial en el Centro Europeo de Empresa de Innovación, en el Parque Tecnológico de Valencia, España, la empresa se establece a partir de agosto de 2004 en Moncofar (Castellón), donde tiene en la actualidad sus instalaciones.

En Biosensores se realizan proyectos de investigación y desarrollo y se materializan en instrumentación analítica de aplicación en la monitorización, "en tiempo real", de moléculas y productos químicos de interés. Tanto en la tecnología, el conocimiento y la instrumentación que la empresa desarrolla aportan los avances y mejoras que caracterizan a la tecnología que comercializa Biosensores.

Productos

Uno de los equipos Biosensores que está entrando al mercado con mucho interés es el equipo automatizado para la monitorización "en tiempo real", de la materia orgánica biodegradable en los estanques de cultivos acuícolas.

El equipo permite un funcionamiento totalmente autónomo. Posibilidad de captura de datos en remoto. En caso de alarma por alcanzar valores límite, la muestra es almacenada en las condiciones necesarias para un posterior análisis en laboratorio. Frecuencia de análisis programable, habitualmente entre 12 y 15 análisis/día. Bajo costo por determinación. Buena correlación con el análisis clásico de laboratorio (DBO5).

Más de 210 mil horas de funcionamiento ininterrumpido avalan la robustez de estos equipos evaluados por distintos organismos de carácter autonómico, nacional y europeo.

Campos de aplicación:

- Tanques de cultivos acuícolas intensivos.
- Estanques de cultivos acuícolas intensivos.
- Laboratorios (hatcheries) de producción intensiva.
- Sistemas de cultivos acuícolas de recirculación de agua.
- Cualquier sistema acuícola donde la medición de oxígeno sea un factor determinante para la viabilidad de la producción.
- Laboratorios y Centros de investigación.

Características del equipo

Flujo de medición: respirometro
Sensor: sonda de oxígeno disuelto con sistema de autolavado.
Tiempo de respuesta del sensor: 3 - 7 segundos.
Rango de medida: 3 - 200.000 mg/L.
Coef. de variación de la sonda: < 3%.
Temp. de medida: 25 °C.
Temp. superior de trabajo: 50 °C.
Calibración: doble, en cada ciclo de análisis.
Consumo de disolvente: nutriente: 32 microlitros.
Consumo de disolvente: de lavado: 40 microlitros.
Tensión: 220.
V consumo eléctrico: 180 W.

Para mayor información contacte con:
Iñaki Diez-Cabelejo Arriola
a las teléfonos +34 96 458 01 24 o a biosensores@biosensores.com
P.O. Ausias March 1
12593 Moncofar, Castellón, España.

la producción acuícola día y noche es posible

Biosensor microbiano para monitorizar la calidad y toxicidad del agua.

Algunos Servicios:

- Multiparamétrica
- Software a medida
- Teledetección
- Alarmas



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María Montolán 2850 Col. del Valle
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Tel: +52 55 5148 1425-5-1424
http://aliance.com.mx



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H. AWARD – “GENOMA ESPAÑA”

The business plan of WOLA Europe was the winner of the award conferred by “Genoma España”.

Genoma España

A la atención de D. Federico Vallmitjana

Madrid, a 6 de noviembre de 2006

Estimado señor Vallmitjana,

Enhorabuena. Nos ponemos en contacto con usted para notificarle que su proyecto de empresa WOLA, ha sido seleccionado como finalista de BioANCES Actúa y que por ello en breve plazo, recibirá la transferencia de la ayuda de 30.000 €

La ayuda otorgada tiene como objetivo facilitar la puesta en marcha del proyecto presentado por lo que debe ser invertida en el arranque del mismo. WOLA podrá invertir la ayuda en aquellas líneas para la mejora de su proyecto que estime oportunas y deberá remitir a Genoma España la documentación que se le solicite para justificar el destino de dichas inversiones en un plazo de seis meses.

Para poder proceder al pago de la ayuda, es necesario que muestre su conformidad a lo expuesto en esta carta y nos remita una copia de la misma firmada.

Nos gustaría que la ayuda concedida no fuera el fin de nuestras relaciones sino que nos mantuviera informados de la evolución de su proyecto empresarial de una manera periódica, pudiendo llegar a colaborar más estrechamente en un futuro. Si WOLA se incorporase a la cartera tecnológica de Genoma España, recibiría el asesoramiento y la financiación necesaria para la comercialización y la búsqueda de alianzas que permitirían desarrollar completamente el proyecto de su empresa.

El pago de la ayuda se realizará mediante transferencia bancaria por lo que rogamos, nos facilite los datos bancarios de la cuenta de la empresa si ya está constituida o en su defecto, los suyos propios.

Quedamos a su disposición para cualquier consulta o aclaración y deseamos que su proyecto empresarial tenga mucho éxito en el mercado.

Reiteramos nuestra felicitación y aprovechamos la ocasión para enviarle un afectuoso saludo

Conforme



Fernando Garcés Toledano
Director Gerente de Genoma España



Federico Vallmitjana

Edificio Eurobuilding
Ortega, 66 - 2ª planta
28016 Madrid, España
www.gen-es.org

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I. PROMOTE-ETV EVALUATION OF THE MB-BOD.

		BOD Biosensores – Verification report	page 3 of 17
ENVIRONMENTAL TECHNOLOGY VERIFICATION (ETV) I. FACT SHEET			
TECHNOLOGY TYPE:	Autonomous, wire less, and at-site BOD5 system		
APPLICATION:	Analysis of water and waste water		
TECHNOLOGY NAME:	BOD Biosensor		
COMPANY ADDRESS:	Av. Ausias March nº1 CP. 12593 Moncofa, Castellón España		
PHONE:	+34 964 579 313		
E-MAIL:	BIOSENSORES@biosensores.com		
Performance claims tested within the EU FP 6 funded RTD project PROMOTE 519074	PROMOTE Project coordinator: DECHEMA c.V., Frankfurt/Main, Germany		
WHAT IS ETV			
<p>The Environmental Technology Verification (ETV) system currently prepared by the European Commission as part of the European Technologies Action Plan, is designed to support the environmental industry by providing credible and independent verification of technology performance claims. The objective of ETV is to enhance the confidence of investors and potential users in promising innovative eco-technologies.</p> <p>PROMOTE is one of 4 research projects funded under FP 6 which are designed to explore the characteristic features of an ETV on different technology sectors. The overall aim of the PROMOTE project is to set up a complete efficiency control and performance verification system for soil-groundwater protection and rehabilitation technologies. The system is based on a stepwise concept including generic testing at reference site and field site scale.</p> <p>The given technology fact sheet, as an extended summary of the verification report, has been elaborated within PROMOTE, following the procedures described by PROMOTE and further laid down in the CEN Workshop Agreement CEN/WS 32 <i>Environmental technology verification – Soil and groundwater site characterization, monitoring and remediation technologies</i> as a first standardisation document in this field.</p>			
<p>DISCLAIMER: Mentioning of trade names or commercial products does not constitute endorsement or recommendation by the European Commission or the PROMOTE consortium for use. Neither the authors nor the parties involved in the execution of the project PROMOTE can be held responsible or are liable for any kind of damage resulting out of the use of information given by the fact sheet. The European Commission is neither responsible nor liable for any written context in this fact sheet.</p>			

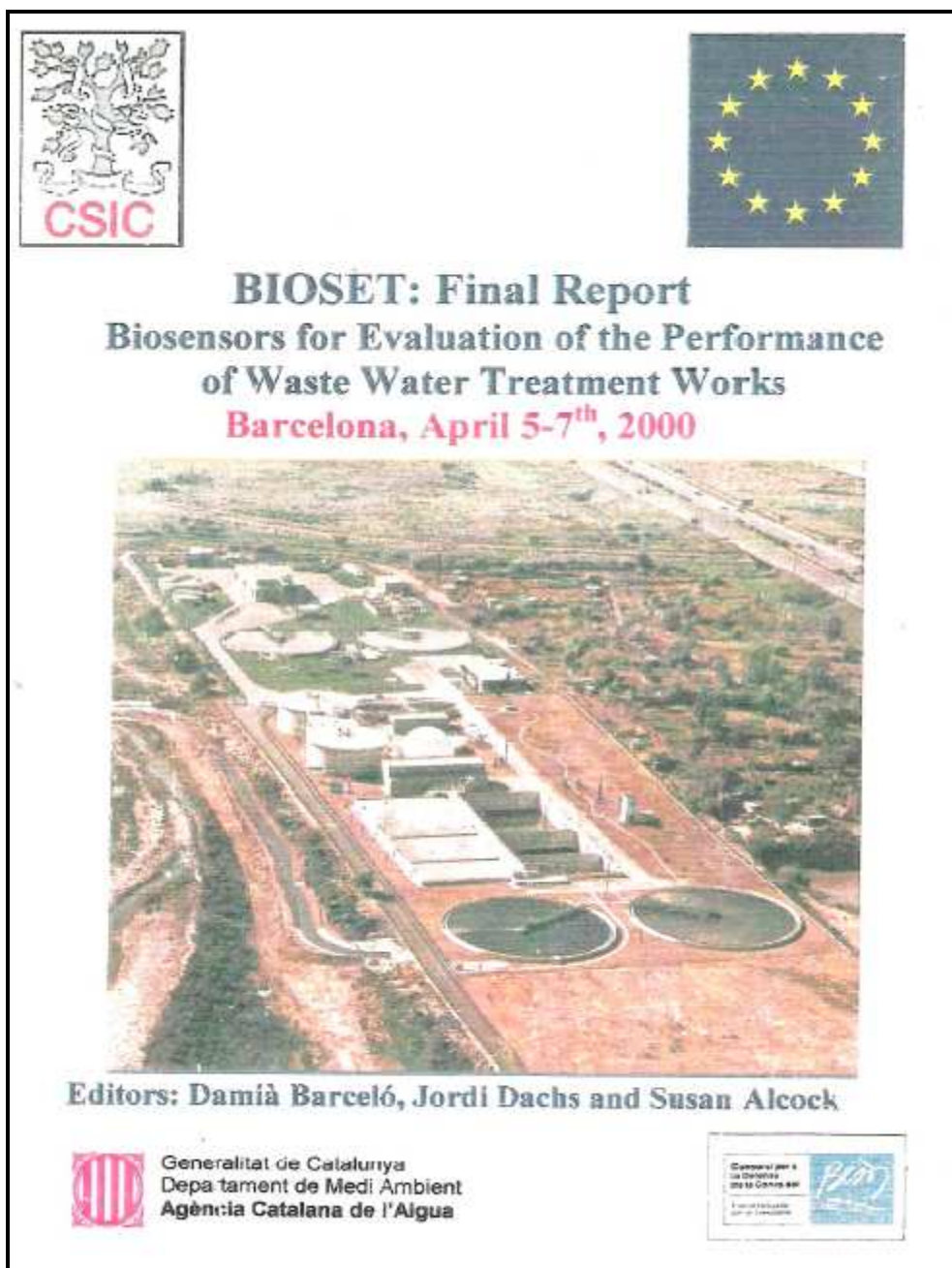


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J. BIOSET



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Sils

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www.silsoffice.cranfield.ac.uk

20 April 2001

Dear Dr. Diez-Caballero

The BIOSET Technical Meeting on Biosensors for Evaluation of the Performance of Waste Water Treatment Works took place in Barcelona, from 5-7 April 2000. It had the specific purpose of achieving a step forward in the use and implementation of biosensors under field conditions by evaluating the performance of two different wastewater treatment works (WWTW). There were 9 biosensor instruments, 40 scientists and 15 end-users, including WWTW operators and public and private water agencies. The Third Technical meeting was jointly organised by CSIC and the BIOSET EU Concerted Action with the technical support and facilities from the Agencia Catalana de l'Aigua and the Consorci per a la defensa del Besos.

An on-line technique for the determination of BOD using equipment transported using a mobile laboratory van was presented by Biosensores. This on-line biosensor device was installed in la Llagosta WWTW and after the Technical Meeting, was installed in two other WWTW for a period of one month. A comparison between the BOD result using the on-line biosensor and the commonly used BOD5 was also carried out, showing good correlation between both measurements. This biosensor is particularly useful for measuring early warning situations since it measures continuously day and night, and it can detect increases in the BOD which can be an alarm to modify the WWTW operation and for preventing the biological treatment. An advantage of the biosensors evaluated, like BOD, is that the measurements can be obtained very rapidly in a maximum of 20 minutes, so the information is available immediately. This is certainly a benefit over conventional methods that require several days eg the BOD-5 method. The implementation of the BOD biosensor device can contribute to solving the real-world problem of routine control of BOD in WWTW.

Yours sincerely,

Susan Alcock

Dr Susan Alcock
Principal Research Officer and BIOSET EU Concerted Action Co-ordinator



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K. LIST OF PROJECTS

1. Penetrating electrodes and hearing: A multitechnical approach for designing a brainstem prosthesis. (2000).
2. Development, optimization and application of genetically optimized microorganism and biosensors for monitoring and biorremediation of polychlorinated byphenyls. (2000).
3. Boron water Remediation by specific microorganisms supported on continuous renewable biofilters (BIOBORON)
4. Ultra-sensitive multianalyte immunoassay based on DNA-sensor. (1994-1998).
5. On-line toxicity monitoring by renewable microorganisms. (1998).
6. Reclamation of industrial sites being contaminated by metal treatment and tanning industries (RISC). (1991-1994).
7. In –situ remediation of industrial sludges (IRIS). (1991-1994).
8. Network of excellence in multifunctional microsystems (NEXUS)
9. Control and monitoring of biofilms for water management with laser assisted measurement techniques (2000).
10. Activated sludge micro flora in municipal wastewater treatment plants (micro flora atlas) (1999).
11. Development of an immune-biosensor with optoelectrics transducers, integrate in an injection flow analytical system for the quantification of tumoral marker in biological fluids.
12. Continuous analyzer of ammonium for the control of water quality, based on the biosensor technology.
13. Development of an immunobiosensor for the detection of pesticides by monoclonal antibodies.
14. Development of both analytical and control system for the detection on line of fungi producers of rotten citric fruit post-harvesting.
15. Development of a prototype of an integrated immunobiosensor in an analytical system by flow injection for the quantification of pesticides in fruits.

16. Immunobiosensor for the quantification of gastric and breast cancer.
17. Integrated ISFET system-immunobiosensor as magnetic renewable surface, as universal sensor for molecular analysis.
18. Automated and continuous determination of biochemical oxygen demand and water toxicity.
19. Toxic factors in the citric cultures.
20. Development of a biosensor for detection of adulteration of milk by mixing products from different animal species.
21. Development of a biosensor for detection aflatoxines in products derived from milk.
22. Automated biosensor for the quantification of chemical toxicity in liquid media.

L. WOLA EUROPE, EXECUTIVE SUMMARY FIVE YEAR BUSINESS PLAN

In the year 1992, the company Biosensores S.L. was founded to develop technology based on biosensors. Now, more than 15 years later, we have developed instrumentation based on the technology of microbial biosensor. WOLA Europe SL is a spin-off of Biosensores S.L. which has the purpose to produce, sell and commercialize this technology and instruments in specific markets throughout Europe.

Our aim is that WOLA Europe SL will become a reference company in the market of water quality, water control and on-line monitoring in the next five years.

The technology which is being developed and commercialized for European markets has the potential to solve important analytical problems in the use and reuse of water in different markets. The technology that WOLA Europe SL will commercialize is described below.

INSTRUMENTATION THAT HAS ALREADY BEEN DEVELOPED AND ARE READY FOR PRODUCTION

MB-BOD: Microbial biosensor for measuring contamination of water

The MB-BOD instrument was designed for the automatic monitoring of the biochemical oxygen demand (BOD) contamination in water. BOD is a measure of any kind of biodegradable matter (mostly organic) in the water and is a good basic indicator of the water quality. Decisions on the use or reuse of water can be based on this fundamental water quality measure.

International standards for the measurement of BOD take five days (called BOD-5). The analysis is manual, requires special technical knowledge and has to be carried out in specially equipped laboratory. On the other hand our MB-BOD instrument is automatic, the measurements take only 30 minutes and they are carried out where the water is being collected (“in situ”).

PolyTox-Res: Microbial biosensor for measuring toxicity of water

The PolyTox-Res instrument measures the integral toxicity of water that affects the metabolic activity of the microorganisms used in the sensor. The output value is a good indicator of the overall toxicity level of the water being measured. The integral toxicity measurements are automatic and carried out “in situ”.

International standards for measuring toxicity of water requires especially equipped laboratory, technical knowledge and the measurements take five hours. The PolyTox-Res on the other hand is automatic and results can be obtained 90 minutes after the sample has been collected.

BioCounter: Microbial biosensor for measuring the quantity of microorganisms in liquids

The concentration of aerobic microorganisms in any kind of water based liquid can be determined with the BioCounter. On the basis of microbial concentration, the user is able to make decisions of water use in “real time”. As with other instruments WOLA will be trading, the measurements are automatic and are carried out “in situ”.

International standard for measuring microbial concentration rely on the cultivation of the microorganisms in a specially equipped laboratory, requiring technical knowledge and take at least 24 hours. With the BioCounter, measurements can be obtained in only 15 minutes.

NATIONAL RECOGNITION

A price awarded in 2006 to Biosensores S.L. by Genoma España (National Board for R&D for genomics and proteomics) for the excellent potential of our technology to reach the market of the water quality control.

The business plan of WOLA Europe SL was awarded the first price for the best business plan for a start-up company by the Valencian Government (CEEI) in 2008.

INTERNATIONAL RECOGNITION

Already in the year 2000 the CONCERTED ACTION of EU (BIOSET, Biosensor for the Evaluation of Performance of Waste Water Treatment Works) said about our technology, which then was only in a prototype stage that “The implementation of the BOD biosensor device [developed by Biosensores S.L.] can contribute to solving the real-world problem of routine control of BOD in WWTW.



WATER ON - LINE ANALYSIS EUROPE

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The basic technology behind the MB-BOD instrument, which is the same for the other two equipments, has recently been approved by the European patent office in Munich.

Also, in the year 2008, the MB-BOD instrument got a very positive review at the newly established PROMOTE-ETV verification system. PROMOTE-ETV (Environmental Technology Verification) is a new EU body – an independent assessment of the performance of a specific environmental product or technology – provides quantitative information on the performance of innovative technologies.

EUROPEAN MARKET AND FINANCIAL ASPECTS

Several different markets can be identified in the area of water quality in Europe. However, WOLA Europe SL will originally concentrate on subsequent markets:

- Waste Water Treatment Plants.
- Fresh Water Treatment Plants for human consumption.
- Polluting industries where companies require continuous control of spills.
- Fish and shrimp farms that can incorporate on-line monitoring of water quality.
- Households in Europe that need to monitor the Waste Water Treatment of septic tanks.
- On-line monitoring of water quality in the natural /environmental natural resources.

We have estimated the size of those markets and put forward a plan for a pessimistic penetration of those in our business plan (**Table 1**).

Table 1. European markets, size and pessimistic accumulative penetration for the five year planning period for different instrumentation commercialized by WOLA Europe SL

Eu market	Size	<i>MB-BOD</i>		<i>PolyTox-Res</i>		<i>BioCounter</i>		TOTAL Number
		Penetration	Number	Penetration	Number	Penetration	Number	
Waste Water	40,000	3.50%	1,400	3.00%	1,200	1.00%	400	3,000
Fresh Water	15,000	1.00%	150	4.00%	600	4.00%	600	1,350
Polluting Ind.	100,000	0.40%	400	0.60%	600	0.24%	240	1,240
Aquaculture	1,500	2.40%	36	2.40%	36	1.80%	27	99
Septic Tanks	10 M	0.05%	5,000					5,000
Env. Monitor.	4,500	0.44%	20	0.89%	40	0.44%	20	80
TOTAL	161,000		7,006		2,476		1,287	10,769

Number of equipments sold over the five year business plan of WOLA is shown in Figure 1. Price of the equipment ranges from 4,000 (septic tanks) to 34,000 (PolyTox-Res with multiparameters) and forecasted profit before taxes can be viewed in Figure 1.

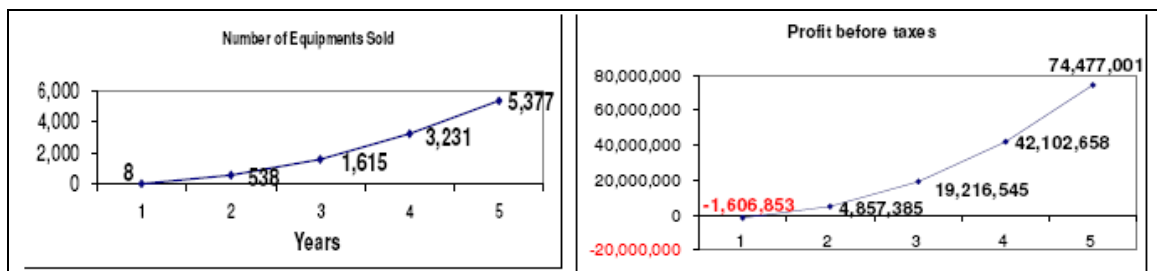


Figure 1. Number of equipments sold (left) and profit before taxes (right) over the five years period planned.

We hope that this initial information about WOLA Europe SL is of interest to you. If that is the case, we can follow up with more detailed information.

Yours sincerely,

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