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AUTOMATED MICROBIAL BIOSENSORS FOR THE CONTINUOUS MONITORING OF: ORGANIC MATTER CONCENTRATION & TOTAL TOXICITY IN WATER SAMPLES. EXAMPLES OF REAL WORLD APPLICATIONS.

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CONCLUSIONS

A new automated technique for the real-time detection of Biochemical Oxygen Demand (BOD) and Total Toxicity has been developed by **BIOSENSORES, S. L** and has been objectively assessed by the scientific community **(EU Concerted Action - BIOSET).**

The BOD Microbial Biosensor (BOD) uses the respirometric activity of a stable population of sensor microorganisms to measure the quantity of organic matter in a water sample.

The double calibration of the device during each analytical cycle, with a standard solution of known BOD5, has been shown to facilitate the detection and quantification of toxic effects using changes in the rate of respiration of a microorganism population.

'Online'measures of BOD, using the **BOD-Toxicity Microbial Biosensor (MB-DBO)**, correlated positively with standard BOD5 assays (r=0.98).

The device has been shown to detect low BOD concentrations, for what it is a useful tool for monitoring soil-ground waters BOD and detects toxicity, not only in the laboratory but also in the outdoors.

The BOD prototype has been used to monitor the BOD and toxicity in WasteWater Treatment Plants (WWTP) and river waters. Its long-term viability has been demonstrated by 3 years of uninterrupted work and "in situ" real time monitoring.

Field experiments carried out on the Rio Jarama are presented and show a high level of utility for this device.

MB-DBO has been demonstrated to be a **viable and efficient online monitor of BOD and Total Toxicity** and is a proven **rapid, accurate and low cost (1 € /analysis for BOD and Total Toxicity)** automated alternative to conventional methods for monitoring organic matter in water.

The **BOD-Toxicity Microbial Biosensor** is a good example of the transition of a biosensor technology, from laboratory development, to a robust device with 'real world' applications.

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INTRODUCTION

The **BOD - Toxicity Microbial Biosensor (BOD-TOX)** system ***(Figure 1)*** allows the continuous, rapid, reliable and simultaneous assessment of Total Toxicity and Biochemical Oxygen Demand (BOD) in soil-ground water, drinking water supplies and industrial and waste water effluents.

Figure 1
The BOD – Toxicity Microbial Biosensor: Previous prototype on the right and currently final product on the left.

BOD-TOX has been objectively evaluated and acknowledged as fulfilling the desired criteria for an **'ideal biosensor'** as drawn up by academic experts, government officials, the waste water treatment industry and the **EU Concerted Action – BIOSET 2000**. Furthermore, this device is currently being introduced by the national government in Spain to assess the efficiency of waste water treatment processes and to ensure that water service providers are meeting their contractual obligations, thus serving the paramount interest of public safety.

MEASUREMENT PRINCIPLE

BOD-TOX uses a novel system that provides a constant supply of a standard microorganism suspension. This stock supply of microorganism is characteristically stable in composition, activity and concentration.

Using the principle of respirometry, **BOD-TOX** measures the amount of oxygen required by a constant volume of the standard microbial suspension to oxidize the organic material present within a water sample. The respirogram, completed within 30 minutes, also provides information on the composition of the biodegradable material i.e. faster metabolism of simple sugars than complex organic compounds.

The decrease in dissolved oxygen as a consequence of bacterial respiration is used to monitor the concentration of organic matter in the water sample (BOD). Inhibition in the microbial respiratory rate for a standard BOD sample is used to detect and quantify the Total Toxicity of an unknown sample. Each measurement cycle uses a renewable aliquot of sensor microorganism suspension.

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DETECTION OF TOXICITY

Figure 6 shows the effect of a toxic water sample (**T**) on the subsequent respirometric signal obtained with the second BOD reference standard. Toxicity is quantified using inhibition (**I**) in the rate of microbial metabolism of the reference standard of BOD (**S2**) following exposure to an unknown water sample.

The BOD and toxicity are simultaneously measured with a high degree of stability without (**cycles 1-6**) or with (**cycles 7-12**) the presence of a toxin ***(Figure 7).***

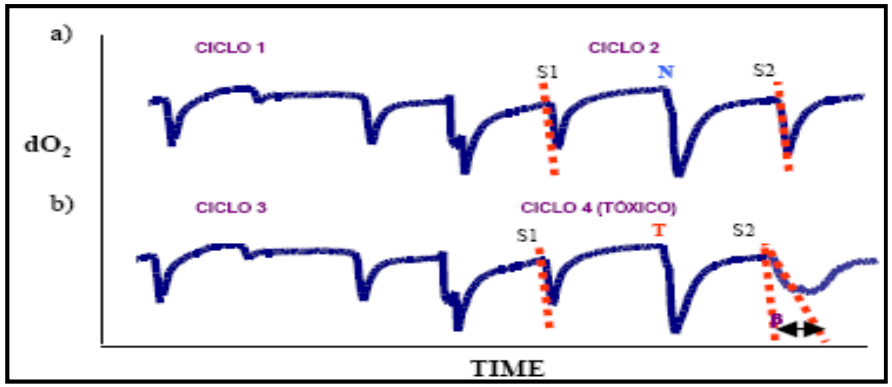

Figure 6.
A comparison of the rate of microbial respiration of the BOD standard following **a)** nontoxic water **(N)** sample and **S b)** toxic water sample **(T).**

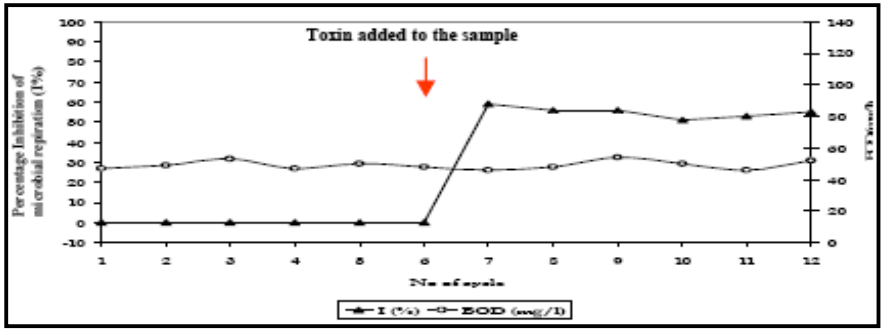

Figure 7.
The detection of sample toxicity using the inhibition of microbial respiratory rates and simultaneous measurement of BOD.

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MEASUREMENT CYCLE

The real-time PC software output for each measurement cycle is shown in **Figure 2**. Two standard samples (of known BOD) are used in each measurement cycle.

The first standard (**S1**) is used to calibrate the system while the second standard (**S2 - identical to the first**) allows the assessment of any toxicity in the intervening 'unknown' sample (**U**) of interest (e.g. wastewater effluent).

The reaction unit undergoes a reconditioning stage between every measurement in order to guarantee equivalent conditions for the measurement of each unknown sample.

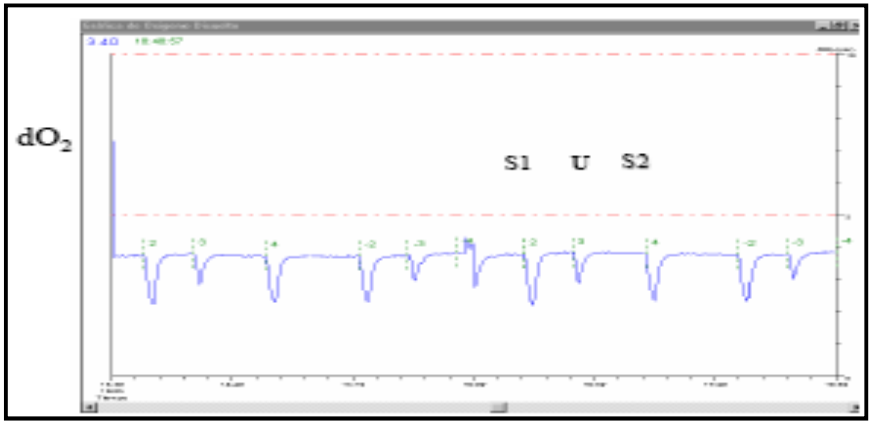

Figure 2.
The PC interface displaying changes in dissolved oxygen levels (over time) as a consequence of microbial respiration during the measurement cycle.

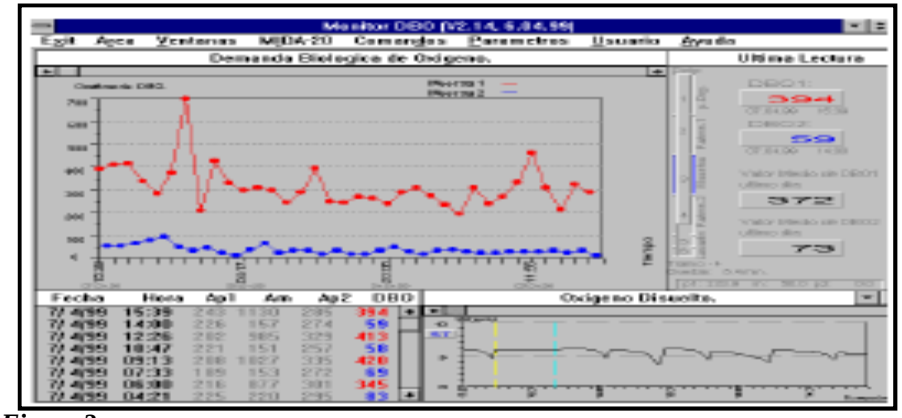

Figure 3.
The display screen of the automated **BOD-Microbiosensor** monitoring the on-line BOD in water samples at an inlet (**red**) and outlet (**blue**) of a waste water treatment plant.

The **BOD-TOX** system then calculates the BOD for a water sample using the correlation of the area of the respirogram for this unknown sample with that produced by a reference standard of BOD5 ***(Figure 3).***

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COMPARISSON WITH BOD$_5$

BOD measurements made at various Waste Water Treatment Plants (WWTP) in Spain using the **BOD-Toxicity Microbial Biosensor** have been assessed objectively **(BIOSET, concerted action meeting, Paris, 2000**).

The measurements of **BOD-TOX** showed a good correlation (r=0.98) with BOD5 (***Figure 4***) and were obtained within **30 minutes rather than 5days** using this traditional BOD5 measurement technique. ***Figure 5*** illustrates the stability of the **BOD-TOX** microbial population relative to BOD5 techniques.

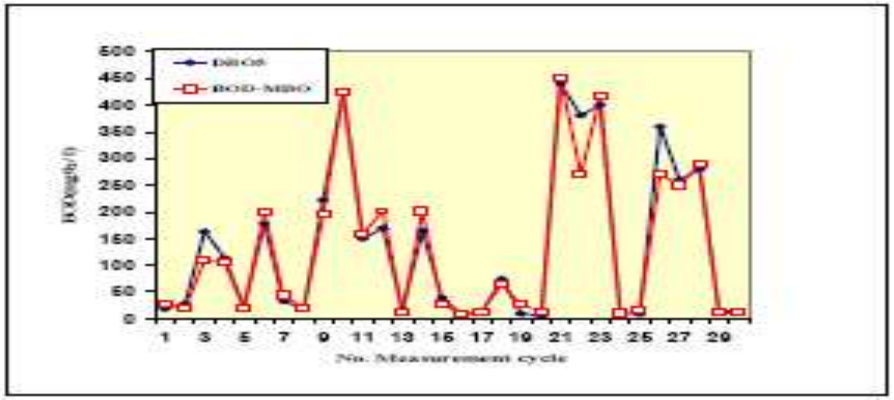

Figure 4.
A comparison of BOD5 and **BOD-TOX** recorded Biological Oxygen Demand (O2 mg/l) for identical waste water samples.

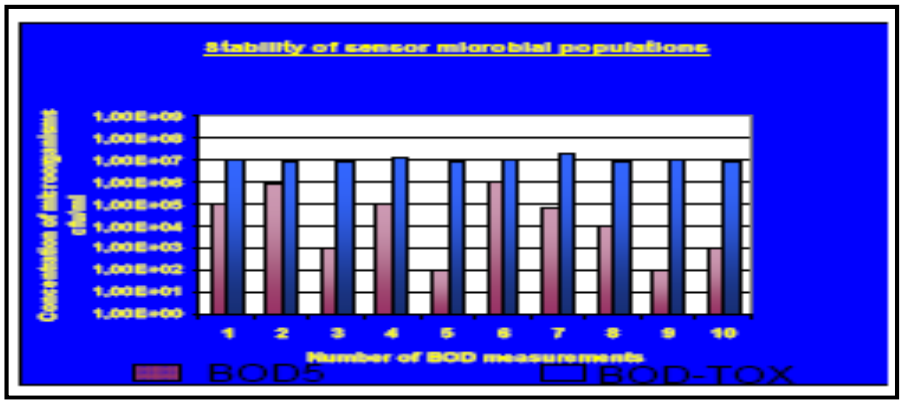

Figure 5.
A comparison of microbial population stability in **BOD-TOX (blue)** and **BOD5 (red)** methodologies.
It is important to note the large variation in microbial number used in the BOD5 in contrast to the stable microbial population continuously made available from **BOD-TOX** stock microorganism.

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FIELD TESTING AND INDUSTRIAL UTILITY

The **BOD-Toxicity Microbial** sensor has been successfully employed in monitoring the water quality of rivers and Waste Water Treatment Plants in Spain.

Figure 8 illustrates how the Biochemical Oxygen Demand (***a***) related to the changes in dissolved oxygen levels due to microbial respiration (***figures b and c*)** during the real-time monitoring of the river Jarama, near Madrid. The double calibration in every cycle ensures that the value of the BOD obtained from the water sample is real and not an artifact.

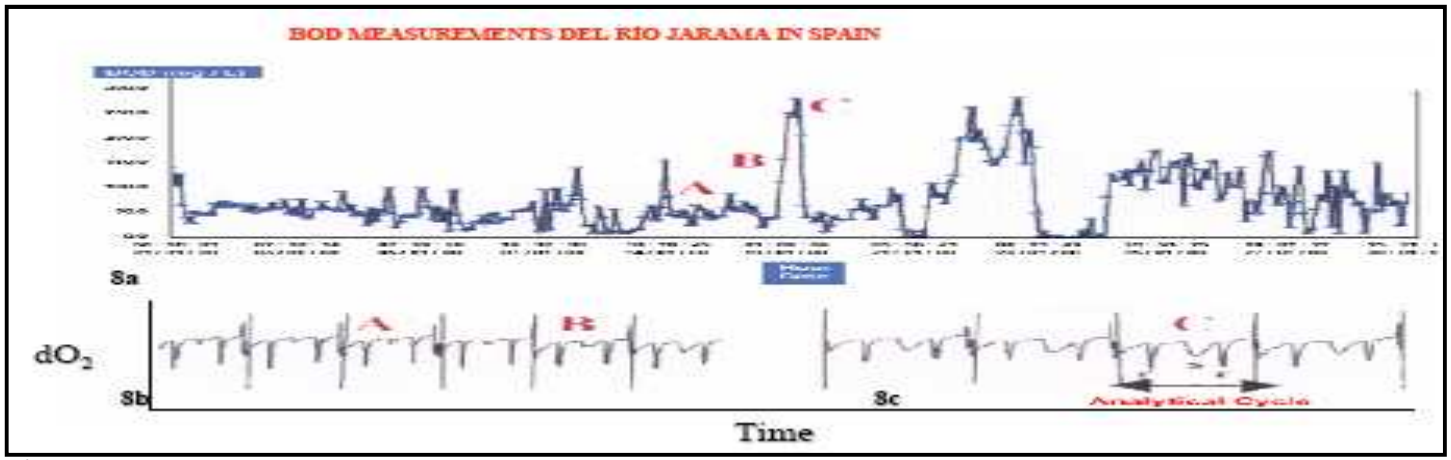

Figure 8.
The online detection of BOD and identification of organic pollution events using the **BOD-Toxicity Microbial Biosensor**.
The real time quantification of BOD is illustrated (**figure a**) in relation to dissolved oxygen levels **(figures b and c).**

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ADVANTAGES OF THE BOD-TOXICITY MICROBIAL BIOSENSOR

BOD-TOX has been independently **PROVEN** to fulfill all the criteria necessary for an accurate, efficient and automated biomonitor:

- ROBUST - Double self-calibration and robust operational design.
- SENSITIVE TO A WIDE RANGE OF BOD VALUES AND VARIETY OF POLLUTANTS
- RELIABLE AUTOMATED FUNCTION
- EASY ANALYSIS OF SAMPLES AND SUBSEQUENT DATA OUTPUT
- SELF-CALIBRATION
- ACCURATE REAL TIME INFORMATION ON WATER QUALITY
- PROVEN INDUSTRIAL UTILITY
- LOW COST ANALYSIS – 1 € per analysis of both BOD and Total Toxicity.
- EASY HANDLING – Simple renewal of consumable liquids.

APPLICATIONS FOR BIOSENSORES, S. L. TECHNOLOGIES

The **BOD-Toxicity Microbial Biosensor** is well suited for incorporation into Biological Early Warning Systems (BEWS).

- SOIL-GROUND WATER
- WWTP & DRINKING WATER
- FOOD AND BEVERAGES INDUSTRIES
- AGRICULTURE AND AQUACULTURE
- LOCAL GOVERNMENT
- LEGISLATIVE BODIES
- BATHING WATERS
- BIOTERRORISM ACTIONS

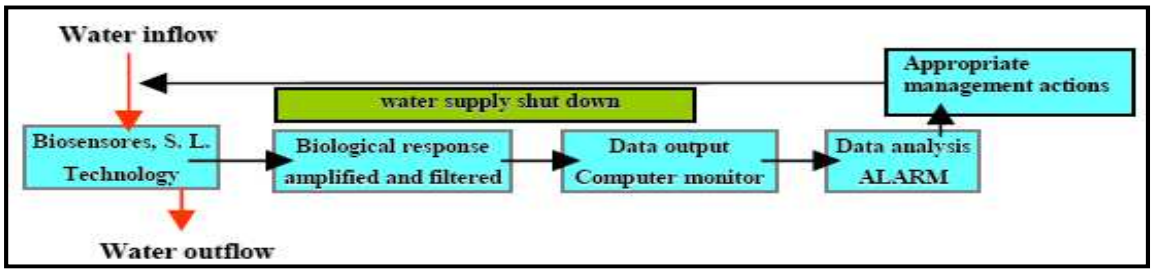

Figure 9.
The general components of a Biological Early Warning System used to detect impending environmental disturbance and breaches of acceptable water quality criteria.

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