Saccharomyces CEREVISIAE, from Wine to **Electrochemical Sen**

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The GIEM has designed an electrochemical sensor to measure the yeast that has given us wine, beer and bread. It has also helped us in the design of drugs and other applications. Although it may seem simple, the sensor is a technological development that opens doors for the study of the behavior of *Saccharomyces cerevisiae* using new methodologies.



Yeast cells arranged on oxidized multi-walled carbon nanotubes after modification of the printed carbon electrode. Photo: courtesy of the project.

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n difficult days when a tiny creature, imperceptible to the naked eye, has sown terror and chaos throughout the planet and has forced us to rethink our daily lives, it is worth highlighting the work of those microorganisms that, on the contrary, have made our existence easier.

Within this microscopic universe, one yeast stands out: *Saccharomy-ces cerevisiae*. It could be considered the queen among the more than 1,500 species of yeast (of which approximately 80% have biotechnological applications) since it is known precisely for its wide use in different bioprocesses and its close relationship with humans. Since ancient times, we have been using this oval microorganism in the preparation of delicious elixirs such as wine and beer, exquisite foods such as bread, or in more complex and sophisticated applications such as the production of proteins and precursors for the manufacture of medicines.



Photo: cortesía del proyecto.

Such has been the importance of *Saccharomyces cerevisiae* that even some anthropologists have come to consider the production of alcoholic beverages as the reason why primitive men decided to settle down and become farmers. They imagine that in about 6000 BC, curious gatherers tasted the contents of harvest containers where grapes fermented spontaneously. From then on, they preferred to consume the grapes transformed into that delicious elixir and enjoy its pleasant effects. Thus, fermentation prevailed and developed through different generations and cultures. During the period 4000-2000 BC, the Egyptians discovered how to make bread with yeast. Although at that time it was not clear how fermentations took place, it is now known that *Saccharomyces cerevisiae* is responsible for these biochemical processes.

This beneficial microorganism belongs to the kingdom *Fungi* and the family Ascomycota. It has an ellipsoid shape, and its size ranges between 5-10 millimeters. It is in constant interaction with us, and we can find it in different environments (in soil, trees, plants and fruits). It has also been, by far, the most studied eukaryote, so it has served to understand the biology of this type of cell.

Saccharomyces cerevisiae is of great biotechnological interest since it not only facilitates fermentation for food preservation but is also key to the development of different bioprocesses, which facilitates the production of high-value-added products such as proteins, amino acids, antibiotics, vaccines, cells, food additives, biopesticides and biofuels. S. cerevisiae is very useful for recognizing the mechanisms of certain diseases and for phytosanitary formulations, bioremediation and biocontrol. It is also used in the production of enzymes and different chemical substances. In addition, it has favored important advances in different fields such as cell biology, genetic engineering and biochemistry.

Given the number of applications that this yeast has, it is important to know aspects of its behavior during bioprocesses to guarantee their development and performance. This implies designing techniques that enable the control and monitoring of the yeast. Although there are many methodologies for these purposes, it is precisely at this point that *Saccharomyces cerevisiae* moves from barrels to electrochemical sensors.

The Challenge of Measuring a Tiny Living Thing

An electrochemical sensor is a device that allows the presence of a substance to be related to a variable, which can be an electrical current, voltage or conductivity. Electrochemical sensors are analytical tools that consist of a working electrode as an element of transduction and a chemical or biological recognition element. Transduction is the process of changing a response signal to a value that can be interpreted, for example, a digital thermometer that receives a thermal signal and transforms it into an electrical impulse that is "translated" into a number in degrees Celsius. The interaction between the recognition element and the analyte of interest results in a chemical change that is translated by the transduction element into an electrical signal, which can be monitored through different electroanalytical techniques. An example of this type of device is the blood glucose meter, which correlates the content of this molecule with the current generated by its reaction on the sensor. This current is translated into a numerical value that allows us to know whether glucose levels are adequate or whether we should take special measures to control them.

The versatility of electrochemical sensors makes them very advantageous since they offer many options: They enable decentralized, sensitive, selective, fast and low-cost measurements without the need for complicated sample pretreatment. Moreover, the sample size is small, even in the order of microliters (μ L), which minimizes costs, as well as the use of reagents and contaminating materials.

Now, electrochemical sensors are usually designed to measure chemicals (glucose, oxygen, dopamine, etc.), but measuring microorganisms is another matter because they are living things! The challenge then is to imagine how a microorganism can cause a chemical reaction that results in an electrical response that can be measured and correlated with the presence of the microorganism. This seems complicated, but if we consider that all living things are reactors that produce billions of chemical reactions per minute, it makes sense that some of these chemicals can be measured by the sensor and, in turn, correlated with the number of microorganisms present in a medium.

A Sensor Tailored to a Yeast

The idea of studying a microorganism from the electrochemical point of view arose thanks to the work carried out in the Electrochemistry line of the Interdisciplinary Group of Molecular Studies (GIEM). This experience made it possible to determine, through the design of electrochemical sensors, biological molecules such as NADH, a biomolecule present in about 300 metabolic processes including ethanolic fermentations and respiration. Mangiferin was another molecule discovered. It is one of the main metabolites found in mango and has antioxidant properties.

The working electrode can be of different materials, configurations and sizes. Currently, printed electrodes (SPE) stand out. They are developed by printing different materials, such as graphite, on a substrate that is generally plastic or ceramic. The resulting electrode integrates the necessary elements to carry out the electrochemical determinations on the same platform. SPEs



Photo: cortesía del proyecto.

have become a striking electrochemical alternative because of their versatility and multiple advantages: They are easy to set up, simple, portable, highly sensitive and disposable. Moreover, their response is fast, and they operate at room temperature with no pre-polishing required. These are just a few advantages. In general, the success of SPEs lies in the possibility of combining their ease of modification, operation and portability with simple and inexpensive electrochemical methodologies.

To modify the electrodes, there are different recognition elements, among which oxidized multi-walled carbon nanotubes stand out. Their physicochemical characteristics improve the immobilization and chemical reactivity of biomolecules, apart from increasing sensitivity and promoting electron transfer. The modification of the working electrodes is carried out to confer selectivity and sensitivity, improve detection and quantification limits, and even obtain signals from species that are not electroactive in principle.



Within the project Electrochemical Study of Fermentations of Industrial Interest, carried out in the GIEM group, a printed carbon electrode was used. Its surface was modified by the addition of carbonaceous nanomaterials and Nafion® (a synthetic polymer with ionic properties). This allowed them to obtain an electrical signal that indicated the presence of yeast, which proved that the electrochemical method can be a valid alternative, especially considering the simplicity and speed of the method compared to other methodologies commonly used to quantify yeast.

These results, besides being useful from the point of view of control and monitoring of bioprocesses, are very interesting if we consider that electrochemical determinations are usually performed on organic or inorganic molecules. However, this result shows that it is also possible to study the behavior of living entities such as *S. cerevisiae*. This is very promising for a large number of processes in which the work with microorganisms is of interest, specifically with *S. cerevisiae*.

In the meantime, while we are studying the behavior of *Saccharomyces cerevisiae* in isolation and during bioprocesses, such as ethanolic fer-

mentations, using the designed electrochemical sensor and electroanalytical techniques, let's enjoy a good book and a delicious glass of wine. **X**

Glossary:

Bioprocess: process in which living microorganisms are involved to bring about physical or chemical changes.

Analyte: chemical substance that can be detected, identified or quantified when the chemical analysis of a specific sample is performed.

Working electrode: place where an electron transfer occurs in an electrochemical reaction.