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Development and characterization of a multiparametric microsensor for yeast cell growth monitoring

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Abstract

In this work, the development and testing of a microfabricated multiparametric sensor for rapid cell growth monitoring is described, especially focused on yeast quality assessment for wine applications. The device consists of two integrated microsensors (pH, impedance), able to monitor extracellular metabolism. Microbial growth has been performed both in standard culture conditions and in presence of ethanol (12% v/v) in order to carry out a common screening of wine yeast strains. Cell growth tests can be performed in just three hours, providing a fast, reliable, sensitive and low cost analysis with respect to the conventional procedures.

Keywords: ISFET, multiparametric sensor , conductivity sensor, cell growth monitoring, wine yeast

1. Introduction

Nowadays, significant oenological research is involved into the screening of wine yeast strains able to survive and carry out difficult fermentation processes at high alcohol concentration. Conventionally, the best performing microbial strains are firstly evaluated by time-consuming and labour-intensive methods such as plating studies or by direct microscopic counts. In the last three decades, many studies have been focused on the microbial metabolism due to the proven correlation between cell growth and both extracellular acidification or ionic conductivity change [1-2], enabling the development of microelectronic sensors for culture monitoring, especially based on ion sensitive field effect transistor (ISFET) sensors [3] and impedance-based microdevices [4]. Moreover, multiparametric chips have been proposed to carry out comprehensive studies about physiological state and dynamic behaviour of cell cultures [5]. In this work, the main goal was to demonstrate the feasibility of an integrated system based on multiparametric microsensors for evaluating ethanol resistance of yeasts, as a test of paramount importance for assessing the quality of yeast strains in wine applications. In particular, pH measurements are compared with the

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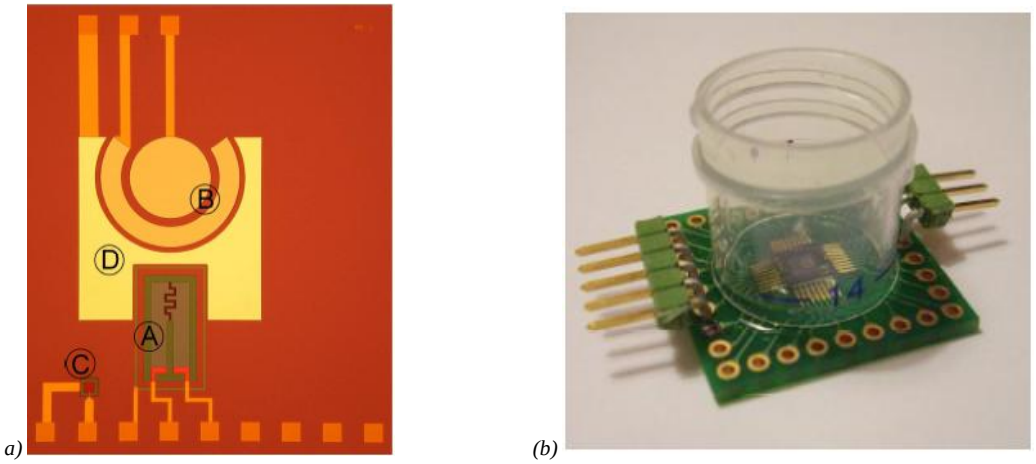
trend of cell proliferation, allowing the detection of the exponential growth phase in the first three hours after culture inoculum and the growth rate variation in presence of ethanol.

2. Materials and methods

The multiparametric sensors have been realized with a non-standard fabrication process derived from a 4µm Al-gate nMOS technology in order to include the realization of double layer SiO_2/Si_3N_4 gate dielectric for pH sensitivity and platinum electrodes. n-channel ISFETs have been realized starting from n-type 4" silicon wafers by using implanted n+ source and drain regions on p-well in order to insulate the devices from the n-type substrate. The technological platform also allows the realization of Pt electrodes exposed to the solution, which can be used for implementing on-chip different electrochemical sensors (e.g. conductivity, voltammetric or chrono-amperometric, Oxidation Reduction Potential (*ORP*) sensors). Electrical contacts to solid state devices and Pt electrodes have been implemented with low resistance Al wires. A Ag/AgCl pseudo-reference electrode has been also realized with an evaporated Ag layer and a post-processing galvanic chlorination from KCl 3M solution saturated with Ag. Fig. 1a shows the microphotograph of the realized multiparametric probe, with (A) the integrated ISFET (W/L = 67), (B) the conductivity electrodes, (C) the diode for temperature measurements and (D) the pseudo-reference electrode. The large central circular electrode is also intended for ORP measurements (not reported in this work). The total chip dimension is 5x5 mm^2.



a) *(b)*

Fig. 1. (a) Microphotograph of the realized multiparametric probe. A) ISFET sensor, B) conductivity sensor, C) diode for temperature sensing, D) on-chip Ag/AgCl pseudo-reference electrode. (b) Chip packaging for experimental testing.

The devices have been packaged with a chip-on-board approach, also including a glued recipient for the solution to be measured (Fig.1B). The characteristics of the device set used in the experiments are reported in Table 1.

To investigate the possibility to use the microsensor for cell growth monitoring, the correlation between cell growth and extracellular metabolic activity in a batch culture has been investigated. *S. cereviasiae* DVS22 (LEVOSPARK®) has been grown in sterile Yeast Peptone Dextrose (2% Bacteriological peptone, 1% Yeast extract, 2% Glucose) Broth (Sigma-Aldrich®). Exponentially growing cultures have been inoculated in 1.2 ml of fresh YPD liquid medium at a concentration of about 10^6 cells ml^{-1}. Yeast cells have been incubated at 28°C overnight to yield a microbial concentration of about 10^7 cells ml^{-1}. In order to evaluate the sensor suitability for the application, an off-line approach has been selected. Every thirty minutes, cells have been harvested by centrifugation (2100 g, 1 min, 25°C) and the yeast culture supernatants have been utilized for analytical measurements in order to monitor the progressive acidification of the culture medium. At the same time, the pellet has been used to determine the total cell number by microscopic counting. Cell growth assays have been carried out in parallel both at 0% and 12% (v/v) of

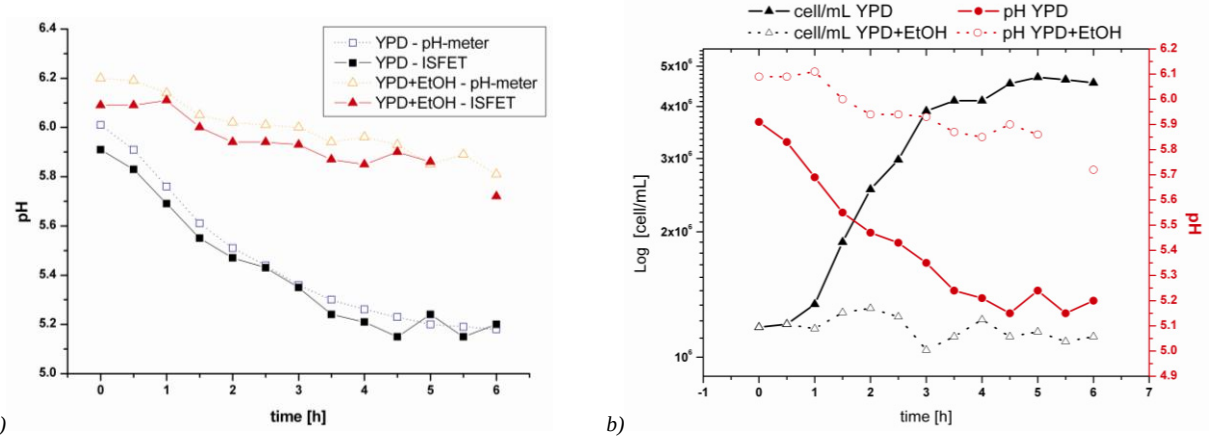
ethanol concentrations in order to detect changes in yeast proliferation throughout a laboratory test of alcohol resistance.

Table 1. Main specifications of the integrated sensors.

Sensor	Parameter	Value
ISFET	Sensitivity	48±3 mV/pH
	Drift	0.3mV/h
	Threshold Voltage	2.1±0.1 V
Conductivity	Range	0.2 10^{-3} ÷ 0.1 S cm^{-1}
	Cell constant	4.3 cm^{-1}
Temperature	Sensitivity	2.3mV/°C
	Tested range	20 ÷ 140°C

3. Experimental results

The pH measurements have been performed by using both the ISFET sensor and a traditional pH-meter (Crison GLP22) in order to verify the sensor response (Fig. 2A). The pH variation over time shows a good correlation with the trend of cell proliferation both in presence and absence of ethanol (Fig. 2A, 2B). The initial cell concentration in both cultures, with and without ethanol, was about 1.2 10^6 cells ml^{-1} and in the first 30 minutes remained almost constant.



a) *b)*

Fig. 2. a) Change of medium pH during cell cultivation, at 0% and 12% ethanol concentration, compared with the response of a traditional pH-meter; b) Comparison of pH and cell concentration vs. time at 0% and 12% ethanol concentration.

Then, as expected [6], after a small common lag phase, yeasts cultivated in fresh medium went through a logarithmic proliferation while most of cells that grew in 12% of alcohol did not duplicate and tried to adapt themselves and survive to this unfavourable condition. The pH curves showed a similar trend by sharp decrease in concomitance with the fast growing culture, while for cells cultivated in presence of ethanol pH initially floated around a constant value and then slowly decreased. As shown in Fig. 2B, the exponential growth phase was detected in the first three hours after culture inoculums, by strongly reducing the time generally required for wine yeasts screening on agar plates (typically in the order of two/three days). Whether pH values can be well correlated to the microbial growth curve, the preliminary impedance response (not reported here) has demonstrated lower robustness to measurement condition and efficiency in growth monitoring.

4. Conclusions

A multiparametric sensor for yeast growth monitoring applications has been designed and fabricated. The preliminary experimental characterization of the device has been performed by using *S. cerevisiae* cultures in presence and absence of ethanol in order to assess alcohol resistance of commercial wine yeast strains. The pH measurement shows the same trend as the reference cell count both at 0% and 12 % (v/v) of ethanol. The selected procedure has allowed the evaluation of ISFET suitability for the application, with a reduction of time required for ethanol resistance test to just three hours. This miniaturized system has shown the possibility to provide a portable platform for fast and automated cell growth monitoring. Future work will deal with the correlation of pH response with impedance measurements in order to increase the system performances and make a great leap forward with respect to the traditional cell cultivation systems.

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