Glucose Uptake in Electrically Stimulated Cultures of *Saccharomyces cerevisiae*
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Glucose Uptake in Electrically Stimulated Cultures of *Saccharomyces cerevisiae*

O.Q.F. Araújo, A.A.C. Oliveira, C.C.O. Torres, M.H. Rocha-Leão, I.C.P. Margarit, M.A.Z. Coelho

Escola de Química, Universidade Federal do Rio de Janeiro - Centro de Tecnologia, Bloco E, Ilha do Fundão, Rio de Janeiro – RJ, Brazil, 21949-900, ofelia@eq.ufrj.br

Abstract

The yeast *Saccharomyces cerevisiae* shows rapid fermentation of sugars to ethanol and carbon dioxide. It is widely known that glucose and other sugars exert a repressive effect on the synthesis of a variety of enzymes. On the other hand, glucose may also exert enhancement of enzyme synthesis. The former are mitochondrial enzymes or enzymes linked to cellular respiration and the second are enzymes linked to fermentative metabolism. The external environment and its changes strongly influence the physiological behavior of yeast. Tight control of the sugar concentration at low threshold values prevents fermentation while high sugar concentrations trigger the catabolite repression, with a decrease of the biomass yield. Of special interest to control metabolic fluxes and direct the production to the aimed application (e.g., biomass or ethanol) is the relationship between glucose uptake and cell cycle. Furthermore, mastering ways of stimulating a culture through exogenous variables is interesting in the development of biotechnological processes. The results obtained in different aeration conditions had evidenced that the applied electric potential increases sugar consumption and impacts the cell cycle through alterations in the fraction of cells in division and in the number of scars per cell.

Keywords: electric potential, *Saccharomyces cerevisiae*, cell cycle

1. Introduction

The yeast *Saccharomyces cerevisiae* is one of the best models of unicellular eukaryotic system. Due to the metabolism, similar to superior eukaryotics with metabolic activation mechanisms (citocromo P450), this microorganism is considered a valuable tool for metabolism and cell physiology study (Soares *et al.*, 2005). This yeast is distinguished because of its fast conversion of sugar to ethanol and CO_2. Changes in environmental conditions have a strong influence in the yeast physiological behavior. The control of the initial sugar concentration in low values favors fermentation, however, high concentration values promotes catabolic repression, leading the reduction of biomass yield. There is a special interest in controlling metabolic fluxes as well as evaluating the relationship between glucose consumption and the cell cycle.

The yeast cell cycle can be determined by the number of divisions that it has carried out. *Sacch. cerevisiae* reproduces asexually by budding and, as consequence of this process, the cell mother holds a scar of chitin in the point of duplication. As subsequent scars do not overlap, the number of scars on the cell surface determines the amount of divisions that it has undergone (Sinclair & Guarente, 1998). Therefore, it is a biological marker that can define the age of one given cell. Such mechanism of reproduction induces the coexistence of two subpopulations: budding and not budding cells (Figure 1).

Eukaryotic cells can respond to a variety of ambient stress such as nutrient availability, among others (pH, temperature, etc.). Cellular responses when submitted to an electric stress have been studied in Biotechnology (Bartlett *et al*., 1997), bioanalitycal techniques (Lötzbeyer *et al*., 1996; Ci *et al*., 1997; Gheorghiu e Asami, 1998) and with medical intentions (Chen *et al*., 1998; Veiga *et al*., 2005). Araujo *et al.* (2004) had evidenced oscillation in *Saccharomyces cerevisiae* growth culture when it was submitted to electric stimulations. Additionally, through optical microscopy smaller cells had been observed when the culture was submitted to electric potential compared to control experiments (without potential application).

Navarathna et al. (2006) report an harmonic generation by budding yeast cells *S. cerevisiae* in response to sinusoidal electric fields. The authors justify their findings on the interiors of intact biological cells negatively charged, and the motions of charged ions and macromolecules in the viscous medium cause them to respond to an applied oscillatory field in a strongly frequency-dependent fashion. Ions gradients across the cellular plasma membrane are controlled by membrane ion pumps, which establish a membrane potential difference that attracts ions dissolved in the extra-cellular medium. An externally applied oscillatory electric field causes these counter-ions to migrate along the cell wall to create a net dipole moment in the direction of the applied field. Membrane-bound proteins and enzymes can effectively harness the electric field energy to perform biologically useful work.

Therefore, the present work aims to evaluate the effect of the application of electric potential in some physiological and morphological responses of *S. cerevisiae* cells. The experiments had been performed in a bioeletrochemical reactor, with and without forced aeration, carried under a sequencing batch mode.

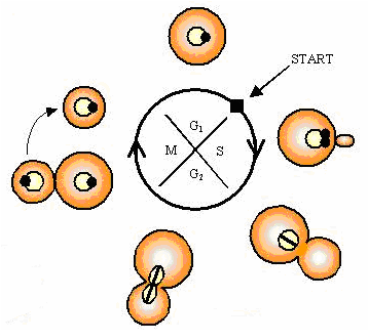

Figure 1: *Saccharomyces cerevisiae* cycle

2. Methodology

Yeast strain and medium: *Saccharomyces cerevisiae* S288C (ATCC 26108, GLC mal gal 2), gently provided by Dr. A.Panek, was used in this work and was preserved at 4 ºC in YPD agar medium. The inoculum was prepared by cultivating the cells in a shaker at 28 ºC and 160 rpm in YPD modified medium (1% yeast extract, 2% peptone and 0.64% glucose) along 15h.

Experimental set-up: The bioreactor was a 4-L glass vase with mechanical agitation and temperature control. On-line monitoring of dissolved oxygen (DO) (Actron OX2000) and pH (Actron PH2000) was connected to supervisory control software (FIX32-GE). A PID controller was responsible for maintaining a constant DO level. The value of applied potential was 0.75 V, according to Araújo *et al.* (2004). The potentiostat (PG-01 Ohnimetra Instruments) used had a working electrode and a counter-electrode of platinum and a calomel reference electrode (SCE), as presented in Figure 2.

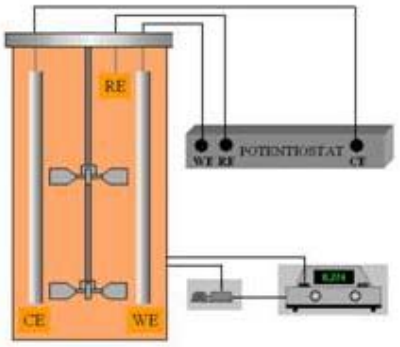

Figure 2: Bioelectrochemical Reator (RE = reference electrode (SCE); CE = counter-electrode (Pt); WE = working electrode (Pt))

Experimental strategy - The cells from the inoculum were inoculated in the bioreactor (YPD modified medium with 0.5% glucose) in sufficient amount to obtain an initial cell concentration of around 0.75 g/L. Experiments with and without aeration were carried out in triplicate with batches cut (i.e. two sequential batches), where the half of the medium volume was removed in the S0/2 phase, with addition of fresh medium in a way to maintain the work volume and the initial substrate concentration (Figure 3). In the experiments performed under electric potential, it was applied always in the second batch.

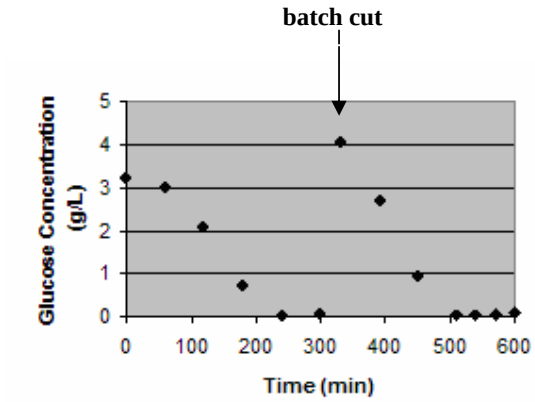

Figure 3: Experimental strategy adopted: sequential batches.

Analytical Methods: Cell growth was quantified spectrophotometrically at 570 nm (Hach, DR400UV) and converted to mg dry weight cells/mL. Glucose was quantified by the glucose oxidase method (diagnostic In vitro). The morphological analysis was carried out by digital images processing in the initial time, in the moment of the batch cut (end of the first batch and beginning of second one) and in the end of the process. The image acquisition was made using an optical microscope (Elipse E200, Nikon) equipped with a CCD camera (Coolpix 990, Nikon) at total magnification of 400x. Three image series was performed along each batch regarding a minimum of 100 cells in each series. The image processing procedure were developed with MATLAB v.6.1 (The Mathworks Inc.) package and were fully automated, as described by Coelho *et al.* (2004), providing the total number of cells, fraction of budding and non-budding cells. The determination of the population histogram was given by counting the number of scars per cell, stained with calcofluor (2µg/ml). The samples were observed in microscope with 1000x magnitude, using fluorescence in 330-360nm band.

3. Results and Discussion

The kinetic profiles of cell growth and glucose consumption achieved under aeration, with and without the application of electric potential are presented in Figures 3 and 4, respectively. The results demonstrate reproducibility in the system performed in batch with cuts, leading to similar kinetic profiles in the first batch. With regard to second batch, where the influence of the electric potential application can be verified, it denotes differentiated glucose consumption comparing both cultures (with and without electric potential application).

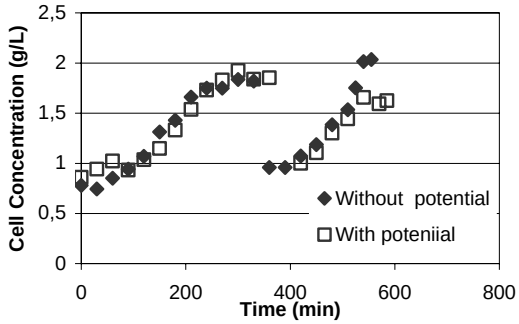

Figure 4: Cell growth profile carried under aeration (with and without electric potential application).

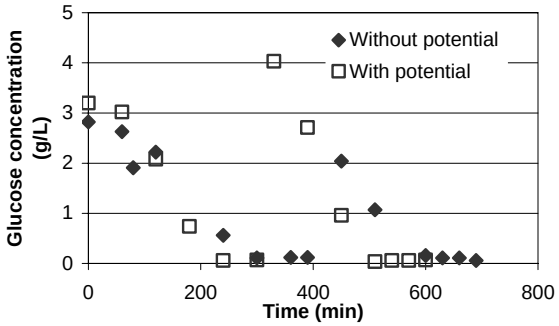

Figure 5: Glucose consumption under aeration (with and without electric potential application)

Figures 6 and 7 present the profiles achieved in the experiments carried without aeration. Similar to what was observed in the experiments with aeration, it is possible to verify the influence of the electric potential application in glucose consumption between the culture submitted to electric potential and the control (without application of electric potential). However, without aeration (where the dissolved oxygen concentration achieved levels next to zero) the application of the electric potential seems to exert some influence in cell growth rate. In order to quantify such influence, Table 1 presents the values obtained for the specific growth rate (μ) and substrate consumption rate (- dS/dt) in the 2nd batch of the experiments with and without aeration.

Table 1: Specific growth rate (μ) and glucose consumption rate (- dS/dt) for experiments with and without aeration

	μ (h^{-1})	**-dS/dt (g/L)**
With Aeration		
Without Potential	0,37 ± 0,04	0,65 ± 0,07
With Potential	0,36 ± 0,04	1,37 ± 0,10
Without Aeration		
Without Potential	0,19 ± 0,02	0,95 ± 0,08
With Potential	0,23 ± 0,02	1,20 ± 0,09

It can be noticed that the application of electric potential lead to an increase (20%) in μ, considering the experiments performed without aeration. Although it was observed an increase in glucose consumption rate with the application of electric potential in all conditions herein studied, the phenomenon magnitude is not the same: cultures under aeration lead to an increase of 100% in glucose consumption rate whereas in the culture carried without aeration, an increase of only 26% was observed.

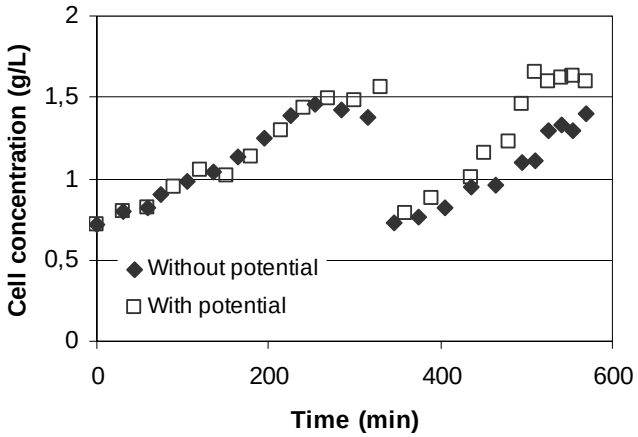

Figure 6: Cell growth profile carried without aeration (with and without electric potential application)

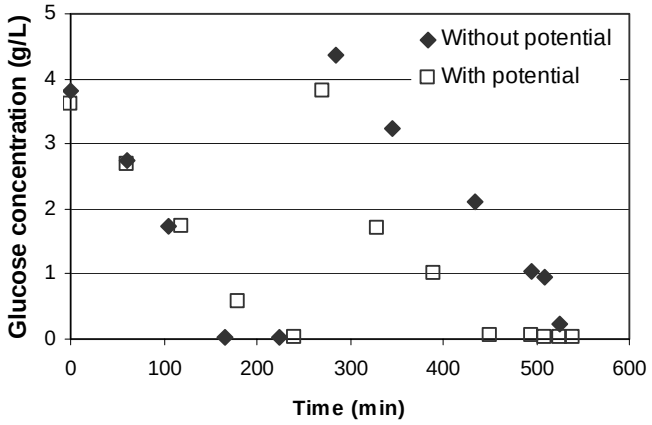

Figure 7: Glucose consumption carried without aeration (with and without the electric potential application).

In all the experiments, independently of the condition of aeration adopted, the number of scars per cell was determined at the beginning of the experiment, soon after the transition between sequencing batches and at the end of the experiment. Figure 8 presents the characteristic distributions for the scars between two distinct

experiments in the end of the first batch, denoting that the populations meet in identical morphological periods.

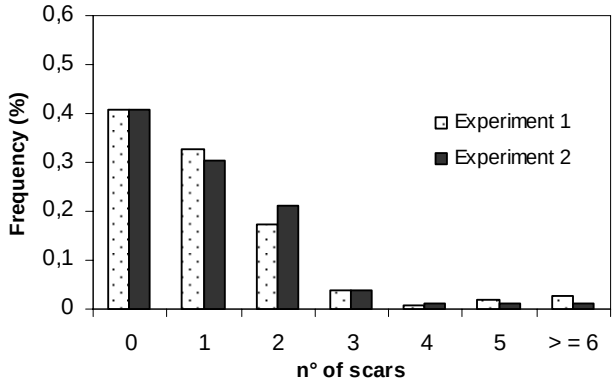

Figure 8: Number of scars per cell in the end of first batch (experiments carried with aeration).

To verify the electric potential effect in cell duplication cycle, scars distributions achieved in the end of experiments carried with and without potential application are depicted in Figures 9 and 10 for systems performed under aerobic conditions and without aeration, respectively. Such distributions demonstrate that profiles are similar and independent of the condition of aeration applied to the culture. However, the application of electric potential leads to an increase of the number of cells that enters in duplication process, indicating a possible acceleration of the biological clock or a reduction of the critical size for division.

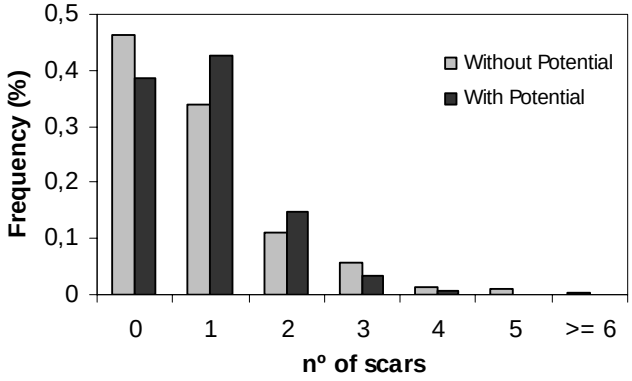

Figure 9: Number of scars per cell in the end of the 2nd batch carried under aeration

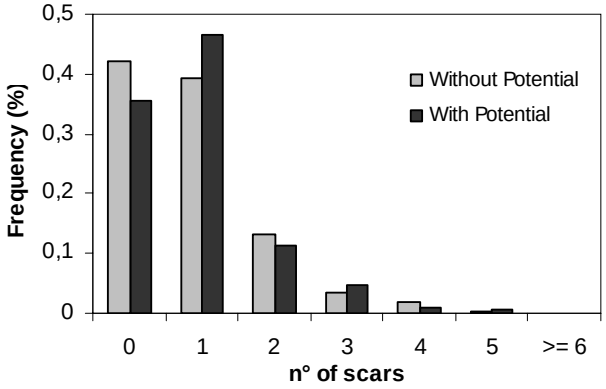

Figure 10: Number of scars per cell in the end of the 2nd batch carried without aeration

Corroborating the results obtained for the scars determination, identical behaviors are observed for the budding cells fraction (Table 2). Independent of the aeration condition, the application of the electric potential seems to induce the process of division denoted by an increase of the budding cells fraction in the end of the experiment, a behavior opposite to the observed for the control experiments (without potential application).

Table 2: Fraction of cells in budding (%)

	Cut	End
With Aeration		
With Potential	49,45 ± 2,5	64,63 ± 3,0
Without Potential	51,23 ± 2,5	16,13 ± 0,8
Without Aeration		
With potential	34,51 ± 1,8	63,45 ± 3,1
Without Potential	66,16 ± 3,0	39,13 ± 2,0

4. Conclusions

Variations herein described for the specific growth rate (μ) and for the substrate consumption rate (- dS/dt) in both the evaluated situations, denote alterations in glucose uptake for cells cultivated with potential application. With respect to the cell duplication process, the electric potential seems to speed up the division process, due to the increase in the budding cells fraction as well as in the number of scars per cell.

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