# BIOEFFECTS OF ULTRASOUND IN YEASTS CELLS SUSPENSIONS.

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**ABSTRACT** -- In this work interesting bioeffects have beem observed as consequence of sonication of cells of **Sacharomyces Cerevisiae**. The irradiation of the cells at frequencies of 1,8mHz and intensities of 200 $mW/cm^2$ caused a slight activation of the enzymes acid phosphatase and ATPase from cellular envelope, however irradiation at frequencies of 20 KHz and intensities of $10W/cm^2$ promoted a great activation of these enzymes. The experimental results confirm that the irradiation both at low and high frequencies submits the cell surface to high compression and rarefation forces that could disrupt the solvatation shell around cells and promote the influx of substance from medium to periplasmic space. When the same cells are sonicated and transfered to distilated water it was observed a great delivering efflux of glicose and ionic salts from cellular envelope suggestion in this condition the yeast cells decrease the rate of development. It was observed that the mean volume of sonicated cells was 35% smaller than not sonicated cells. The observed bioeffects suggest that ultrasonic irradiation of cells in suspensions is a powerful tool to study its complex behaviour and may generate important biotechnological process in a near future.

## INTRODUCTION

If enough ultrasonic energy is directed into any biological material, it may occur heating or disruption by cavitation or both, Willians, Palma (1983). Thus, some biological effects can be produced in virtually every living organisms or structure if it is supplied with enough acoustic energy.

Interesting bioeffects have beem observed as consequence of sonication of cells in suspensions and there are many practical advantages to be gained by irradiating living cells in suspensions in "vitro". The experimentalist has complete control over the ultrasonic exposure conditions and can choose to use a standing wave field or a plane travelling wave, or to irradiate in the near or far field of the transducer. Thus, one can obtain a homogeneuous population of cells which can be asynchronous or a variety of techniques can be employed to

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induce synchrony so that the cells may be irradiated at any stage within their life cycle. The cells are free to move in suspension and the acoustic streaming aided by any additional stirring ensure that the irregularities in exposure due to the non-uniforme nature of the ultrasonic beam are evened out, Willians (1983)). If the ultrasonic irradiation conditions permit the occurence of a stable acoustic cavitation the most of cells are kept alive and the system is exposed to beioffects, Willians, Bucalon, Palma (1989). The biological parameters generally influenced are: morphology, function, biochemical mechanisms and genetic changes, Bucalon (1989). Thus, irradiation of living cells in suspensions provides a convenient model system after which the cells are amenable to a battery of sophisticated tests to evaluate subtle changes in most of the surviving cells under stressing conditions induced by ultrasound.

The yeast **Sacharomyces Cerevisiae** seems to be a suitable system for this purpose, since its cells may be took as a rigid particle composed of cell wall and plasma membrane, in which the cellular invelope play an important role in the uptaking of metabolites and defensive mechanisms, Vilanueva (1978). We have studied the bioeffects of ultrasound irradiation in yeasts cells suspensions, Bucalon (1989). Sonic irradiations at frequencies of 20KHz have been used both to disrupt cells during extractive procedures, Goldman, Thacker (1971) and cell wall polymers degradations, Goldman (1952). Low frequency ultrasound has promoted enzymatic activation as consequence of transiente cavitation (collapsa of vapour filled microbubles) against the cellular envelope, Palma (1989). In a previous work was described some modifications at level of cellular envelope, induced by a linear pulsation of microbibles in low amplitude sound fields know as stable acoustic cavitation, without cell disruption, Palma (1983). It has been pointed out that cells in suspension exposed to frequencies of 20KHz and intensities about $10W/cm^2$ are routinely used for extraction of material from cells, Goldman, Tarker (1971). It has been observed that biopolymers degradation occurs as consequence of sonochemical reactions mediated by sonic cavitation, Palma (1987). In order to elucidate some molecular process that occur at cell envelope of yeasts during cavitation we are investigating the molecular interactions betweem the cell envelope and acoustic waves.

## MATERIAL AND METHODS

### Sonication condictions

Cells suspensions ($1.10^6$ cells/ml)were maintained in perspex flasks protected against light incidence. The sonication was performed at 20 KHz (intensity of 10 $W./cm^2$) by the use of a commercially-avaiable apparatus equiped with a pre-loaded piezoelectric transducer coupled to an accoustic transformer of catenoidal geometry; the irradiation at 1.8 MHz (intensity of 250 $mW./cm^2$) was performed with a piezoeletric transducer matched to the liquid medium through a metalic quarter wave ressonant plate. Ultrasonic intensity measurements have been performed in a radiation pressure balance used to measure the ultrasonic power emitted by therapeutic devices.

**Biological**

The yeast **Saccharomyces cerevisiae** was grown in a yeast extract-peptone-dextrose medium with aeration at 28°C, during 18 hours. The initial number of cells was determined by microscopic examination on NeuBauer Lamina.

**Enzymatic Activities**

Acid phosphatase activity was assayed incubating 0.5 ml of cell suspension in a solution containing 12 moles p-nitro phenylphosphate and 200 moles sodium, pH 5.5, in a final volume of 2.0 ml at 37°C during 30 minutes; the reaction was stoped by addition of 1 ml 0.1M Sodium hydroxide and centrifuged at 1200xg during 10 minutes. The optical density was measured at 405 nm and the concentration of p-nitrophenoxide was calculated by using the coefficient of molar absortivity of 17800 $M.cm^{-1}$. The ATPase activity was assayed incubating 200 moles sodium maleate pH 3.3, 25 moles sodium fluoride, 17 moles ATP in final volume of 2.0 ml during 30 minutes at 37°C. The reaction was stoped by addition of 1.0 ml 10% (w/v) TCA and centrifuged at 1200xg during 10 minutes.

Inorganic phosphate delivered as product of reaction was measured by the phosphomolybdenic acid complex method.

**Determination of cellular volume**

Samples (5 ml) were colleted from cultivation medium and filtered through a selective set of membrances in a "coulter counter".

**Analytical Procedures**

Immediately after sonications, samples were collected (10 ml), centrifuged at 500 xg during 10 minutes and suspended in destilated water. Aliquotes of 0,5 ml were taken, the conductivity was measured and then glucose was analysed by gas-chromatography analysis.

The **S.cerevisiae** cells were sonicated immediately after inoculation and cultivated during 18 hours under aeration. After this time of cultivation the cellular volume and its frequency of distribution in cells population was investigated.

## RESULTS AND DISCUSSIONS

**Properties of yeast cells as rigid particles in suspensions**

Irradiation of cells in suspensions both at frequencies at 20KHz and 1,8MHz under stable cavitation do not cause apreciable cell disruption (less than 5%) Palma, Bucalon, Luchesi (1983).

The irradiation of cells in suspensions at frequencies of 1,8MHz and intensities of 200 $mW/cm^2$ caused a slight activation of the enzymes acid phosphatase and ATPase from cellular envelope, however irradiation at frequencies of 20KHz and intensities around 10 $W/cm^2$ promoted a great activation of these enzymes (Figures 1 and 2). It must be emphasized that the effect of low frequency sonication is immediate, promoting a "burst" of activity, this activation desapperar after 36 hours of cultivation. The enzyme activation desappear after 36 hours of cultivation. The enzyme activation may be resulting from ionization and/or conformational changes caused by sonication.

The recovery of original (control) activity suggests that the effects are only surfacial and do not affected the cytoplasmic components, Bucalon (1989). Yeasts cells suspended in aquous solutions are envolved by solvatation shells, Vilanueva (1978) and in this condition may be considered as rigid particles subjected do the action of a ultrasounic field. The irradiation in this condition, both at low and high frequencies, submits the cell surface to high compression and rarefation forces that could disrupt the solvatation shell around cells and promote the influx of substances from medium to periplasmic space. When S.C. cells are sonicated in a culture medium and them transfered to distilated water it was observed a great delivering efflux of glicose and ionic salts from cellular envelope, these substances were present originally in the cultivation medium and entered inward cellular envelope suggesting that during yeasts cells irradiation occurs the formation of a gradient of concentration inside the cellular envelope, as schematized in Figure 3.

The high metabolite concentration inside the periplasmic space may have affected the conformation and/or have been misunderstood by the biochemical receptors of growing elicitating a false stimulus of a hypertonic culture medium.

The yeasts cells answered to this condition decreasing the rate of cell development, Bucalon (1989). It was observed that the mean volume of a normal yeast cell in YEPD medium is 330 $um^3$ meanwhile sonicated cells at 20KHz presented the mean volume of 215 $um^3$. Therefore, the cellular volume of sonicated cells was 35% smaller than not sonicated cells.

A major difference between cells in a tisue and cells in suspensions is that the cells within the tissue are contact inhibited and are therefore not actively preparing for their next cell division. Cells in suspension are not subject to the complex feedback mechanisms which prevent unrestrainde growth within a tissue and are therefore actively growing and replicating themselves at the maximum rate permited by enrivonmental factors such as temperature, pH, oxigen and nutrients avaiability and absence of toxic products, Lorincz, Carter (1979). When viable non-dividing single cells are introduced in a fresh culture medium, the growing curve a may be described as a sigmoide. However, when the cells are irradiated at 20KHz it was observed a linearization of growing curve (Figure 4). In yeasts the cell volume plays an important role in the "start" of bud formation, Lorincz, Carter (1979).

The reduced cellular volume observed for the cells after ultrasonic irradiation may have influenced the rate of budding, as consequence of acoustic microstreaming fields which can damage the cell surface.

Thus when non-dividing and viable yeast cells are introduced into a fresh growth medium it is observed a characteristic delay (called the lag phase) before those cells begin to divide. This delay is followed by a period of regular growth when an exponential rate of populational growing (called the log phase) is observed, Willians, Lorincz, Carter (1979). The cells submited to ultrasonic irradiation in the lag phase changed the property of exponential growing and grew linearly, Bucalon, (1989).

The observed bioeffects presented above suggest that the ultrasonic irradiation of yeast cells in suspensions may be used as a very usefull tool to study the complex behaviour of cells under stressing conditions and to creat special systems that may generate important biotechnological process in a short future.

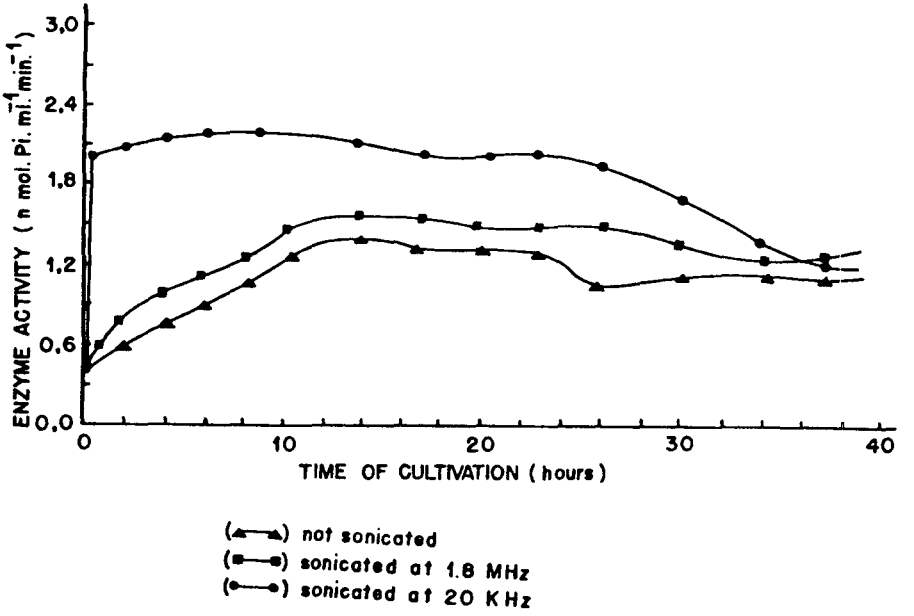


Figure 1. Periplasmal ATP ase activity (pH 3.5) of intact cells.

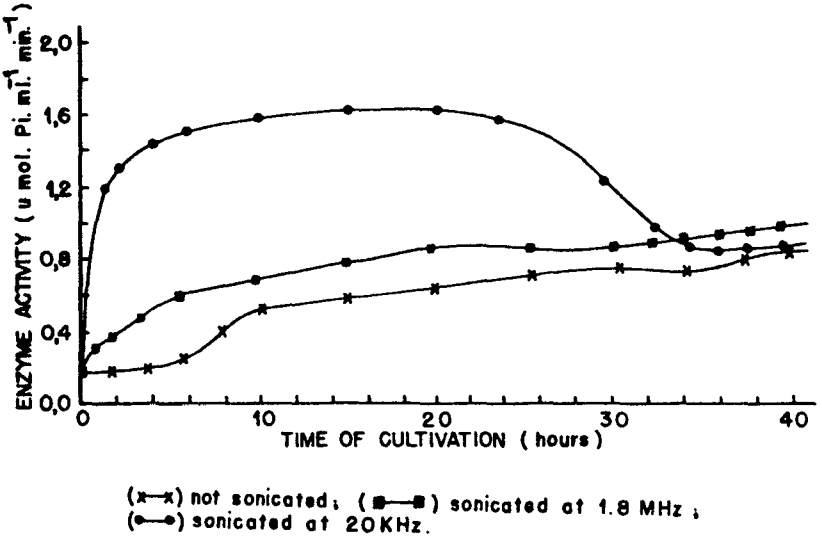


Figure 2: Periplasmal acid phosphatase activity (pH 5.5) of intact cell.

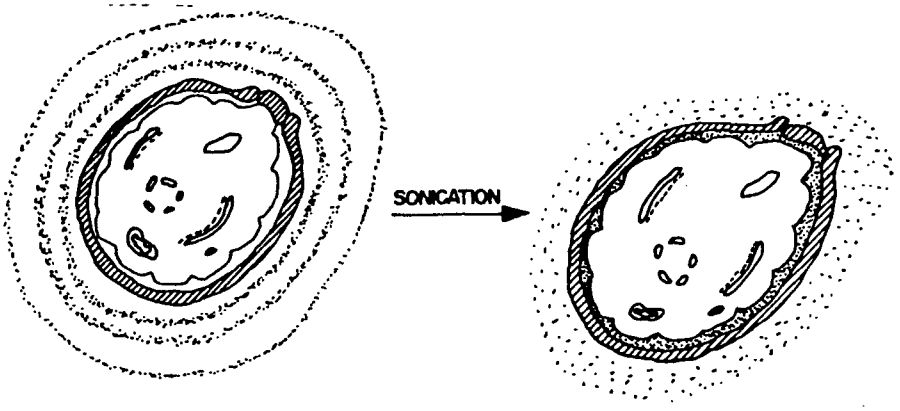


Figure 3. Schematic diagram ilustrating the effect of sonication on yeast cell, suggesting the formation of concentration gradiente.

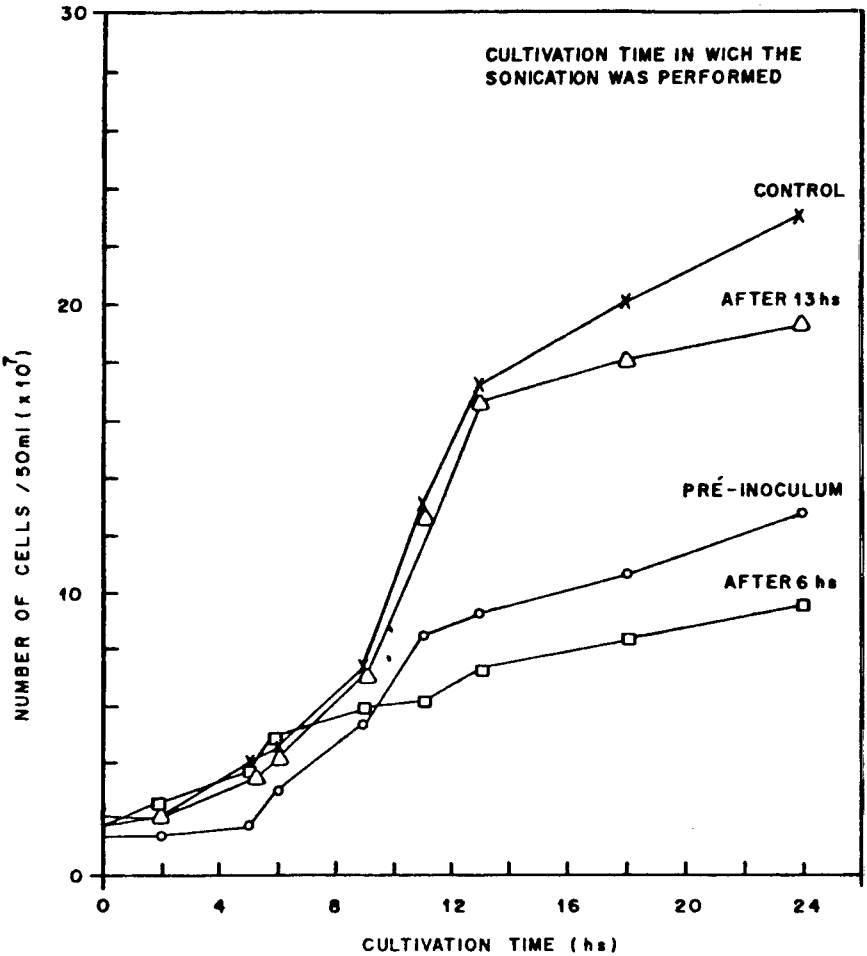


Figure 4. Effect of ultrasound on populational growing of saccharomyces cerevisiae. The cells were sonicated at 20 KHz (10w/cm$^2$) during 15 minutes.

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