



## Studies of the *Saccharomyces cerevisiae* Cultivation under Oscillatory Mixing Conditions

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Received: November 11, 2005

Accepted: November 30, 2005

Published: December 16, 2005

**Abstract:** *Saccharomyces cerevisiae* was cultivated under non-aerated conditions in a 5 l laboratory bioreactor. Using the experimental data and the regression analysis method, some mathematical correlations for stirrer rotational speed oscillation frequency and the reaction of the yeast were established. It has been found that different growth parameters are influenced variously by stirrer rotational speed and stirrer rotational speed oscillation frequency. Stirring oscillations can be among the methods for stimulation of biotechnological processes. The obtained results can be used for designing bioreactors and optimizing working conditions.

**Keywords:** *bioreactor, mixing, stirrer rotational speed oscillations, Saccharomyces cerevisiae*

### Introduction

Mechanically agitated bioreactors are widely used in biochemical processes. The growth of microorganisms as well as their products' biosynthesis and quality depend greatly on the hydrodynamic situation in the bioreactor [1]. It is known that, in a real industrial bioreactor, it is not practically possible to realize a completely equal distribution of the introduced energy throughout the volume. The distribution of energy in real chemical and biochemical reactors is more or less unequal because some heterogeneity is present in them [2, 8]. Therefore, the cultivated cells are moving with broth streams in closed circulation loops, periodically passing in different zones of aeration and mixing intensity. Hence, there are different characteristics of micromixing in different zones of a bioreactor. Non-ideal mixing (mainly mixing in large-scale reactors) can lead to remarkable concentration gradients of nutrients and, in aerobic fermentation, also oxygen gradients [7]. During batch cultivations of *Saccharomyces cerevisiae*, the stirrer rotational speed has a significant influence on growth [9]. The use of the computational fluid dynamics technique to simulate the behaviour of flow patterns shows that particles (or cells of microorganisms) reside mainly in the region of considerably lower shear rates [6]. In such bioreactors, different-size spatially non-delimited circulating profiles exist simultaneously.



Therefore, this cannot identify the influence of the specified frequency oscillations of turbulence and aeration on the cultivating cells development and metabolism process.

Mixing characteristics are determined by the design and scale of a bioreactor, as well as the stirring regime. The study of rigorously cellular responses to less-than-ideal mixing requires the knowledge of the circulation time [7]. Circulation time is one of the important parameters of a bioreactor with a stirrer. It is defined as the time between the consecutive passages of a particular cell through the well-mixed, nutrient-rich or oxygen-rich zone. Circulation time depends on the rotational speed of the stirrer and the scale of the bioreactor. With increasing stirrer rotational speed and decreasing scale of the reactor, this time decreases. The inverse value of circulation time is frequency. The frequency of the broth passing through the active regions is very important for the desired biosynthesis productivity [5,10].

Our earlier investigations of aerated submerged bioreactors having aeration pulses [12] by a special stirring intensity measurements technique [1, 2, 11, 13, 14] do not comprehensively reflect the hydrodynamic situation and do not enable to solve scale-up problems in geometrically dissimilar bioreactors.

Therefore, the aim of the present study was to develop a method for physical modelling in a laboratory bioreactor the turbulence oscillations observed in a real industrial bioreactor, and, from the experimental results, to derive a mathematical expression of the process in the circulation loop. It enabled to explain the influence of the mixing amplitude and frequency on the essential characteristics of the biosynthesis process.

### **Materials and methods**

To obtain oscillating stirring conditions, a bioreactor was equipped with a control unit, which changed the stirrer rotational speed from maximal to zero with a variable period [3].

At each fermentation process, one maximal stirrer rotational speed ( $n_{max}$ ) and one stirrer rotational speed oscillation frequency ( $f$ ) was used.

The maximal stirrer rotational speed was changed from 0 to 500 rpm and oscillation frequency from 0 (without mixing) to  $0.5\text{ s}^{-1}$ .

A commercial strain of *Saccharomyces cerevisiae* was used for experiments. The medium used for the cultivation process of the yeast contained 20 g/l glucose, 10 g/l yeast extract and 10 g/l peptone.

Experiments were carried out in a 5 l bioreactor FU-8 equipped with a double staged Rushton turbine with the working volume 3 l.

During the fermentation, the following parameters were kept constant:

- maximal stirrer rotational speed;
- stirrer rotational speed oscillation frequency;
- temperature.



During the experiments, samples were taken, which were analyzed for:

- concentration of glucose;
- concentration of biomass;
- concentration of ethanol.

Biomass was determined by measuring the optical density at 540 nm after centrifugation and resuspension in distilled water, and converted to biomass using a dry-weight calibration curve.

A gas chromatograph CHROM 4 (glass column 3 mm x 1.2 meter; filled with Gaschrom Q 100-120 mesh + 3% PEG M 6000; temperature of the evaporator 200°C; temperature of the column 80°C; volume of the sample 1 microlitre; detection – flame ionization) was used to determine the concentration of ethanol.

Software package STATSOFT 2 (Randec Ltd.) was used for processing of experimental data.

## Results and discussion

To investigate the influence of the cyclic turbulence intensity on *Saccharomyces cerevisiae*, equipment was devised for simulation in a laboratory bioreactor the conditions similar to those in which cultivation of cells would be realized in an industrial bioreactor, which pass through different zones of the turbulence intensity cyclically with the liquid stream. The control unit of a bioreactor stirrer was created, which periodically changed the stirrer rotational speed and therefore influenced microorganisms as they occurred in different zones of a bioreactor. To achieve exact and standard oscillations, we used a frequency generator HP-3312A [3]. The impulse with the given frequency passes from the generator to a special drive control unit, which forms a stirrer-driven oscillation motion with definite frequency and definite maximal rotational speed. The control unit changes the voltage and therefore the stirrer rotational speed from the maximal defined one to zero and up to the maximal one with the generated frequency [4].

The experimental results were used to obtain multiparameter non-linear mathematical models, which characterized the dependence of the process parameters on the oscillation intensity amplitude and frequency.

From the experimental results, the mathematical expressions were derived:

$$Y_{x/s} = -0.141 + \frac{0.503}{0.5 + 2f} + \frac{0.0524(0.5 + 2f)^2}{(0.0125n - 2)^2} \quad (1)$$

where:

$Y_{x/s}$  – yield of biomass of *Saccharomyces cerevisiae* from glucose, g g<sup>-1</sup>

$n$  – stirrer rotational speed, rpm

$f$  – stirrer rotational speed oscillation frequency, s<sup>-1</sup>



$$\begin{aligned} dX/dt = & 1.7 - 0.16(0.0125n - 2)(0.5 + 2f)^3 - \frac{0.676}{0.5 + 2f} - \frac{0.3}{(0.0125n - 2)} + \\ & + \frac{0.0347}{(0.0125n - 2)^2 (0.5 + 2f)^2} + \frac{0.0238(0.0125n - 2)^3}{0.5 + 2f} \end{aligned} \quad (2)$$

where:

$dX/dt$  - biomass growth rate,  $g\ l^{-1}h^{-1}$

$t$  - time

$$\begin{aligned} dS/dt = & 13.89 - 3.1(0.5 + 2f)(0.0125n - 2) - \frac{7.9}{0.5 + 2f} + \frac{2.79(0.0125n - 2)}{0.5 + 2f} + \\ & + \frac{0.176}{(0.0125n)^3 (0.5 + 2f)} - 0.31(0.5 + 2f)^4 \end{aligned} \quad (3)$$

where:

$dS/dt$  – glucose consumption rate,  $g\ l^{-1}h^{-1}$ .

Figures 1-3 show graphic dependencies of the growth parameters on the stirrer rotational speed and oscillation frequency, derived from the experimental results using equations (1-3).

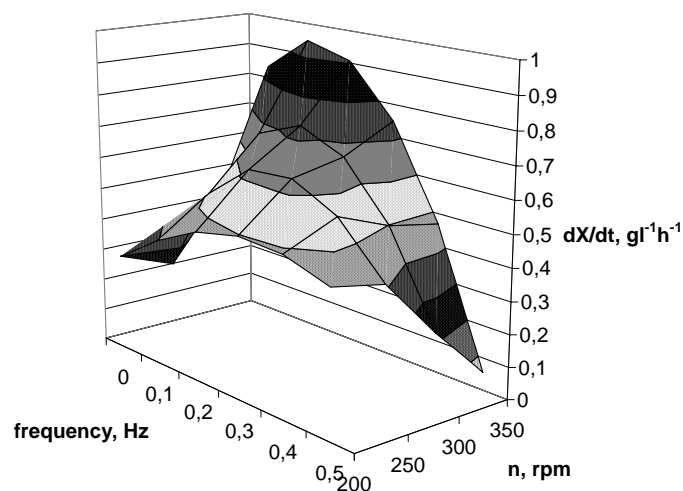


Fig. 1 Dependence of biomass growth rate  $dX/dt$  on stirring conditions.

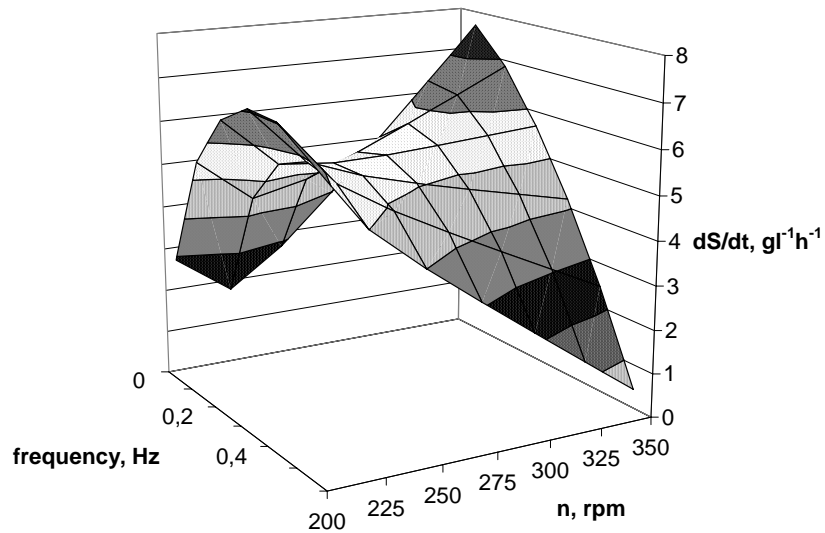


Fig. 2 Correlations between substrate consumption rate  $dS/dt$  and stirring conditions.

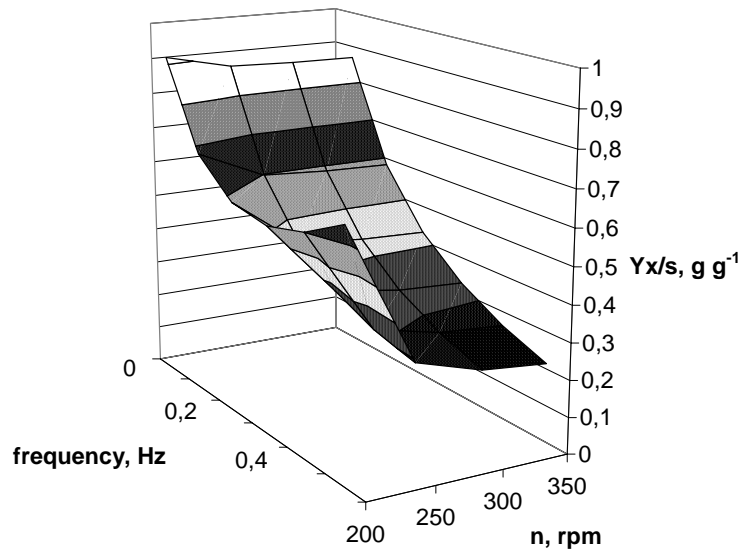


Fig. 3 Dependence of biomass yield  $Y_{x/s}$  on stirring conditions.

As can be seen from the graphs, different growth parameters are influenced variously by the stirrer rotational speed and the stirrer rotational speed oscillation frequency. In the bioreactor, liquid circulation loops with different diameters exist at a time. Therefore, different bioreactor and stirring regime are necessary for each process.



Fig. 1 shows that the maximal biomass growth rate at the stirrer rotational speed values 350 rpm and 200 rpm was at the stirrer rotational speed oscillation frequencies  $0.4\text{ s}^{-1}$  and  $0.1\text{ s}^{-1}$ , respectively.

With increasing stirrer rotational speed, the biomass growth rate maximum moves to the direction of the decreasing stirrer rotational speed oscillation frequency (increasing circulation time and, therefore, optimal bioreactor dimensions).

Fig. 3 demonstrates that the maximal biomass yield occurs at stirrer rotational speed oscillation frequencies close to  $0\text{-}0.1\text{ s}^{-1}$  (long circulation loop with decreased shear rate). With increasing stirrer rotational speed oscillation frequency to  $0.5\text{ s}^{-1}$  (short liquid loop and frequent changes of zones with different mixing regimes), the maximal biomass yield was at the stirrer rotational speed 200 rpm. With increasing stirrer rotational speed, the biomass yield decreased very fast at 250 rpm.

At the same stirrer rotational speed (oscillation frequency 0.3), a curve occurs for the dependence of the substrate consumption rate on the stirring conditions (Fig. 2).

It can be concluded that, with increasing stirrer rotational speed for *Saccharomyces cerevisiae*, very important is the length of the circulation loop of liquid (the frequency at which cells pass through zones with different mixing intensities) and, respectively, also dimensions of the bioreactor.

## Conclusions

1. Oscillations, for example, stirring speed oscillations, can be among the methods for stimulation of biotechnological processes.
2. The obtained results can be used for designing bioreactors and optimizing working conditions.

## Acknowledgements

*This work was supported by the Latvian Council of Science (Project No. 05.1558) and the EU Project QLAM-2001-00360 WOODPRO (QLK5-CT-2002-30360).*

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