Disintegration of yeast Saccharomyces cerevisiae in the vertical perl mill with a bell-shaped impeller

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Abstract Investigation of disintegration of yeast Saccharomyces cerevisiae in the laboratory batch perl mill with a bell-shaped impeller was carried out. The number of non-damaged cells, changing in time was determined using hemocytometer (Thorn’s chamber).

To describe kinetics of the disintegration process the differential equation was applied:

\[
\frac{dN_p}{dt} = k \cdot N_p^m,
\]

where

- \( N_p \) the number of non-damaged cells in the sample, [number of cells/ml]
- \( t \) time, [s]
- \( m, k \) constants

The effect of three operating parameters: rotation frequency of the impeller shaft \( n \), filling of the mill with disintegrating elements (ballotini) \( S_k \) and the initial concentration of yeast cells in the suspension \( C_0 \) on the process of disintegration was analyzed.

For \( S_k = 0.5 \), \( m = 1 \) and dependence of constant \( k \) on the rotation frequency of the impeller and suspension concentration were obtained. For \( S_k = 0.6 \) and 0.7 the values of \( m \) were higher than 1. The effect of rotation frequency of the impeller and filling of the mill, with ballotini on constant \( k \) and exponent \( m \) was determined.

List of symbols

- \( a, b \) constants
- \( a_1, b_1, c_1, d_1 \) constants
- \( C_0 \) initial concentration of suspension [g/ml]
- \( C \) concentration of cell suspension [g/ml]
- \( k \) constant disintegration rate \( 1 \left( N_0^{1-m} \right)/s \)
- \( m \) variable in the equation

1 Introduction

Disintegration of microorganisms is one of the technological operations that enable or facilitate to separate components contained in the cells. In the case of some microorganisms microbiological products such as proteins, amino acids, vitamins, fats, enzymes can be separated only after breaking cell walls.

Cell walls can be broken either by biological methods using enzymes, or by physical methods among which the most efficient are mechanical methods.

A variety of microorganisms as far as their size and morphological forms are concerned, makes it necessary to design and construct possibly most efficient equipment for effective disintegration of particular microorganisms.

Mechanical disintegrators have very different constructions among which perl mills are of significant importance.

Perl mills were designed for very fine grinding of solids in a suspension. When applied to disintegration of microorganism cells their applicability was largely extended.

Disintegration of yeast S. cerevisiae in the laboratory perl mill with a horizontal multi-disk impeller was investigated among others by Hedenskog and Mogren [5], H. Schütte, K. Kromer and M.R. Kula [6], J. Limon-Larson et al. [7], F. Marfly and M.R. Kula [8], and J. Rehaček [9]. These authors determined the effect of selected operating parameters of the mills such as rotation frequency of the impeller shaft, filling of the mill with ballotini, initial concentration and flow rate of yeast suspension on the disintegration intensity.

Recently, new mill constructions have been developed which are characterized by a relatively small volume of the working chamber. They include Dyno-Mill [10], a Japanese mill with a horizontal cylindrical impeller [11], a vertical mill with
a closed cylindrical impeller [12] and a mill with a bell-shaped impeller [13].

In this paper the investigation of disintegration of yeast S. cerevisiae in the laboratory perl mill with a bell-shaped impeller operating batch-wise will be discussed. The aim of the study was to evaluate the effect of selected operating parameters on the disintegration intensity and to investigate process kinetics in the mill of this construction.

2 Experimental

A schematic diagram of the mill is shown in Fig. 1. The working space of the mill was filled with ballotini 1.0 to 1.25 mm in diameter, highly resistant to abrasion and impacts. Since earlier investigations of microgrinding in the mills equipped with cylindrical impellers [14, 15] revealed that due to process efficiency the filling of the mill with ballotini should not be lower than 0.5 (at an increase of this parameter above 0.8 the energy input grows rapidly), three values of S, 0.5, 0.6 and 0.7 were used in our study.

The selection of rotation frequencies of the impeller was based on our own investigations of novel constructions of vertical perl mills [16, 17, 18], which proved that because of the obtained grinding effects the rotation frequency of the impeller should not be lower than 16.7 s⁻¹ and should not exceed the value of 34.0 s⁻¹ at which a significant wear (in some cases even damage) of disintegrating elements and a remarkable increase of energy input were observed at a slight increase of grinding effects. Thus, three values of rotation frequency n: 16.7 s⁻¹, 25.0 s⁻¹ and 33.3 s⁻¹ were assumed.

In the investigations fresh baker’s yeast Saccharomyces cerevisiae was used. Disintegration was performed for four initial concentrations of \( C_0 \): 0.09, 0.12, 0.14, 0.16 g d.m./ml. These values are optimal for this medium: they were determined on the basis of results obtained by other authors [3, 5, 6, 7].

The concentration of cells in the suspension was determined on the basis of the yeast dry matter. After defining the concentration of the suspension obtained, the suspension of initial concentrations \( C_0 \), assumed for the investigations, were prepared for disintegration using solutions in distilled water. For each sample the initial number of cells \( N_0 \) was determined additionally. The number of cells was calculated in hemocytometer (Thom’s chamber). The value of \( N_0 \) was assumed as a mean of 10 calculations. Differences in the number of cells for the same initial concentrations \( C_0 \) can be explained by properties of biological material, e.g. a rapid growth of cells during storage.

To determine the disintegration intensity samples of the suspension were taken at determined time intervals. Counting the non-damaged cells by a hemocytometer (Thom’s chamber) using a microscope, the disintegration was calculated from the formula:

\[
X(t) = \left( \frac{N_0 - N_t}{N_0} \right) \cdot 100\%.
\]  

For each analyzed sample 20 to 25 calculations were made. If the difference between disintegration level calculated for every single counting of a sample exceeded ±5% of the average value of 20 countings, the analysis of the given sample was repeated. Parallel to some experiments the disintegration was evaluated by measuring the concentration of nucleic acids in the supernatant liquid after centrifuging the biomass, by spectrophotometric technique. The concentration of nucleic acids in the supernatant liquids was calculated from the formula:

\[
C_{\text{DNA} + \text{RNA}} = \frac{E_{260}}{6 \times I \times \text{Rozc}},
\]

where: \( E_{260} \) – extinction at wavelength 260 nm,
\( \varepsilon = 10800 \ (\text{M}^{-1}\text{cm}^{-1}) \) – calibration constant [19],
\( l \) – cuvette width,
\( \text{Rozc} \) – dilution.

The disintegration efficiency was calculated as a ratio of nucleic acids \( C_{\text{DNA} + \text{RNA}} \) released to the maximum content of nucleic acids \( C_{\text{DNA} + \text{RNA}}^{\text{max}} \) in the biomass volume:

\[
X(t) = \frac{C_{\text{DNA} + \text{RNA}}}{C_{\text{DNA} + \text{RNA}}^{\text{max}}} \cdot 100\%.
\]

The maximum concentration of nucleic acids in yeast cells was determined by the spectrophotometric method after alkaline hydrolysis (0.3 M KOH 60 min., 37°C) and dilution of cells 0.5 M HClO₄. As compared to the generally applied hydrolysis with perchloric acid, and determination of nucleic acid concentration by the orcinolic method, the applied technique [20] gives smaller measuring errors (0.9 to 1.1%).

The disintegration process was carried out for ten minutes in each run. The final disintegration values are shown in Table 1.

3 Analysis of results – mathematical model of process kinetics

At the first stage of the analysis of results, to describe the disintegration kinetics, the classical equation proposed by other authors [6, 7, 8, 21] was adopted:

\[
- \frac{dC}{dt} = k \cdot C.
\]