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Disintegration Kinetics of Microbial Cells

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Abstract

Results of the disintegration of yeast *Saccharomyces cerevisiae* in the bead mill with a multi disk impeller are presented. The degree of disintegration was specified on the basis of absorbency measurements at the wavelength 260 nm. The process was investigated by two integrated methods. The experimental values of maximum absorbency A_{m2} appeared to be smaller than theoretical ones A_{m1}, which resulted from searching for the highest values of correlation coefficient between variables t and $\ln[A_{m1} / (A_{m1} - A)]$. A significant increase of the process rate constant was observed when the slurry concentration increased in the range from 0.05 to 0.20 g d.m./cm^3. This phenomenon was explained by an additional mechanism of cell destruction, which was induced by fragments of ground walls. The rate constant changed during the process due to a change of inner process conditions, and not directly as a result of a changing number of microbial cells. Modeling of the process in which the first-order differential equation is used to describe the kinetics is correct, with the process rate constant being a function of parameters that describe inner conditions changing during the process.

Keywords: Random transformation of dispersed matter, disintegration of microorganisms, bead mill, kinetics of the process

1. Introduction

Chemical compounds contained in microorganisms provide the opportunity, often the only one, to satisfy a whole range of human needs. Commercial importance was gained by, among others, intracellular enzymes used to shape and preserve functional properties of foodstuffs, conducting clinical analyses, antibiotic conversion, or therapy applied during cancer diseases [1]. The genetic modification of microorganisms allows for a further increase in the range of

intracellular compounds' utilization. Such a possibility should lead to obtaining valuable components from microorganisms generally considered safe for people on an industrial scale [2].

The separation of compounds contained in microorganisms generally requires the destruction of cell walls and cytoplasmic membranes. The process is conducted in high pressure homogenizers and bead mills on a semitechnical and technical scale. The device type depends on the type of destroyed microorganisms. Homogenizers are recommended for the disintegration of some bacteria and yeasts, while mills are for yeasts, fungi, and algae [3]. High-energy consumption of the process carried out by mechanical methods is the cause to seek more economical technical means of its implementation. The use of chemical, biological, and other physical methods at the present stage of technology development is not economically justified. It is expected that the use of mechanical methods, combined with others, for the disintegration of more resistant microorganisms in future will be beneficial [3].

The research carried out on the process improvement realized in bead mills is difficult to a large number of phenomena occurring in circulating mill filling and their specificity. During the disintegration of microorganisms, there occurs disruption of cell walls and membranes, releasing and dissolving intracellular compounds; organelle disruption; milling fragments of cell walls, cytoplasmic membranes, and other cellular fragments; interaction between the released compounds and microorganisms; and unprotected organelles and their mutual interaction. While conducting the process, rheological properties change, both of a suspension and its continuous phase. Mutual relativity of occurrence times of separate events during disintegration can have very small (cell disruption—dissolving intracellular compounds) or very large values (cell disruption—cell autolysis). The process course is affected by a very large number of parameters: instrumental (e.g., mill design and size, filling ball diameter, filling degree, materials used, and surface condition), raw material (e.g., conditions for microorganisms growth, their size and morphological form), and process (e.g., mixer rotation speed, duration of the process, temperature, microorganisms' suspension flow rate under conditions of continuous operation, and initial concentration of microorganisms' cells). So far, there are several models leading to the mathematical description of the disintegration of microorganisms in bead mills by a first-order linear differential equation [1–10]. A dependency of the release rate of enzymes on their distribution in a cell has been shown [11]. Taking into account the consequences of events: disruption of the cells - release of intracellular compounds, led Melendres et al. [12] to a nonlinear description of the overall process assuming the linear course of component processes. Some researchers showed a dependency of the process rate constant on the initial microorganism concentration. Marffy and Kula [4] presented the results of the disintegration of brewer's yeasts *Saccharomyces carlsbergensis* in a horizontal mill with a multiple disc mixer with a working chamber volume of 600 cm^3 and indicated an almost twofold increase in the process rate constant at an increase in the suspended matter concentration described as the final amount of released protein from about 10 to 60 mg/cm^3. This effect was confirmed by Heim and Solecki [5] for the process carried out in a bead mill at a large concentration of *Saccharomyces cerevisiae* yeast suspension (0.14–0.20 g d.m./cm^3) and high rotational speeds of the mixer (2500–3500 rpm). On the other hand, the analysis of the presented by Currie et al. [6] values of rate constants dependent on the size of bed balls for the increased concentrations of *S. cerevisiae* yeast suspension in the range of 0.3 to 0.75 g yeasts/cm^3 indicates a continuous decrease in the obtained disintegration results. A rate constant decrease in a

horizontal mill with a steel mixer at an increase in concentration from 0.15 to 0.30 kg yeasts/m^3 was demonstrated by Limon-Lason et al. [7]. The same tendency was demonstrated by the same authors in a horizontal mill with a polyurethane mixer with an increase in concentration from 0.15 to 0.75 kg yeasts/m^3. A dependency of the disintegration of microorganisms that results in changes in their concentration is explained by the authors by a change in rheological properties of the suspension [4, 7] or an additional mechanism to destroy microorganisms [5]. Of course, some researchers show in their publications that there is no effect of microorganism concentration on the process course [2, 8].

For researching, describing, and managing processes of random transformation of the dispersed matter in a limited space, the theory basics were developed [13–15]. They include the assumed possibility of shaping and transforming material set elements during mass exchange between dispersed types of volume differing in properties. It was assumed that these processes can occur as a result of physical, chemical, and biological factors in specified limited volumes, and their separate types are generated randomly in the space of material medium. For the assumed paradigm, there is a possibility to build phenomenological and mathematical process models. The basic description of matter formation assumes the form of a system of differential equations. Modeling methodology enables taking into account many phenomena and mechanisms appropriate for the investigated processes in the conducted analyses. The kinetics of matter formation description allows to recognize the effect of many factors and to develop the model of a given process at the desired level of complexity. Application areas of the theory, with its great potential, include numerous domains, such as medicine, biology, agriculture, environmental protection, and industry. The theory of random transformation of the distributed matter was already used for the description of the disintegration of microorganisms, inter alia, for the description of the effect of yeast cell size on the process course [13, 14]. The aim of this study is to apply the theory of a random transformation of the distributed matter in order to explain the dependency of the process rate constant on the initial microorganism concentration.

2. Theory

A bead mill is a tank filled with beads set in circulating motion by a rotating impeller. Microorganisms dispersed in liquid are broken as a result of the filling elements' impact on them. The type of mechanism to destroy cells results from the system of combined normal and tangential loading. Its three basic forms can be distinguished as caused by crushing, grinding, and rolling by means of balls. Generally, cell destruction mechanisms do not occur in a pure form in the circulating chamber filling. The process of the disintegration of microorganisms, a very complex one as it was presented in the introduction, is a random transformation of matter. In order to enable the specification and analysis of phenomena occurring during its course, the following phenomenological model of the disintegration of microorganisms in the circulating mill filling was developed [13–15]. The suspension of microorganisms constituting a set of material objects N occupies space in a mill V. In volume V_{ati}, being its part, volumes of transformation $V_{\gamma ji}$ are generated. It was assumed that the ith cell from the set N is destroyed

after a relative transfer from volume V_α, where conditions are safe for it, to the jth volume $V_{\gamma ji}$. The transfer from volume $V_{\alpha ji}$ to volume $V_{\gamma ji}$ takes place through a boundary surface $F_{\gamma\alpha\rightarrow}$. The mathematical description result of the phenomenological model is a first-order differential equation (Eq. 1) [15], in which the rate constant is determined by Eq. (2).

$$dN_d = \frac{F_{\gamma\alpha\rightarrow}}{V_\alpha} u \left(N_0 - N_d \right) dt \tag{1}$$

$$k = \frac{F_{\gamma\alpha\rightarrow}}{V_\alpha} u \tag{2}$$

The increase in the process rate of the disintegration of microorganisms in suspensions with high concentrations presumably is caused by the occurrence of the additional mechanism to destroy cells [5]. As a result of high concentration, microorganisms affect each other. By blocking cells between colliding balls with high kinetic energy, they cause an increase in volume $V_{\gamma ji}$. Thus, greater values of the process rate constant for large concentrations and high impeller rotational speeds may result.

If the above hypothesis is true, then during the process with a reduction in the number of live cells, volume $V_{\gamma ji}$ should decrease, and consequently rate constant k as well. Its value describing the process for suspensions with large concentration, after obtaining a sufficiently large degree of disintegration, should be the same as in the case of suspensions with small concentration.

3. Bead mill

The process of disintegration was investigated in a horizontal bead mill with a multidisk impeller. The capacity of the working chamber 80 mm i.d. was 1 dm^3. Circular, full disks of the impeller of diameter 66 mm were placed every 30 mm. All parts of the mill were made from acid-resistant steel. The inside of the mill was filled in 80% with balls of diameter ranging from 0.8 to 1.0 mm made from leadless glass of density 2500 kg/m^3.

The shaft of the multidisk impeller was coupled with a d.c. motor. A voltage control system enabled smooth change of the impeller speed in the range from 0 to 3600 rpm. The cooling jacket of the mill was connected to a Fisherbrand FBC 735 thermostat (Fisher Scientific GmbH). The temperature of 50% water solution of ethylene glycol cooling the mill was 4°C at the inlet to the tank jacket. The slurry temperature was about 4°C.

4. Biological material

Investigations were made using the suspension of commercially available baker's yeast *S. cerevisiae* produced in the Silesian Yeast Factory "Polmos" at Wołczyn. Biological material from

a single fermentation was transported to a laboratory after cooling for 36 h, which completed the technological process. All experiments were carried out within 14 days. Yeast cubes of 100 g were stored at a temperature of 4°C. The continuous phase of microbial slurry was a 0.1% aqueous solution of β-mercaptoethanol containing 0.01 M EDTA and 0.001 M PMSF. A pH of 7 was ensured by a phosphate buffer (NaH_2PO_4, Na_2HPO_4).

5. Range of investigation

Experiments were carried out batchwise (constant feed) at the rotational speed of the impeller ranging from 1000 to 3500 rpm. Thirteen experimental series were made for a suspension prepared from living yeast cells at concentrations ranging from 0.05 to 0.20 g d.m./cm^3. The series consists of two experiments carried out at the same parameters. In the experiments, a slurry was used for which the mean concentration value determined for five measurements did not differ from an assumed value by more than 5%.

Series	n	Slurry 1		Slurry 2		Slurry mixture	
		S	X	S	X	Concentration of living cells	Total biomass concentration
	rpm	g d.m./cm^3	%	g d.m./cm^3	%	g d.m./cm^3	g d.m. /cm^3
10	1000	0.1410	0	0.1974	99.5	0.0618	0.1727
11	2500	0.1393	0	0.1974	99.5	0.0599	0.1724
12	3500	0.1407	0	0.1940	99.5	0.0594	0.1711

Table 1. Parameters of a slurry made from living and previously disintegrated cells.

Three experimental series were carried out for a mixture of slurries characterized by total biomass concentration of about 0.17 g d.m./cm^3 and living cell concentration of about 0.06 g d.m./cm^3. The parameters of the slurry mixture and its components as well as the impeller speed during the process are given in Table 1. The slurry mixture was prepared by combining slurry 1 and slurry 2 in proper proportions. Microorganisms in slurry 2 were subjected to disintegration in the bead mill until reaching 99.5% disintegration degree. Rotational speed of the impeller during the disintegration of microbial cells in slurry 2 corresponded to the impeller speed used during the disintegration of yeast contained in the slurry mixture.

6. Methodology

Changes in the slurry state were analyzed on the basis of spectrophotometric measurements of light absorbency in the supernatant at the wavelength 260 nm. Near this value, there are maximum spectra of spectral nucleic acids. The measurements were carried out using a

Lambda 11 spectrophotometer (Perkin Elmer GmbH). The supernatant was obtained after 20 min centrifugation of slurry samples at the temperature 4°C with a centrifugal force of 34,000g. A 3K30 ultracentrifuge (B. Braun Biotech International GmbH) was used. The degree of microorganism disintegration after time t of the process duration (Eq. 3) was calculated from the ratio of relative absorbency A, determined for a sample to the maximum relative absorbency A_m, which was observed at total yeast cell disintegration. A reference liquid was the supernatant obtained from the slurry prior to the process.

$$X = \frac{A}{A_m} = \frac{A' \cdot r - A_0' \cdot r_0}{A_m' \cdot r_m - A_0' \cdot r_0} \tag{3}$$

Depending on the cell disintegration degree, the slurry samples were diluted 0, 100, 200, or 400 times so that the measured absorbency did not exceed boundary values. Because of difficulties in using two parameters when describing the process kinetics, it was decided to convert the absorbency obtained at a given concentration into pure nucleic acids RNA released from the cell inside. Mean values for nucleotides used by Benthin et al. [16] were applied in the calculations. When solving the problem presented in this study, this simplifying assumption had no negative effects; however, the determined quantities of RNA could not be treated as precise values because of pollution of the supernatant with other intracellular components. In order to estimate the supernatant pollution, A/A_{250} and A/A_{280} ratios were studied. They were determined on the basis of the measurement of absorbencies A, A_{250}, and A_{280} identified for each sample in all experiments at the wavelengths 260, 250, and 280 nm, respectively. The effect of process conditions, and first of all the applied inhibitors of serine proteases and metaloproteases, was investigated by carrying out electrophoresis on 8% polyacryloamide gel SDS taken during slurry sample disintegration. The investigations were made for extreme concentrations of microorganisms at the impeller speed 2500 rpm.

The disintegration was investigated at different stages of the process, carrying out two experiments for fixed combinations of variable parameters. During the first experiment, 10 samples were taken in the time interval determined by the disintegration degree ranging from 0% to about 90%. On the basis of these data, a maximum amount of RNA possible to release, determined by the symbol C_{m1}, was obtained. At this amount, the correlation coefficient between variables t and $\ln[C_{m1}/(C_{m1} - C)]$ was the highest. From the process description obtained in this way, using the regression line passing through 0 (Eq. 4), the process time in which disintegration degrees ranged from 98.0% to 99.5% was calculated. These were boundary values of the time interval in which 9 slurry samples were taken during the second experiment. Having the measured absorbency values and disintegration degrees calculated on the basis of k_1, a maximum amount of RNA C_{m2} was calculated for a given concentration, which would be observed after the disruption of yeast cell walls. Along with the data from the first experiment, this value was used to prepare again the process description by the regression line which passed through 0 (Eq. 5).

$$\ln \frac{C_{m1}}{C_{m1} - C} = k_1 \cdot t \tag{4}$$

$$\ln \frac{C_{m2}}{C_{m2} - C} = k_2 \cdot t \tag{5}$$

The first part of the methodology, involving a maximum amount of the intracellular compo‐
nent determined as a result of the tendency to reach the best description of the process by the
first-order differential equation, was used by Currie et al. [6]. It was also employed to describe
part of the experiments with yeast *S. cerevisiae* disintegration in the vertical bead mill. Accord‐
ing to the researchers, such necessity followed from the fact that it was impossible to obtain a
satisfactory description of the process on the basis of a maximum amount of protein possible
to release R_m, determined for microorganisms disintegrated in a high-pressure homogenizer.
The authors quote that in five cases, they had to reject the data referring to the released protein
R defined by the ratio R/R_m above 0.6, so that the first-order description was possible. In the
subsequent six cases, such a description was not possible at all. A similar methodology was
used by Limon-Lason et al. [7], who revealed the lack of any possibility to describe correctly
the process carried out in the bead mill of volume 5 dm^3 basing on the amount of protein R_m
specified in the mill of volume 0.6 dm^3. On the other hand, van Gaver et al. [8] in all investigated
cases used only the values of R_m determined as a result of tending to achieve agreement
between the experimental data and the assumed first-order process description. This decision
was probably caused by much worse results obtained for experimentally determined maxi‐
mum quantities of protein R_m. According to the authors, the experimental value of R_m was
obtained after 10 cycles of slurry transition through the mill. In most publications, the maxi‐
mum amount of measured intracellular components was determined experimentally.

7. Results and discussion

Results of the disintegration experiments performed in order to characterize biological
material and the confirmation of its stability are given in Table 2. Strength properties of yeast
cells derived from given fermentation were determined by means of parameters characteristic
of the course of the disintegration process conducted for the extreme values of changed process
parameters (*S* and *n*) in the assumed wider research plan [5, 9, 17]. Experimental series from
the first to the fourth one were carried out at the beginning of the investigations, while those
from the thirteenth to sixteenth at the end. In the two first columns, the values of variable
parameters are quoted. In the subsequent columns, results obtained for the first (based on the
value of C_{m1}) and second methods (based on the value of C_{m2}) are presented. The maximum
concentrations of nucleic acids determined for 100% microorganism destruction, correlation
coefficient, process rate constant, and its standard deviations are given in subsequent columns.
On the basis of these results, it can be concluded that during storage, there were no changes
in yeast cell sustainability to mechanical destruction in the bead mill significant for the
investigations and changes that could distort the results of measurements.

Results of the investigations of process kinetics obtained by the first method revealed that the
first-order differential equation, according to the present theory, described very well the

Series	S	n	C_{m1}	R_1	$k_1 \cdot 10^3$	C_{m1}	R_2	$k_2 \cdot 10^3$
	g d.m./cm³	rpm	mg/cm³	---	1/s	mg/cm³	---	1/s
1	0.0513	1000	2.631	0.9983	1.307	2.317	0.9955	1.698
2	0.0491	3500	2.626	0.9984	6.024	2.434	0.9961	7.156
3	0.1991	1000	9.352	0.9937	1.817	8.725	0.9764	2.438
4	0.1991	3500	9.622	0.9971	9.632	8.745	0.9836	13.420
13	0.0485	1000	2.450	0.9997	1.348	2.263	0.9962	1.656
14	0.0485	3500	2.173	0.9996	6.986	2.252	0.9993	6.488
15	0.1957	1000	8.071	0.9907	2.149	7.935	0.9898	2.320
16	0.1957	3500	8.651	0.9962	10.429	8.106	0.9854	13.413

Table 2. Characteristics of biological material.

process run. The correlation coefficients for most of the experiments exceeded 0.9960. Only in two cases for slurry concentration 0.20 g d.m./cm³ and impeller velocities 1000 rpm they were slightly lower (Table 2). Experimental results along with regression lines obtained for different slurry concentrations are shown in Fig. 1. On the basis of these results, it can be concluded that the process rate increases with an increase of the initial yeast slurry concentration. Rate constants for the highest slurry concentrations determined at the impeller speed 2500 and 3500 rpm are higher by over 60% than those obtained for the lowest concentrations. In the process performed at the impeller speed equal to 1000 rpm, the differences between the values of constant k_1 obtained for slurries at concentrations 0.05 and 0.20 g d.m./cm³ are much smaller (about 40%) (Table 2). Standard deviations of the process rate constants for method 1 given in Table 3 are in the interval from 0.46% to 2.55%. In general, for higher slurry concentrations, higher values of the standard deviation were obtained. In the case of changes in the rotational speed of the impeller, the results are opposite.
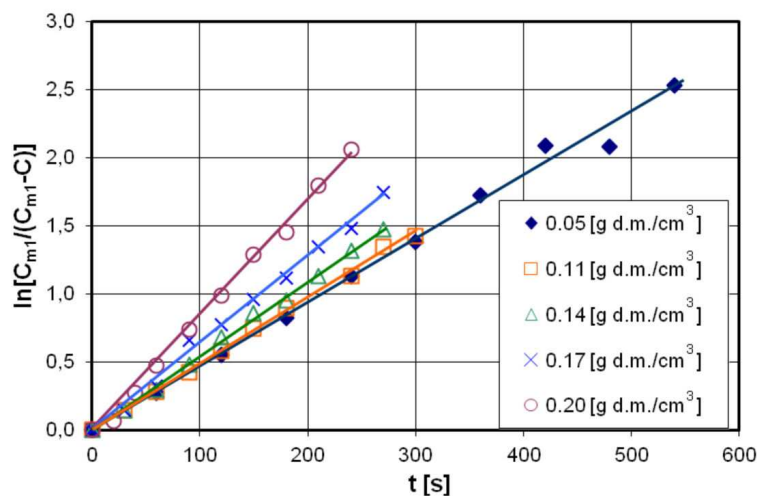

Figure 1. Microbial cell disintegration at different slurry concentrations (method 1, n = 2500 rpm).

Series	S	n	C_{m2}/C_{m1}	Standard deviation	
				Method 1	Method 2
	g d.m./cm^3	rpm	---	%	%
1	0.0513	1000	0.881	1.21	2.07
2	0.0485	1000	0.924	0.46	1.64
5	0.0491	2500	0.980	1.51	1.70
3	0.0491	3500	0.927	1.01	1.63
4	0.0485	3500	1.036	0.57	0.72
6	0.1102	2500	0.926	0.84	1.19
7	0.1431	2500	0.910	0.88	1.54
8	0.1645	2500	0.933	1.32	2.87
9	0.1991	1000	0.933	2.01	4.27
13	0.1957	1000	0.983	2.55	2.72
14	0.1974	2500	0.959	1.26	1.48
15	0.1991	3500	0.909	1.46	3.80
16	0.1957	3500	0.937	1.55	3.31

Table 3. Characteristics of experimental results.

Results of electrophoretic research carried out for the largest suspension concentration demonstrated clear separation of macroparticles, confirming the maximum limit of cutting proteins by enzymes released from highly specialized organelles. An increase of the disintegration effect with an increasing slurry concentration is not caused by an intensified supernatant pollution. Such a result was also confirmed by constant relations between absorbances being measured. Examples of the results obtained for experimental runs made for extreme parameters are shown in Table 4.

Series	S	n	A/A_{250}		A/A_{280}	
			Mean value	Standard deviation	Mean value	Standard deviation
	g d.m./cm^3	rpm	---	---	---	---
1	0.0513	1000	1.142	0.0036	1.797	0.0129
2	0.0491	3500	1.127	0.0073	1.748	0.0240
3	0.1991	1000	1.153	0.0022	1.790	0.0058
4	0.1991	3500	1.147	0.0032	1.767	0.0111
10	0.0618	1000	1.133	0.0039	1.721	0.0135
12	0.0594	3500	1.151	0.0030	1.789	0.0090

Table 4. Characteristics of the supernatant pollution degree.
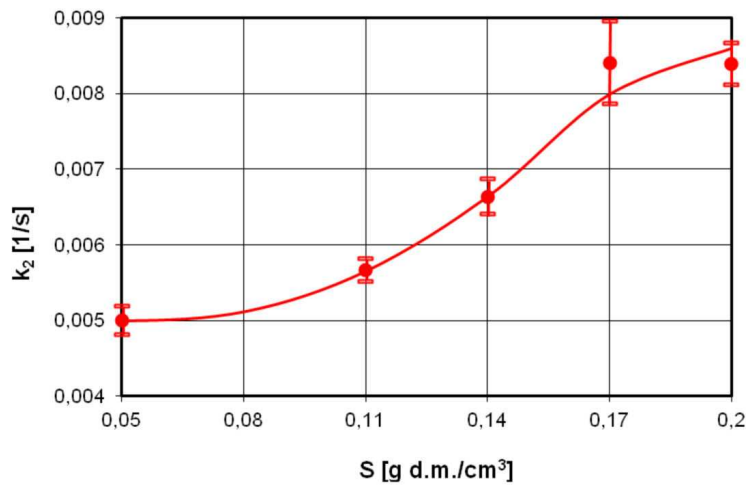
Figure 2. The effect of yeast slurry concentration on process rate constant at rotational speed of the impeller n = 2500 rpm (method 2, bottom and upper limits are marked for 95% of the confidence interval).

Changes of rate constants obtained by the second method, which take place at an increasing initial yeast cell concentration (Fig. 2, Table 2), are close to the ones obtained using method 1. Significant differences between values k_1 and k_2 were found. The process rate constants obtained by method 2 are from 7% to 39% higher than those obtained by method 1. An exception are the values obtained for slurry concentration 0.0485 g d.m./cm^3 at the impeller speed 3500 rpm (Table 1). The correlation coefficients obtained when describing the process by Eq. (5) are lower than R_1 (Table 2) and are in the range from 0.9764 to 0.9993. Much less advantageous are also standard deviations of the process rate constant (Table 3).
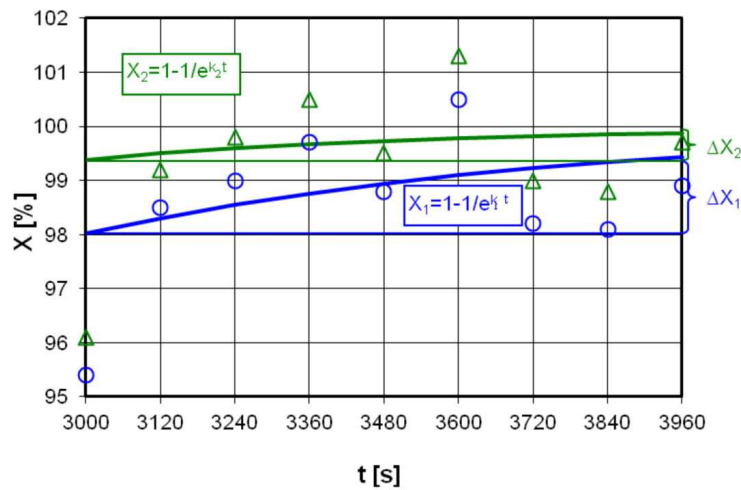
Figure 3. Changes of disintegration degree on the second stage of the process (S = 0.05 g d.m./cm^3, n = 1000 rpm).

Higher values of constant k_2 are due to the fact that values C_{m2} are lower than C_{m1}. They are compared in Table 3. The amount of nucleic acids C_{m2} for the slurry with disintegration degree

X=100% was determined based on the result of an experiment planned and carried out on the basis of the rate constant k_1. Therefore, values C_{m1} and C_{m2} should not differ very much and should reveal a typical statistical dispersion in relation to the real value. As it is not so, it was checked if the points used to determine C_{m2} were better described by the rate constant k_1 or k_2. Since the values of rate constant k_2 are higher than k_1, then in time interval Δt (time of process investigation in the second experiment), differences of the disintegration degree ΔX_2 are smaller than ΔX_1 (Fig. 3). For k_1 and k_2 in time, which determines slurry sampling, the disintegration degrees X_1 and X_2 were specified. On this basis and taking into account the measured values of absorbency A, A_{250}, and A_{280}, such values that would occur at 100% microorganism disintegration were identified. Only for three experiments, rate constant k_1 describes better absorbencies A, A_{250}, and A_{280}. In one experiment, the values of A are better described by rate constant k_1 and the values of A_{250} and A_{280} by rate constant k_2. In all other experiments, the values of standard deviation are lower than when rate constant k_2 is used to determine the value of relevant absorbencies, which occur when the total content of microbial cells is released. As differences between ΔX_2 and ΔX_1 were insignificant as compared to measuring errors of absorbency for the disintegration degree close to 100% [18], this result was assumed sufficient to claim that the rate constant k_2 described better the process tested in the second experiment. It follows that the values of C_{m2} should be closer to the real values at $X = 100\%$. The correlation coefficient for linear regression between variables C_{m1} and S was 0.9873, and between C_{m2} and S, it was 0.9949 (Fig. 4).
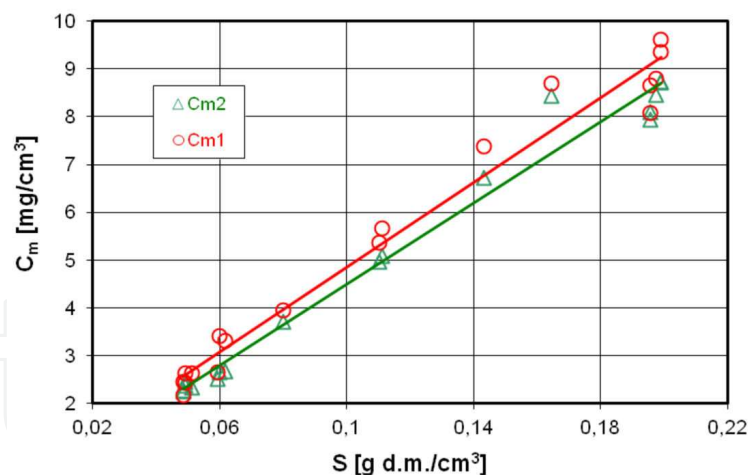
Figure 4. The effect of changes in the slurry concentration on determined values of C_m.

If in two separate time periods the process run can be described more precisely using different values of rate constant, then it may be assumed that the disintegration rate constant changes during the process. Fig. 5 shows experimental changes in the disintegration degree described on the basis of C_{m2}. The blue line denotes changes that result from the process description by Eq. (6) ($\Delta t = 0$).
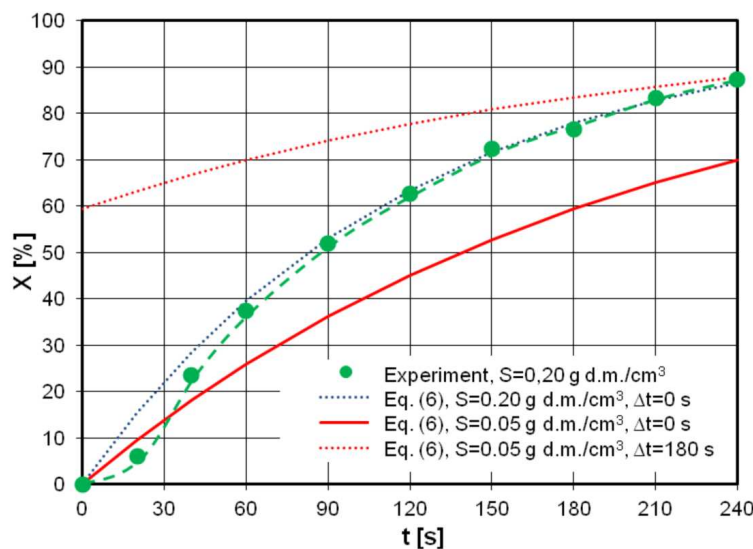
Figure 5. Changes of disintegration degree in the process carried out at slurry concentration 0.20 g d.m./cm^3 and impeller speed 2500 rpm.

$$X_2 = 1 - e^{-k_2(t + \Delta t)} \tag{6}$$

In first part of the process (from 0 to 120 s), the experimental points are located below the theoretical line, while in the second one, the results obtained are consistent. The position of the experimental points can be hardly taken as random. Real changes of the disintegration degree are close to the changes marked by the green line. Fig. 5 contains the process model obtained for the suspension concentration of 0.5 g d.m./cm^3 (red line). In this case, the dependency presented in Eq. (5) described the process course very well (Fig. 1).

During the cell disintegration in a suspension with concentration of 0.20 g d.m./cm^3, the process initially (up to 20 s) proceeds at a considerably slower pace than the model disintegration (blue line) determined at this concentration and at even slower pace than the process determined for 0.05 g d.m./cm^3. This can result from very large cell density at their largest concentration. The analysis of changes in the distance between geometrical centers of yeasts followed by an increase in their concentration was presented [5, 17]. In these considerations, the assumption of distributing cells in a close-packed hexagonal network was adopted because such a distribution provides the largest distances between microorganisms. The determined distances at such a distribution are boundary distances, the most favorable for minimizing interactions between microorganisms. Any other distribution of cells, including the actual one, causes a further increase in the intensity of their interaction. In accordance with the stated dependencies, at concentration of 0.20 g d.m./cm^3, the interaction between neighboring microorganisms is very large. The cell size distribution for yeasts used for the research in this work (Fig. 6) was very similar to the size distribution of microorganisms used in earlier works [5, 9, 13, 14, 17]. The comparison of results obtained in tests by means of laser particle size analyzers, with results obtained as a result of the computer analysis of microscopic images is included in the published work [19]. A cell before being disrupted is compressed between balls. Its

dimensions in a plane perpendicular to the compression axis increase. This is a consequence of neighboring microorganisms being driven out from the danger zone when, e.g., two balls approach them. The result of this visible in Fig. 5 is the reduction in the cell disruption rate at the initial stage of the process. The described mechanism does not cause changes in the transformation volume $V_{\gamma ji}$ nor volume unavailable to a live ith cell $V_{\beta ji}$. In the developed theory basics [15], in order to simplify the initial considerations, independence of transformation events was assumed. In the case described above, the disruption of one cell increases the chance of saving the neighboring cell. The result of such impact will be deviations of the process course from linearity. Changes in the process rate constant at the time of its duration are shown in Fig. 5.
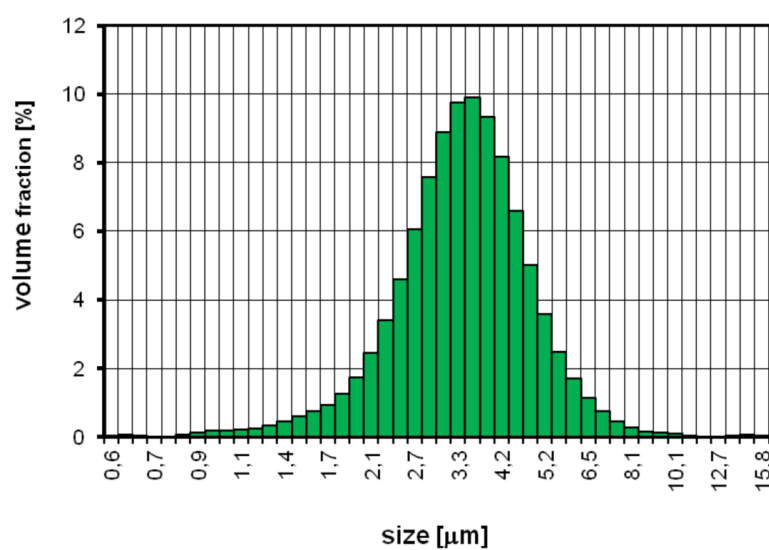

Figure 6. The yeast particle size distribution in the suspension.

In the suspension with a concentration of 0.20 g d.m./cm³, the rate of microorganism disruption and release of intracellular compounds increases significantly from about 20 to 120 s of the process duration (Fig. 5). Is it greater than the cell disintegration rate in a suspension with concentration of 0.05 g d.m./cm³ and also greater than the process course rate determined from Eq. (5) for the disintegration of microorganisms in the suspension with concentration of 0.20 g d.m./cm³. Such an effect can result from an increase in the transformation volume $V_{\gamma ji}$ and volume $V_{\beta ji}$ unavailable to the live ith cell. Hypothetically, it is the result of blocking a cell in the danger zone by neighboring unthreatened microorganisms [5].

At the final stage of disintegration, the course of the process of the yeast cells disintegration carried out in the suspension with concentration of 0.20 g d.m./cm³ is not in accordance with the course obtained for the suspension with concentration of 0.05 g d.m./cm³. At the end of the process being carried out for the higher biomass concentration, there is a significant reduction in the number of viable cells. The rate constant is much higher than specified for the disintegration of the yeast cells at a concentration of 0.05 g d.m./cm³.

Fig. 6 shows the graphic analysis of the process course for the cell disintegration in a mixture of slurries (Table 1, Series 11) analogical to the one presented above. In this case, due to less intensive interactions between neighboring yeast cells, there was probably no pushing out of cells from the danger zone by compressed microorganisms. The obtained values of rate constant (Table 5) are large over all the investigated time frame. They are much greater than those obtained with a suspension concentration of 0.05 g d.m./cm³ (Fig. 7, red line). The values of the rate constant are similar to those obtained for the concentration of 0.17 g d.m./ cm³ (Fig. 2).
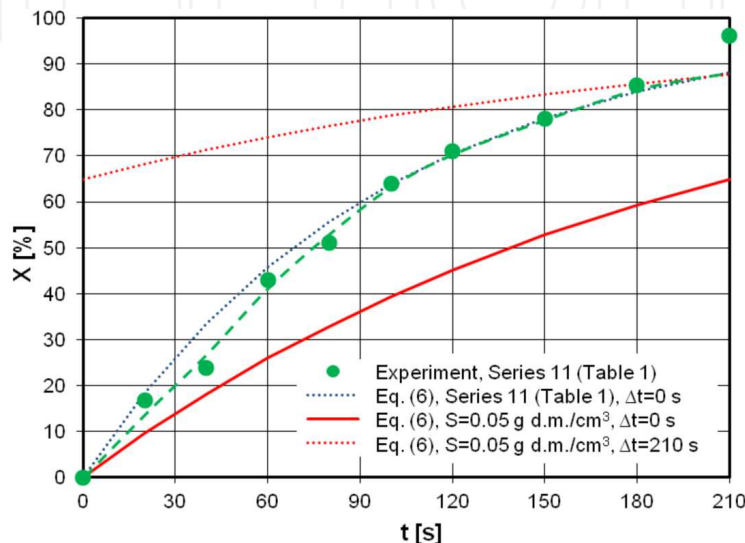
Figure 7. Changes of disintegration degree in the process carried out at biomass concentration 0.17 g d.m./cm³, living cell concentration 0.05 g d.m./cm³, and impeller speed 2500 rpm (Series 11, Table 1).

Such a process run, which is more or less distinct in all experiments performed, provides the evidence that the additional cell destruction mechanism is a result of the action of comminuted cell wall fragments, and not of the living microorganisms. The deviation of experimental points similar to the ones presented above was obtained by Melendres et al. [10], who investigated the process of disintegration on the basis of living cells specified using a hemocytometer. The results obtained by the researchers were a basis to develop a first-order mathematical model of the process based on the analogy to the theory of gas kinetics. Using their own data, Middelberg et al. [18] proved better agreement of the disintegration degree determined on the basis of the absorbency measurement ($\lambda = 260$ nm) and the number of living cells than that obtained from released proteins and living cells. According to Limon-Lason et al. [7], the process of disintegration is related to two phenomena: cell wall disruption and protein release. The latter phenomenon is much slower than the first one. According to the results obtained by Middelberg et al. [18], the difference between cell wall disruption rate and the rate of nucleic acid release is negligible.

Fig. 8 shows a graphical interpretation of disintegration kinetics described by Eq. (5). The regression line is not in agreement with experimental points. The real process run is not linear. Constant k_a at the onset of the process ($t \to 0$) is lower than the constant determined for time $t = 200$ s, and this in turn is smaller than constant k_m determined for time 1200 s. Changes in
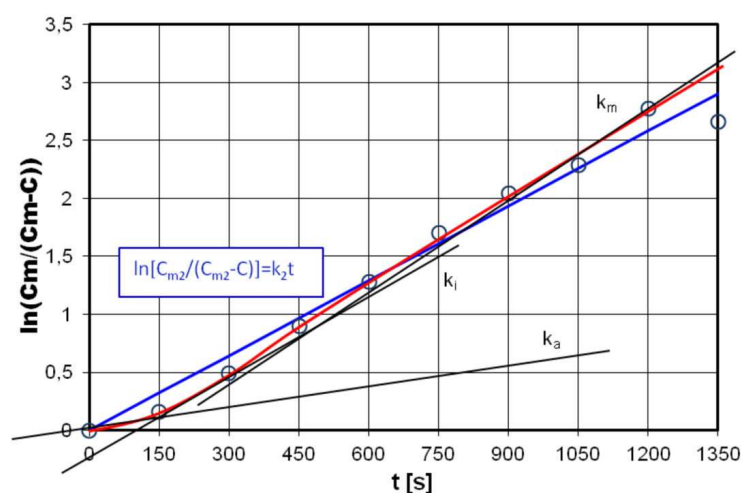
Figure 8. Yeast disintegration kinetics in the slurry at concentration 0.05 g d.m./cm³ and impeller speed 1000 rpm.

the rate constant during the microorganism disintegration can be explained by an increase of the concentration of very fine fragments of cell walls. Irrespective of the initial slurry concentration, the rate constant at the beginning of the process k_a is always the same at lower concentrations. This is confirmed by the position of first experimental points obtained after starting the process. The mean value of k_a for slurries made exclusively from living cells of yeast disintegrated at the impeller speed 2500 rpm is 0.005146, and its standard deviation is 6.21%. For high concentrations of microbial slurry, the process rate increases significantly as a result of formation of numerous fine fragments of cell walls. The further run of the process is affected by their concentration, which depends on the rate of microgrinding and the amount of material being ground.
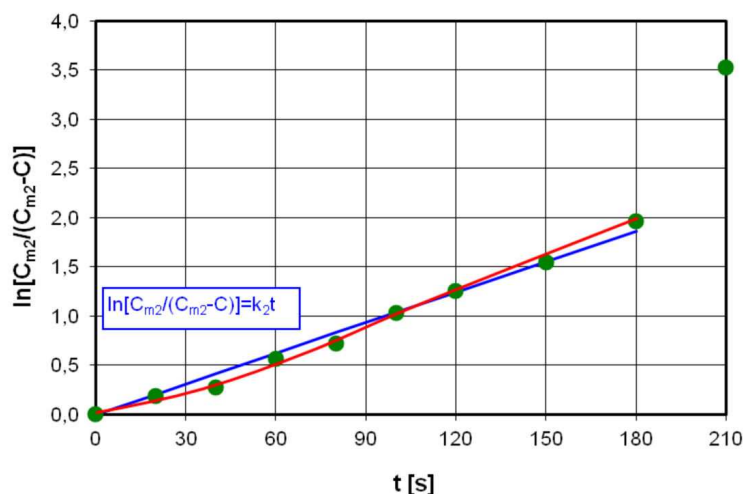
Figure 9. Yeast disintegration kinetics in the slurry mixture (biomass concentration 0.17 g/cm³, living cell concentration 0.05 g/cm³, n = 2500 rpm).

Series	n	C_{m1}	R_1	$k_1 \cdot 10^3$	C_{m2}	R_2	$k_2 \cdot 10^3$
	rpm	mg/cm³	---	1/s	mg/cm³	---	1/s
10	1000	2.803	0.9942	2.651	2.647	0.9912	3.203
11	2500	2.950	0.9970	8.078	2.621	0.9936	10.375
12	3500	2.742	0.9835	13.049	2.511	0.9682	19.964

Table 5. Results of microorganism disintegration in the slurry mixture.

In the case of the disintegration of microorganisms contained in the slurry mixture (experimental series 10, 11, and 12), much higher values of k_1 and k_2 (Table 5) were obtained than in the case of ordinary slurries with a comparable concentration of living cells. The change of conditions that define the increase of constant k during the process is determined not only by the presence of cell wall fragments but also by an increasing level of their comminution. Nonlinearity of the process kinetics (Fig. 9) is the evidence that the boundary value of concentration of small cell fragments has not been reached yet. The rate constant after starting the process is higher by 25% than constant k_a obtained for slurries made exclusively from living microorganisms. The correlation coefficients smaller than in other cases result from insignificant changes of absorbency during the process as compared to the initial absorbency.

8. Conclusions

At low concentrations of the suspension, cell disruption in the mill filling is an independent event. The process course is described very well by a linear first-order differential equation. During the disintegration of the microorganisms, disruption of the cells, release of intracellular compounds, and grinding of the fragments of broken walls take place.

An increase in the yeast suspension concentration in the range of 0.05 to 0.20 g d.m./cm³ causes a significant reduction in distances between geometrical centers of neighboring cells and significant intensification of interactions between them.

At very high concentrations of the suspension, at the initial stage, that is, from the disintegration level 0% to 5%, the process course reveals deviations from linearity. An event consisting of cell disruption can affect the fate of neighboring cells. A cell being deformed as a result of compression pushes out cells adjacent to it from the danger zone. Thus, transformation events of objects from set N are not independent events. This effect does not cause an increase of the transformation volume and volume unavailable to the live ith cell. It seems that this phenomenon causes a significant decrease of the rate of cell disruption and release of intracellular compounds.

At high concentrations of the suspension, the process course reveals deviations from linearity. The cell disruption and the intracellular compound release rate are greater than the rate determined from the linear model for smallest concentration. At high initial concentration of microbes, reducing the number of cells during the disintegration does not reduce the rate

constant of the process. Increasing the transformation volume and the volume inaccessible to the *i*th cell is to increase the rate of disintegration. It appears that increasing the amount of the intracellular compounds released or increasing the grinding degree of cell walls increases the volume of the transformation, and the volume is inaccessible to the *i*th cell.

At very high concentrations of the suspension, at the initial stage, that is, from the disintegration level 0% to 5%, the disintegration rate is influenced by both mentioned phenomena. In the case of the maximum concentration, the second mechanism, causing a significant reduction of the disintegration rate in comparison to the rate determined from the linear model, predominates.

Nomenclature

A; absorbency at wavelength $\lambda = 260$ nm

A_m; maximum absorbency determined for wavelength $\lambda = 260$ nm

A_{250}; absorbency at wavelength $\lambda = 250$ nm

A_{280}; absorbency at wavelength $\lambda = 280$ nm

A'; absorbency of the supernatant as related to distilled water

C; amount of pure nucleic acid corresponding to absorbency A, mg/cm³

$F_{\gamma\alpha\rightarrow}$; slurry flow area from volume V_α to $V_{\gamma ji}$, m²

k; process rate constant, 1/s

n; rotational speed of the impeller, rpm

N_d; number of disintegrated cells

N_o; initial number of living cells

S; concentration of microorganism slurry, g dry matter/cm³

t; process duration, s

u; slurry transition rate from volume V_α to V_β, m/s

V; maximum slurry volume in working chamber of the mill, m³

V_α; mill volume at physical conditions safe for microorganisms, m³

$V_{\beta ji}$; volume not available for nontransformed *i*th object from set N, m³

$V_{\gamma ji}$; the *j*th transformation volume of the *i*th material object from set N, m³

X; cell disintegration degree, %

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