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High voltage electrical discharges (HVED) and high pressure homogenization (HPH) were compared for extracting intracellular compounds from yeasts. The efficiency of cell disruption was evaluated by measurements of electrical conductivity, UV-spectroscopy, etc. It was shown that the HPH permits better extraction than HVED. The filtrations of yeast suspension were studied in a dead-end stirred cell. The filtration of untreated, HVED and HPH treated yeast suspensions without stirring was governed by cake formation mechanism. The filterability of HVED and especially HPH-treated yeast suspension was very low. Under stirring, different filtration mechanisms were observed for untreated and treated yeast suspensions.

Keywords cell disruption; dead-end microfiltration; high pressure homogenization; high voltage electrical discharges; *Saccharomyces cerevisiae* yeast suspension; stirring

INTRODUCTION

The cytoplasm of (*Saccharomyces cerevisiae*) yeast cell is a rich source of bio-products (proteins, cytoplasmic enzymes, polysaccharides, etc.) valuable in the biotechnology, the pharmacology, and the food industry. For the good recovery of these intracellular bio-products, the efficient breakage of the cell walls preserving cell compounds and effective separation of cell debris are essential.

Numerous techniques have been proposed for cell disruption (1,2). High-pressure homogenization (HPH) has been shown to be one of the most effective ways for the mechanical disruption of microbial cell walls and is employed for the large-scale industrial recovery of intracellular products. It has been a subject of continuing research interest for the past two decades. This method results in considerable breakage of cells and high recovery of bio-products. However, HPH is restricted by temperature elevation, requires multiple product passages with supplementary cooling, and the final products contain a large quantity of cell debris, which complicates the downstream processes of purification.

Nowadays, applications of the electrically induced extraction technologies for the production of homogeneous and heterogeneous proteins are very promising (3). High voltage electrical discharges (HVED) have found application for inactivation of microorganisms (4) and extraction of soluble materials from the cellular tissues (5,6). Underwater HVED (40 kV) of microsecond duration create an electric arc in liquid, powerful light radiation, pressure shock waves, bubbles cavitation, and other physical phenomena, which provoke the mechanical disintegration of cell walls and cell membranes (7,8). It was shown (7) that the HVED treatment of β-lactoglobulin solutions provokes secondary and tertiary changes of the protein structures; however, protein aggregation was not observed. HVED seems to have no negative effect on the quality of proteins extracted by this method (9,10). Recently, the application of combined HVED – HPH for yeast disruption was reported (11). It was shown that even a small cell damage initially induced by HVED pretreatment results in a higher yield of extracted proteins. Loginov, M (12) also obtained effective extraction of intracellular proteins from yeast cells by using HVED.

Extraction of soluble protein from cell debris is an inherently difficult operation (13–15). High-speed centrifuges have been traditionally used for the recovery of soluble protein from cell debris (16–18) and for the isolation of inclusion bodies from cellular debris and soluble protein (19–21). Liquid–liquid extraction has been used for the recovery of protein after solubilization of the inclusion bodies (22). Membrane filtration has been used as an alternative for the separation of soluble intracellular protein from cell lysates (23–26). The application of the membrane

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filtration technique allows filtrate recovery and cell debris removal with satisfactory efficiency both in cross-flow and dead-end systems (27–31).

In the microfiltration technique, the main focus is on flux and the quality of the product. Application of filtration for treatment of bio-suspensions is hindered by membrane fouling and formation of a cake layer with high compressibility (32,33). Fouling substantially decreases the filtration flux rate and transmission of bio-molecules through the membrane. The apparition of fouling can be evidenced by analyzing the filtration rate through the Hermans-Bredée's equation. Various mechanisms of filtration, including cake formation and models of standard, complete, and intermediate pore blocking, were proposed for the description of the membrane fouling (34,35).

Stirring can be considered as a good means to reduce the membrane fouling and improve the filtration process. For instance, Lora and Arnal (36) had studied the effect of agitation on reverse osmosis desalination, the results reflected the marked improvement in fluxes compared with unstirred operation and some increase in salt rejection was noted. Reihanian (37) used unstirred and stirred devices for separation of proteins by ultrafiltration and found different fouling mechanisms.

While several examples of membrane filtration of cell debris have been presented in the literature, the influence of the cell disintegration degree achieved by different emergent techniques on yeast suspension characteristics and dead-end microfiltration behavior was not studied enough.

Two methods were used in this paper for yeast cells disruption: HPH and HVED. These methods enable to obtain complex suspensions with different content of disrupted and undisrupted cells, cell debris, and extracted biomolecules. Influence of the yeast desintegration degree and suspension stirring on filtration efficiency and fouling mechanisms was investigated.

MATERIALS AND METHODS

Yeast Suspension

Wine yeast *S. cerevisiae* (bayanus) cells (Vitilevure DV10, Station Oenotechnique de Champagnc, Epernay, France) were used throughout this study. Considering the increase of electrical conductivity of liquid after the treatment, suspensions of 0.5% w/w were prepared by mixing the dry yeast cells and distilled water under magnetic stirring (rotation speed was 100 rpm) at room temperature for 0.5 h.

Analysis Instruments

The size distributions of aggregates in suspensions were recorded using a laser diffraction instrument (Mastersizer X 6618, Malvern Instruments GmbH. UK). The optical system allowed the detection of particles sized within 1–1000 μm. The particle size distribution was calculated by the original Malvern software.

For absorbance mesurement, a 15 mL sample of suspension was centrifuged for 5 min at 4000 g after treatment, and the suspension was collected. The turbidity of the supernatant was measured by Ratio/XR Turbidimeter (Hach, Loveland, USA). The UV absorption spectrum of the supernatant was measured by UV-spectrophotometer Biochrom Libra S 32 (UK). Prior to absorption measurements, the supernatant was diluted 100 times with distilled water. The wavelength range was within 190–360 nm (±1 nm). The path length of the SUPRASIL quartz cuvette was 10 mm (Hellma, Müllheim, Germany).

The electrical conductivity of suspensions was measured at 25°C using a conductivity instrument InoLab pH/cond Level 1 (WTW, Weilheim, Germany) with conductivity probe WTW Tetra Con 325. The electrical conductivity of suspensions is related to the extraction of ionic intracellular components from damaged cells. The degree of yeast damage can be evaluated by the conductivity disintegration index Z (11,38):

$$Z = \frac{\sigma - \sigma_i}{\sigma_{\max} - \sigma_i} \tag{1}$$

where σ is the electrical conductivity of yeast suspension after HVED or HPH treatment, σ_i is the initial electrical conductivity of yeast suspension before treatment, and σ_{max} is the electrical conductivity of yeast suspension with maximum of disintegrated cells. The above equation gives $Z = 0$ for the untreated yeast and $Z = 1$ for a maximally disintegrated material. The value of σ_{max} was estimated by measuring the electrical conductivity of the suspension treated by HPH technique after 20 passes at a fixed pressure of 800 bar. After such treatment by HPH, the electrical conductivity reached its maximal value and the disintegration index was $Z \approx 1$.

HPH Treatment

The yeast suspensions were homogenized in a two-stage high pressure homogenizer at a fixed homogenizing pressure of 800 bar. The flow rate was 10.0 L/h, and 250 mL of suspension in total was processed. Only the first HPH valve with a ceramic ball (190015, Niro Soavi S.p.A., Parma, Italy) and stainless steel seat (0126311, Niro Soavi S.p.A., Parma, Italy) were used in this study (Fig. la). The temperature of the suspensions was measured by thermocouple immediately after homogenization. Then the suspensions were cooled to room temperature for their further characterization or for the next pass through the homogenizer.

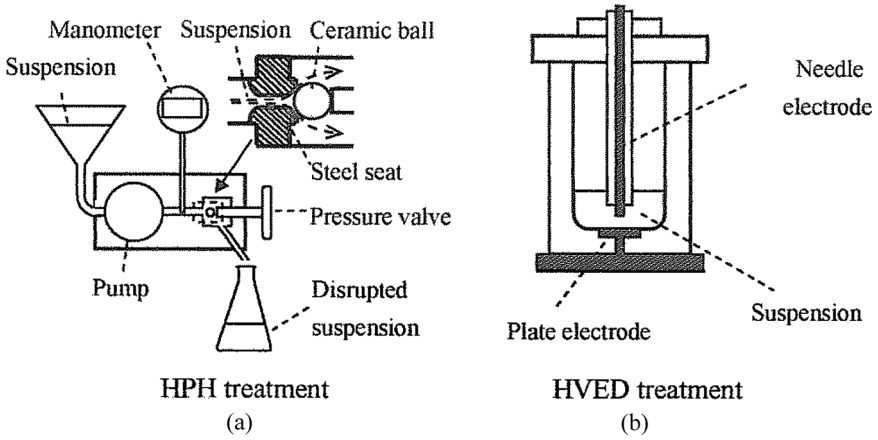

FIG. 1. (a) Scheme of the HPH treatment; (b) Scheme of HVED treatment.

HVED Treatment

Experimental HVED apparatus (Tomsk Polytechnic University, Tomsk, Russia) consists of a high voltage pulsed power supply and a laboratory treatment chamber with electrodes of a needle-plate geometry (Fig. lb). A stainless steel needle of 10 mm in diameter was used. The grounded plate electrode was a stainless disk of 25 mm in diameter. A positive pulse voltage was applied to the needle electrode. The high voltage pulse generator provided 40 kV–10 kA discharges in a one-liter chamber. The distance between the electrodes was 10 mm, and the peak pulse voltage was 40 kV. It corresponds to the mean electric field strength of 40 kV/cm. The electrical discharges were generated by electrical breakdown in the yeast suspension. Energy was stored in a set of low-inductance capacitors, which were charged by a high-voltage power supply. Damped oscillations with a frequency of 0.5 Hz were thus obtained, the effective pulse duration corresponded to a few microseconds (2 μs) (10). The treatment chamber was initially filled with 500 mL of yeast suspension (0.5% w/w). The HVED treatment lies in the application of N_{HVED} successive pulses ($N_{HVED} = 1$–500). Disrupted yeast suspension characteristics were measured between successive applications of the HVED pulses. A sample of 5 ml after each 100 pulses was taken for further measurements.

Dead-End Microfiltration

Dead-End Microfiltration Module

Dead-end microfiltration was performed in a stirred cell (Fig. 2) (filter area 31.65 cm^2 and total volume 180 mL) (Millipore, Billaica, USA). Given the size of the cell debris, a nylon membrane (nominal pore size 0.2 μm) was used as the filtration membrane, polypropylene filter cloth 25302 AN (pore size 25 μm, SEFAR FYLTTS. Lyon, France) was used as a filter support. All filtrations were carried out at constant pressure of 1 bar at room temperature and a new membrane was used for each filtration. Filtration was started immediately after the filter-cell was poured with freshly treated yeast suspension. The permeate was collected in a vessel placed on an electronic balance and the permeate weight was recorded by computer software (Service électronique UTC, Compiègne, France). Different rotation speeds between 0 and 500 rpm were tested.

Determination of the Specific Cake Resistance α

The specific cake resistance α is often used to characterize the hydrodynamic resistance of the cake during the dead-end filtration of particulate suspensions. The specific cake resistance can be calculated by using the following equations (39):

$$t/V = k_1 V + k_2$$
$$k_1 = \frac{\mu\omega\alpha}{2\Delta P A^2}, \quad k_2 = \frac{\mu R_m}{2\Delta P} \tag{2}$$

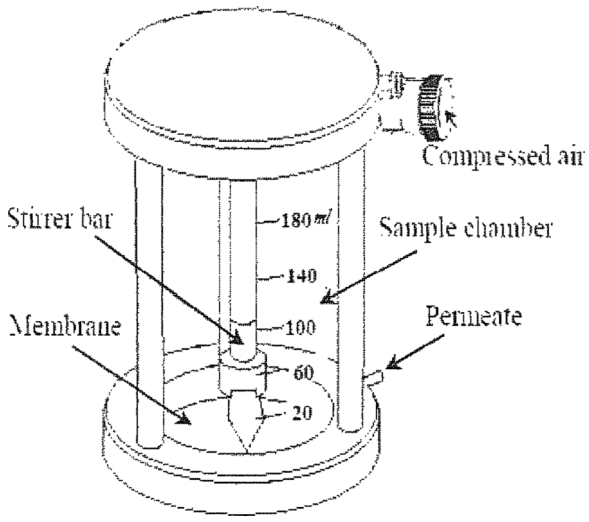

FIG. 2. Schematic diagram of dead-end microfiltration apparatus.

where t is the filtration time, V is the filtrate volume, k_1 and k_2 are the slope and intercept of the curve t/V vs. V, respectively, α is the specific cake resistance, μ is the viscosity, ω is the mass of solid particles deposed per unit volume of filtrate, P is the filtration pressure, A is the membrane surface, R_m is the membrane resistance.

General Characteristic Form

The filtration laws can be written into one characteristic form presented in Eq. (3). This equation represents the resistance (inverse flux, dt/dV) related to the change in resistance (d^2t/dV^2) (40)

$$\frac{d^2t}{dV^2} = k\left(\frac{dt}{dV}\right)^n \qquad \frac{d^2t}{dV^2} = -\frac{1}{J^3A^2}\frac{dJ}{dt}, \quad \frac{dt}{dV} = \frac{1}{JA} \tag{3}$$

where k and n are constants and J is the permeate flux. The exponent n characterizes filtration mechanism: $n=0$ for the filtration with cake formation, $n=1$, 1.5, and 2 for intermediate, standard, and complete pore blocking mechanisms, respectively.

All the experiments were repeated at least three times. The mean values and standard deviations were calculated.

RESULTS AND DISCUSSION

Comparison of HVED and HPH Modes of Treatment

The mechanisms of disruption of yeast cells after application of different modes of physical treatment may be rather different. HVED treatment generates pressure shock waves and air bubbles cavitation contributing to the mechanical damage of yeast, disintegration of cell walls, and membrane rupture (5,6). HPH treatment results in mechanical breakage of cell walls, disintegration of cell aggregates, suspension homogenization, etc. (41).

The efficiency of cell disruption can be estimated by the conductivity disintegration index Z. Evolutions of the conductivity disintegration index Z and the temperature of suspension T during HPH and HVED treatments are presented in Fig. 3. The values of Z and T increase gradually with the number of treatment cycles N both for the HPH and HVED methods. Increase of Z during the treatment is related to the extraction of ionic intracellular components from damaged cells and it is proportional to the disruption degree. Disintegration and extraction attain their maximal level after long-term treatments. For the HVED treatment, the highest level of yeast disintegration ($Z \approx 0.75$) required 500 pulses with effective treatment time of 1000 μs, while for HPH treatment, 20 passages under pressure of 800 bar with a duration of 14400 s were needed to attain the value of $Z \approx 1$. The temperature elevation resulting fiom the HPH treatment was significantly higher than those resulting from HVED treatment (Fig. 3). The final temperature of suspension treated by HPH exceeded 50–55°C, which is critical for the preservation of intracellular bio-products (proteins and cytoplasmic enzymes) from thermal degradation. So the yeast suspension cooling was needed for the HPH with multiple passes.

Various methods were carried out to characterize the yeast disruption caused by HPH and HVED. The evolution of particle size distribution is shown in Fig. 4. For untreated suspensions, the size distribution function (SDF) was approximately log-normal with maximum d_{max} located at 4.7 ± 0.1 μm and with a noticeable fraction of cell aggregates with the mean total diameter higher than 10 μm. Disruption of yeast by HPH and HVED techniques resulted in decrease of the average particle size. The values of d_{max} were located at 3.5 ± 0.1 μm and 4.3 ± 0.1 μm for

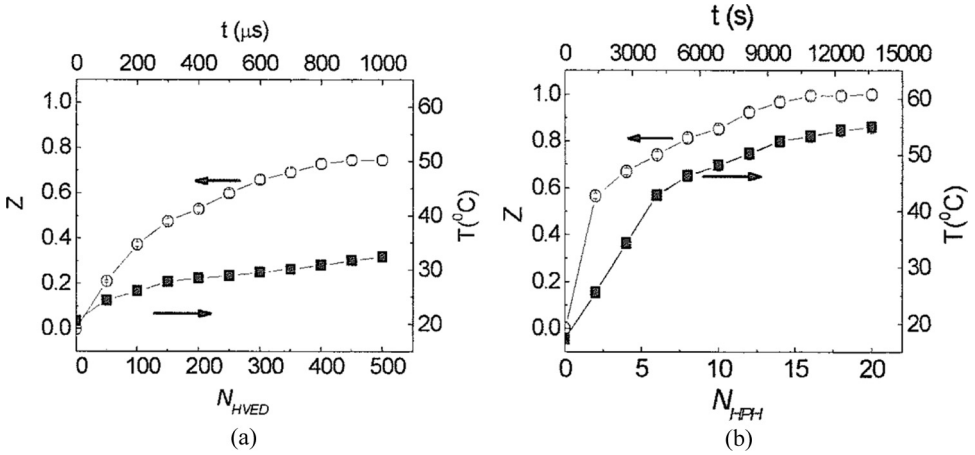

FIG. 3. (a) Z and T vs. number of HVED pulses (N_{HVED}) and effective HVED duration t (μs); (b) Z and T vs. number of HPH passes (N_{HPH}) at 800 bar and HPH duration t (s).

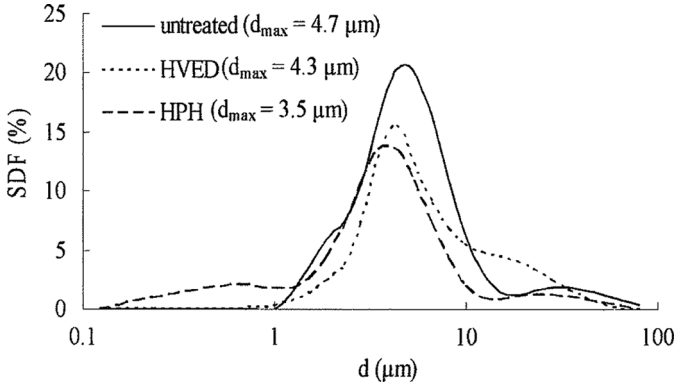

FIG. 4. Size distribution function of untreated ($Z=0$) and maximally disrupted by HVED ($Z\approx0.75$) and HPH ($Z\approx1$) yeast suspensions.

yeast suspensions treated by HPH and HVED respectively, which evidences disaggregation and damage of cells. Particles smaller than 1 μm were observed for HPH treatment, and the formation of cells aggregates with the mean total diameter higher than 10 μm was clearly observed for HVED treated suspensions. Loginov, M. (l2) also reported the formation of the aggregate after the HVED treatment. These differences in the SDF shape and location of d_{max} can reflect different mechanisms of disruption of the yeast cells, as well as distinctions in the aggregation of cells and cell debris.

In order to estimate the efficiency of extraction of the high molecular weight compounds, the absorbance spectra of the supernatant solutions of yeast suspensions were measured. In accordance with literature data (42) the peak observed at the wavelength $\lambda\approx210$ nm corresponds to absorption by bonds of peptides and proteins, which are the main intracellular constituents of the *S. cerevisiae,* and peak at $\lambda\approx260$ nm corresponds to nucleic acids. Both absorbances gradually increased with the increase of treatment cycles, The similar tendency is reported by Ohshima, T. (43) who observed a correlation between the absorbance increase and the treatment cycles. Data also show that the absorbance of the supernatant is much higher for the HPH-treated suspensions than for the HVED-treated suspensions for all disintegration index (except $Z=0$) (Fig. 5a). Hence, the disruption of yeast cells by the HPH method seems to be more effective than the HVED-treatment and results in higher release of proteins. This conclusion agrees with data on the turbidity of the treated suspensions (Fig. 5b). Increase of turbidity with increase of Z reflects the release of proteins and fine cell debris during the treatment. Furthermore, turbidity is much higher after homogenization of yeast suspension than after HVED treatment at the same level of Z. These results are in agreement with the studies reported by Shynkaryk, M. et al. (11) in which a higher extraction of proteins from yeast cells treated with HPH was described.

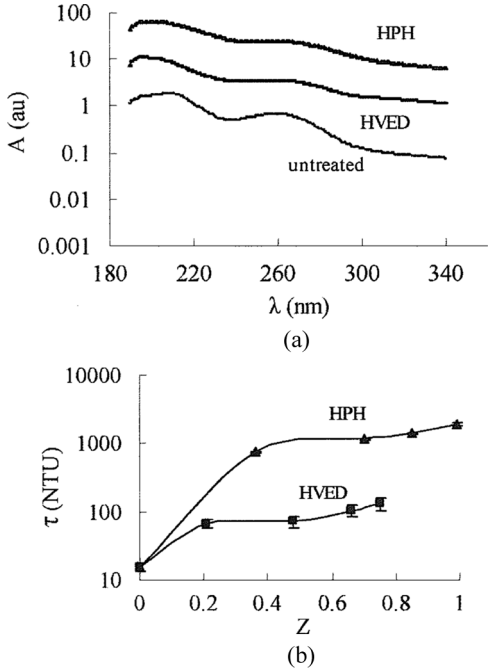

FIG. 5. (a) Absorbance spectra for untreated, and maximally disintegrated by HVED ($Z\approx0.75$) and HPH ($Z\approx1$) yeast suspensions; (b) turbidity vs. Z for yeast suspensions after HPH or HVED treatment.

Filtration Behavior of Yeast Suspensions Without Stirring

For the filtration experiments, the standard deviations were not shown in the figures. The deviations are within the range of 3–5%. Figure 6 compares the filtration behavior of intact and disrupted yeast suspension under the pressure of 1 bar without stirring. The results show that the filtration rate for the untreated yeast suspension is very high, and the filtration was finished in a few seconds. However, after the HPH and HVED treatments, the filtration rate becomes much slower, especially for the yeast suspension treated by HPH (Fig. 6a). In order to examine the underlying fouling mechanism, the data were re-plotted in conventional cake filtration coordinates t/V vs. V. The classical cake filtration models (Eq. 2) predict the linear behavior for curves t/V vs. V. This is in good agreement with the experimental filtration data presented in Fig. 6b for the untreated, but also for the HVED ($Z\approx0.75$) and HPH treated ($Z\approx1$) yeast suspensions under 0 rpm.

In cake filtration operations, the specific cake resistance α can be considered to be a measure of the filterability of suspensions as its value determines to a large extent the efficiency of the process. According to Ruth–Carman's classical cake formation model (Eq. (2)), the slope k_1 of t/V vs. V curve (Fig. 6b) is directly proportional to specific cake resistance α. The values of α, calculated using Eq. (2), are listed in Table 1. A great increase of α is observed after both treatments; however, the HPH leads to the much

TABLE 1
Specific cake resistance α of yeast suspensions for filtration at 1 bar without stirring

Disruption method	Specific cake resistance α ($\times 10^{12}$ m/kg)
Untreated	2.8 ± 0.1
HVED ($Z \approx 0.75$)	408 ± 3
HPH ($Z \approx 1$)	4000 ± 300

higher values of α than HVED. Increase of the specific cake resistance α is associated with disaggregation and damage of cells and the formation of cell debris.

Filtration Behavior of Yeast Suspensions With Stirring

Agitation was used as a method for filtration improvement. From graphs (Fig. 7a), it is possible to see that the kinetic of the permeate flux decline is accelerated under a stirring velocity of 200 rpm compared to the results in the previous section. For instance, 75 ml of the filtrate was obtained during 2000 s of filtration under 200 rpm for HVED-treated yeast suspension, while just 53 ml of the filtrate was obtained for the same filtration time under 0 rpm (Figs. 6a and 7a). The same tendency was observed for untreated and HPH-treated suspensions. Experimental filtration curves in coordinates t/V vs. V are also presented in Fig. 7b. For the untreated yeast suspension, the curve is approximately linear ($R^2 = 0.9977$) except the part of the curves at the end of filtration (Fig. 7b). This means that the filtration of untreated yeast suspension was governed by cake formation even under stirring conditions. Stirring leads to the retardation of particles deposition on the cake surface. If one supposes that the specific cake resistance α is not modified by stirring, then the values of ω (mass of solid particles deposed per unit volume of filtrate) can be calculated for different rotation speeds by using Eq. (2). The results are listed in Table 2. It is shown that ω decreases with the increase of stirring speed, which supports the supposition about the retardation of particles deposition by stirring.

For the filtration behaviors of the maximally disintegrated yeast suspensions by HVED ($Z \approx 0.75$) and HPH

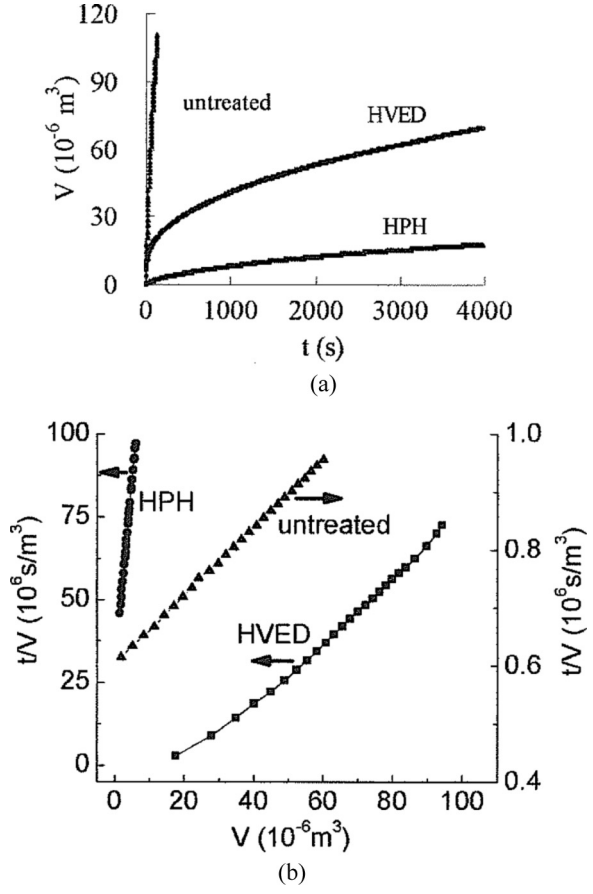

FIG. 6. Filtration behavior for untreated and maximally disintegrated yeast suspensions by HVED ($Z \approx 0.75$) and HPH ($Z \approx 1$) under 0 rpm: (a) filtrate volume V vs. time t; (b) t/V vs. filtrate volume V.

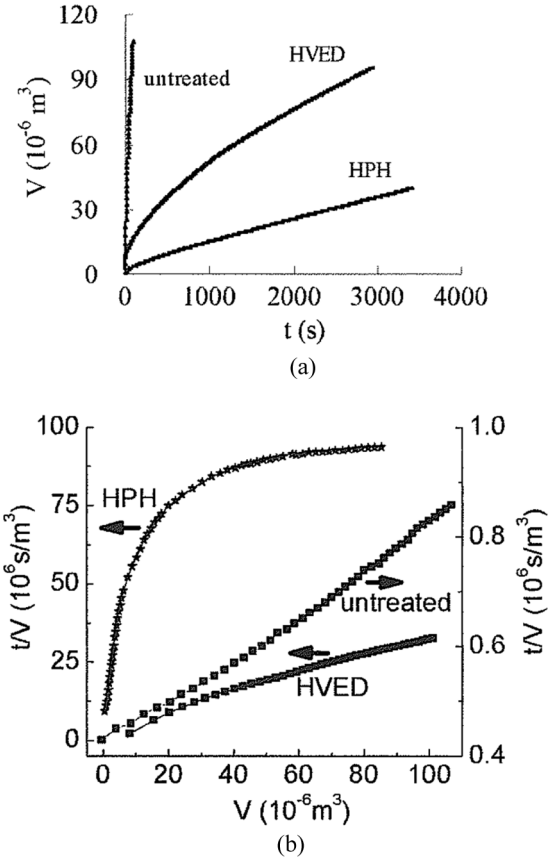

FIG. 7. Filtration behaviour for untreated and maximally disintegrated yeast suspensions by HVED ($Z \approx 0.75$) and HPH ($Z \approx 1$) under 200 rpm: (a) filtrate volume V vs. time t; (b) t/V vs. filtrate volume V.

TABLE 2
Effect of stirring velocity on ω (mass of solid particles per unit filtrate volume) for untreated yeast suspensions

Agitation velocity	ω (kg/m^3)
0 rpm	5.0 ± 0.3
100 rpm	4.9 ± 0.2
200 rpm	2.2 ± 0.2
500 rpm	1.1 ± 0.1

($Z \approx 1$) under stirring velocity of 200 rpm, the results show complex filtration mechanisms under suspension stirring, which do not follow a cake filtration model (Fig. 7b). Suspensions treated by both HVED and HPH present two distinguished filtration stages: on the first filtration stage the curves in t/V vs. V coordinates are approximately linear ($R^2 = 0.9943$ for HPH and $R^2 = 0.9944$ for HVED) and on the second stage the curves decline from straight lines and tend to decrease their slope. This tendency is more pronounced for the filtration of yeasts treated by HPH when the curves t/V vs. V approach to horizontal lines. Such two-staged filtration behavior is often produced during the cross-flow or dynamic filtration (44). On the first stage of filtration, the cake layer is formed on the membrane surface. Afterwards the liquid flow rate through the low-permeable cake becomes very slow and the energy of stirring becomes sufficient to maintain the smaller yeast debris suspended. As a result, the particles deposition is nearly stopped on the second filtration stage and the liquid flow rate becomes nearly constant. The smaller particles obtained by HPH form the cake with very high specific resistance (Table 1). Therefore, the flow rate through this cake is low enough to maintain nearly all disintegrated yeast particles suspended and to produce a nearly constant flow rate.

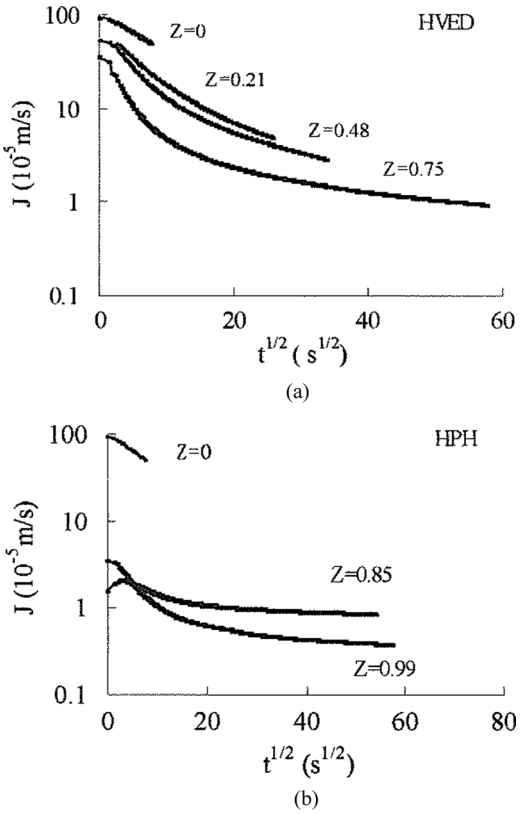

FIG. 8. Filtration curves as a function of Z under stirring velocity of 200 rpm; (a) HVED-treated yeast suspensions; (b) HPH-treated yeast suspensions.

Filtration Behavior of Yeast Suspensions With a Different Disintegration Degree

The filtration behavior under the pressure of 1 bar and impeller speed of 200 rpm was systematically studied for differently damaged yeasts. The evolution of filtration curves with different disintegration index Z is shown for yeast suspensions treated by HVED and HPH (Fig. 8). Filtration kinetics is clearly decelerated for higher yeast damages attained by HVED or HPH treatments. Increase of Z results in noticeable decrease of the flux for both HVED and HPH. The decrease is more pronounced for suspensions treated by HPH. Evidently, the values of Z characterize the yeast damage degree just incompletely, based on the electrical conductivity evolution. The higher values of Z correspond to the increased release of ionic species from interior of damaged cells. The main impermeable barrier for extraction of intracellular compounds is a cell membrane, which can be effectively removed by both HPH and HVED treatments. However, the cell wall structure is more destroyed for yeasts treated by HPH, which leads to a slower filtration kinetics comparatively to yeasts treated by HVED due to smaller particle size.

Fouling Mechanism Analysis

In order to distinguish the different filtration mechanisms, the data shown in Figs. 6, 7 are replotted as (d^2t/dV^2) versus (dt/dV). As suggested by Eq. (3), plotting of a filtration curve as $\log\left(\frac{d^2t}{dV^2}\right)$ versus $\log\left(\frac{dt}{dV}\right)$ yields a linear relationship with a slope equal to n. The re-plotted filtration curves for yeast suspensions are presented in Fig. 9. For the filtration without stirring (0 pm), the slope of the experimental curves remains $n = 0$ for the untreated and yeast suspensions treated by HVED and HPH (Fig. 9a). This result is consistent with a previous conclusion about the mechanism of filtration with cake formation, which characterizes the filtration of untreated and treated yeast suspensions without agitation.

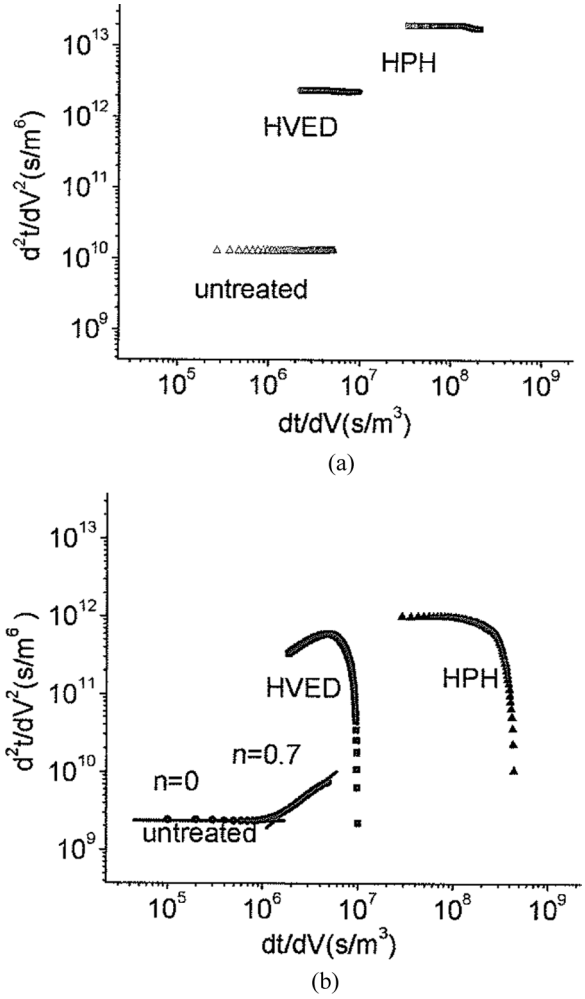

FIG. 9. Flux decline analysis for untreated and maximally disintegrated yeast suspensions by HVED ($Z \approx 0.75$) and HPH ($Z \approx 1$): (a) without stirring; (b) under stirring velocity of 200 rpm.

For the filtration of untreated yeast suspensions under stirring (200 rpm), the filtration mechanism changes from the filtration with cake formation ($n \approx 0$) to the pore blocking mechanism ($n \approx 0.7$) at the end of the process. It can be explained by the retardation of particles deposition on the cake surface due to the stirring. It is well known that the pore blocking mechanisms are typical for the filtration of low concentrated suspensions (45).

For the filtration of maximally disintegrated yeast suspensions by HVED ($Z \approx 0.75$) and HPH ($Z \approx 1$) under stirring (200 rpm), the filtration curves changes from the horizontal ($d^2t/dV^2 \approx \text{Const}$) at the beginning of the process to the vertical ($dt/dV \approx \text{Const}$) at the end of process (Fig. 9b). It clearly indicates the cake formation mechanism on the first filtration stage of filtration, which tends to the filtration at the constant rate through a nongrowing filter cake. Note, that the slope of the curves is negative ($d^2t/dV^2 = 0$ for $dt/dV = \text{Const}$).

CONCLUSIONS

The HPH treatment is found to be more effective for the extraction of intracellular compounds (proteins and nucleic acids) than the HVED treatment. However, the HPH-treated suspensions have higher specific cake resistance. Stirring is confirmed to be an effective method to enhance the filtration of intact and damaged yeast suspensions. The filtration of untreated, HVED and HPH treated yeast suspensions without stirring was governed by the cake formation mechanism. Under the stirring, just a first stage of filtration of untreated and treated yeast suspensions was governed by the cake formation mechanism. On the second stage of filtration, after the initial cake layer formation, the filtration had a pore blocking mechanism for the untreated yeast suspension, and tended to a constant rate for the maximally disintegrated yeast suspensions by HVED and HPH.

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REFERENCES

1. Geciova, J.; Bury, D.; Jelen, P. (2002) Methods for disruption of microbial cells for potential use in the dairy industry – a review. *Int. Dairy J*, 12 (6): 541.
2. Middelberg, A. (1995) Process-scale disruption of microorgansims. *Biotechnol. Adv.*, 13 (3): 491.
3. Gancva, V.; Galutzov, B. (1999) Electropulsation as an alternative method for protein extraction of from yeast. *FEMS Microb. Lett.*, 174: 279.
4. Zuckerman, H.; Krasik, Y.E.; Felsteiner, J. (2002) Inactivation of microorganisms using pulsed high-current underwater discharges. *Innovative Food Sci. & Emerg. Technol*, 3 (4): 329.
5. Gros, C.; Lanoisellé, J.-L.; Vorobiev, E. (2003) Towards an alternative extraction process for linseed oil. *Chem. Eng. Res. Des.*, 81 (9): 1059.
6. Barskaya, A.V.; Kuretz, B.I.; Lobanova, G.L. (2000) Extraction of water soluble matters from vegetative raw material by electrical pulsed discharges. Proceedings of the 1st International Congress on Radiation Physics, High Current Electronics and Modification of Materials, Tomsk, Russia, 533.
7. Kimeldorf, M.; Gleizer, S.; Krasik, Ya E. (2003) Effect of underwater high-current discharge on the properties of low-concentration β-lactoglobulin solutions. *Innovative Food Sci. Emerg. Technol.*, 4151.
8. Mikula, M.; Panak, J.; Dvonka, V. (1997) The destruction effect of a pulse discharge in water suspensions. *Plasma Sources Sci. Technol.*, 6: 179.
9. Vishkvaztzev, L.I.; Kuretz, B.I.; Lobanova, G.L.; Filatov, G.P.; Barskaya, A.V. (1998) Use of electrical discharges for the treatment of soya beans (in Russian), Vestnik Rossiyskoy Akademii Selskohozyaistvennih Nauk (in Russian), 6: 71.

10. Gros, C. (2005) Extraction aqueuse et athermique de l'huile de lin assistee par dechargcs electriques de haute tension, These presentee pour l'obtention du grade de Docteur de l' UTC.
11. Shynkaryk, M.V.; Lebovka, N.I.; Lanoisellé, J.-L.; Nonus, M.; Bedel-Clotour, C.; Vorobiev, E. (2008) Electrically-assisted extraction of bio-products using high pressure disruption of yeast cells (*Saccharomyces cerevisiae*). *J. Food. Eng.*, 92 (2): 189.
12. Loginov, M.; Lebovka, N.; Larue, O. (2009) Effect of high voltage electrical discharges on filtration properties of *Saccharomyces cerevisiae* yeast suspensions. *J. Membr. Sci.*, 346 (2): 288.
13. Becker, T.; Ogez, J.R.; Builder, S.E. (1983) Downstream processing of proteins. *Biotechnol. Adv.*, 1 (2): 247.
14. Bui, P.T. (1983) Recovery and purification of biologically active polypeptides from rDNA microorganisms. *Nat. Biotechnol.*, 488.
15. Kroner, K.H.; Schuette, H.; Hustedt, H.; Kula, M.R. (1984) Cross-flow filtration in the downstream processing of enzymes. *Process Biochem.*, 19 (2): 67.
16. Higgins, J.J.; Lewis, D.J.; Daly, W.H.; Mosqueira, F.G.; Dunnill, P.; Lilly, M.D. (1978) Investigation of the unit operations involved in the continuous flow isolation of β-galactosidase from *Escherichia coli*. *Biotechnol. and Bioeng.*, 20 (2): 159.
17. Le, M.S.; Spark, L.B.; Ward, P.S.; Ladwa, N. (1984) Microbial asparaginase recovery by membrane processes. *J. Membr. Sci.*, 21 (3): 307.
18. Marston, F.A.O.; Lowe, P.A.; Doel, M.T.; Schoemaker, J.M.; White, S.; Angal, S. (1984) Purification of calf prochymosin (prorennin) synthesized in *Escherichia coli*. *Biotechnol.*, 2: 800.
19. Schoner, R.G.; Ellis, L.F.; Schoner, B.E. (1985) The isolation and purification of protein granules from *E. coli* cells overproducing bovine growth hormone. *Biotechnol.*, 3: 151.
20. Titchener-Hooker, N.J.; Gritsis, D.; Mannweiler, K.; Olbrich, R.; Gardiner, S.A.M.; Fish, N.M.; Hoare, M. (1991) Integrated process design for producing and recovering proteins from inclusion bodies. *BioPharm.*, 4 (4): 34.
21. Meagher, M.M.; Bartlett, R.T.; Rai, V.R.; Khan, F.R. (1994) The extraction of rIL-2 inclusion bodies from *Escherichia coli* using cross-flow filtration. *Biotechnol. Bioeng.*, 43: 969.
22. Hart, R.A.; Lester, P.M.; Reifsnyder, D.H.; Ogez, J.R.; Builder, S.E. (1994) Large scale, in situ isolation of periplasmic IGF-1 from *E. coli*. *Biotechnol. Appl. Biochem.*, 12: 1113.
23. Bailey, S.M.; Meagher, M.M. (2000) Separation of soluble protein from inclusion bodies in *Escherichia coli* lysate using crossflow microfiltration. *J. Membr. Sci.*, 166 (1): 137.
24. Quirk, A.V.; Woodrow, J.R. (1984) Investigation of the parameters affecting the separation of bacterial enzymes from cell debris by tangential flow filtration. *Enzyme Microb. Technol.*, 6 (5): 201.
25. Forman, S.M.; DeBernardez, E.R.; Feldberg, R.S.; Swartz, R.W. (1990) Crossflow filtration for the separation of inclusion bodies from soluble proteins in recombinant *Escherichia coli* cell lysate. *J. Membr. Sci.*, 48 (3): 263.
26. Bailey, S.M.; Meagher, M.M. (1997) Crossflow microfiltration of recombinant *Escherichia coli* after high pressure homogenization. *Biotechnol. Bioeng.*, 56: 304.
27. Zokaee, F.; Kaghazchi, T.; Zare, A. (1999) Cell harvesting by microfiltration in a dead end system. *Process Biochem.*, 34 (8): 803.
28. Becht, N.O.; Malik, D.J.; Tarleton, E.S. (2008) Evaluation and comparison of protein ultrafiltration test results: Dead-end stirred cell compared with a cross-flow system. *Sep. Purif. Technol.*, 62 (1): 228.
29. Riesmeier, B.; Kroner, K.H.; Kula, M.R. (1990) Harvest of microbial suspensions by microfiltration. *Desalination*, 77: 219.
30. Parnham III, C.S.; Davis, R.H. (1995) Protein recovery from cell debris using rotary and tangential crossflow microfiltration. *Biotechnol. Bioeng.*, 47 (2): 155.
31. Parnham, C.S.; Davis, R.H. (1996) Protein recovery from bacterial cell debris using crossflow microfiltration with backpulsing. *J. Membr. Sci.*, 118 (2): 259.
32. Knutsen, J.S.; Davis, R.H. (2006) Deposition of foulant particles during tangential flow filtration. *J. Membr. Sci.*, 271 (1–2): 101.
33. Belfort, G.; Davis, R.H.; Zydney, A.L. (1994) The behaviour of suspensions and macromolecular solutions in crossflow microfiltration. *J. Membr Sci.*, 96 (1–2): 1.
34. Iritani, E.; Mukai, Y.; Tanaka, Y.; Murase, T. (1995) Flux decline behavior in dead-end microfiltration of protein solutions. *J. Membr. Sci.*, 103 (1–2): 181.
35. Giiell, C.; Davis, R.H. (1996) Membrane fouling during microfiltration of protein mixtures. *J. Membr. Sci.*, 119 (2): 269.
36. Lora, J.; Arnal, J.M. (1990) The effect of agitation on reverse osmosis desalination. *Desalination*, 79 (2–3): 261.
37. Reihanian, H.; Robertson, C.R.; Michaels, A.S. (1983) Mechanisms of polarization and fouling of ultrafiltration membranes by proteins. *J. Membr. Sci.*, 16: 237.
38. Vorobiev, E.; Lebovka, N.I.; in Raso, J.; Heinz, V. (2006) Extraction of intercellular components by pulsed electric fields. Pulsed electric field technology for the food industry: Fundamentals and applications, Springer, Berlin, 153.
39. Mahesh Kumara, S.; Royb, S. (2008) Filtration characteristics in dead-end microfiltration of living *Saccharomyces cerevisiae* cells by alumina membranes. *Desalination*, 229 (1–3): 348.
40. Grenier, A.; Meireles, M.; Aimar, P.; Carvin, P. (2008) Analysing flux decline in dead-end filtration. *Chem. Eng. Res. Des.*, 86 (11): 1281.
41. Kleinig, A.R.; Middelberg, A. (1998) On the mechanism of microbial cell disruption in high-pressure homogenisation. *Chem. Eng. Sci.*, 53 (5): 891.
42. lida, Y.; Tuziuti, T.; Yasui, K.; Kozuka, T.; Towata, A. (2008) Protein release from yeast cells as an evaluation method of physical effects in ultrasonic field. *Ultrason. Sonochem.*, 15 (6): 995.
43. Ohshima, T.; Sato, M.; Saito, M. (1995) Selective release of intracellular protein using pulsed electric field. *Journal of Electrostatics*, 35: 103.
44. Hwang, K.-J.; Liao, C.-Y.; Tung, K.-L. (2007) Analysis of particle fouling during microfiltration by use of blocking models. *J. Membr. Sci.*, 287 (2): 287.
45. Iritani, E.; Katagiri, N.; Sugiyama, Y. (2007) Analysis of flux decline behaviors in filtration of very dilute suspensions. *AIChE J*, 53 (9): 2275.