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DISRUPTION OF YEAST CELLS *XANTHOPHYLLOMYCES DENDRORHOUS (PHAFFIA RHODOZYMA)* BY VIBRATION RESONANT LOW-FREQUENCY CAVITATOR

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Abstract. The goal of the study is to optimize the mode of disruption of the yeast *Phaffia rhodozyma* KNH 1 by a vibration-resonant low-frequency cavitator (VLC). The destruction of the cell biomass of yeast culture *P. rhodozyma* strain KNH 1 was carried out in VLC with water cooling, the capacity of 800 W, and resonant frequencies of vibrations of 30 Hz, 35 Hz, 37 Hz, 37.8 Hz, 39 Hz, 50 Hz, and in the presence of nitrogen in the reaction medium. Our data suggest that the yield of processed biomass by the treatment of yeast culture in VLC depends on the culture age and the mode of the treatment. Thus, for the six-day culture, we got the highest yield by its processing in VLC at 35 Hz for 75 min. The highest yield from the five-day culture was obtained after the treatment in VLC for 1 h at 37–37.8 Hz. The lowest yield of the disrupted yeast cells was obtained after 5 h of treatment in VLC at 37.8 Hz. The high level of yeast cell disruption can be used for the preparation of glucans aqueous solutions. Our data show that for such a level of disruption to treat five-day culture of *P. rhodozyma* in VLC at 37 Hz resonance frequency with nitrogen gas, bubbling through the reaction medium is economically profitable. For the first time, this study demonstrates the established optimal mode of destruction of yeast cells of *P. rhodozyma* strain KNH1 for the action of the vibration-resonance low-frequency cavitator or VLC. Analysis of the presented data indicates that the claimed method is convenient, efficient, and technologically justified.

Keywords: yeast, *Phaffia rhodozyma*, vibration resonant low-frequency cavitator.

1. Introduction

Yeast biomass *Phaffia rhodozyma*, enriched with carotenoids, is often used as a probiotic for health care purposes.[1-3] However, their tough cell wall makes it difficult to release the carotenoids.[4-9] Damage to the branched glucans of the cell wall and the formation of pores in yeast cells improves the bioavailability of carotenoids in the organism. Moreover, beta-glucans of different origins (plants, microorganisms) are used as sorbents of toxins.[10-13] Thus, it is important to search for methods of destroying yeast cells, which promote the facilitated release of carotenoids from biomass and the simultaneous formation of pores in the beta-glucan layer's structure.

The basis for stable self-disturbance vibratory resonance and for the long-lasting cavitation process in liquids is a periodic energetic influence on cavitation initiation points, which always exist in liquids. This influence takes place with a frequency that is equal to or a multiple of the natural oscillation frequencies of cavitation initiation points and an amplitude that exceeds their size.[14-15] The advantage of vibration resonant low-frequency cavitators (VLC), along with energy saving, is that the processed fluid is exposed to magnetic processing by powerful variable magnetic fields simultaneously with cavitation, providing a significant synergistic effect on the quality of final products. The saturation of liquid with gases increases the number of cavitation initiation points and thus reduces the strength of intermolecular bonds, lowering the threshold for spontaneous cavitation phenomena. Cavitation processing of fluids is conducted mainly in closed-type cavitators without access to the air. This leads to the degasification of the treated liquid, which results in a rapid reduction in the number of cavitation initiation points decreasing the intensity of the cavitation field and the efficiency of cavitation processing. In order to reduce the duration of the VLC use and energy consumption in the technical process, it was proposed to perform a cavitation processing of liquid under the application of gas.[15] The molecules of dissolved gas significantly reduce the strength of intermolecular bonds in the liquid, which leads to the increased formation of cavitation initiation points. The mechanisms of the synergistic action of gas in the cavitation field have been described by Shev-

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chuk *et al*.[15] The authors suggested that individual clusters of molecules and atoms of dissolved gas form an independent oscillatory system separated from the liquid, *i.e.*, invisible micro-bubbles. The system of oscillating micro-bubbles in the liquid needs to be put into a so-called resonance mode by the external periodic energy influence of about the same vibration frequency; this is accompanied by an active increase in the intensity of the micro-bubble movements in the liquid. With available technology, it is difficult to keep a volume of liquid in a steady and stable resonant mode due to the structure of water, which is closer to being quantum than a molecular one. At the instant when the bubble becomes a cavitation initiation point, instantaneous pressure changes occur inside the bubble, provoking its collapse, which is accompanied by the formation of a shock wave. If an external energy influence supports the resonance phenomenon, and new dissolved gas molecules compensate for degasification, the process becomes self-sustaining, creating a cavitation field. In this case, the amount of energy required to maintain the micro-bubbles steady resonant vibration cavitation state is significantly less than in the case when resonance phenomena do not appear.

The aim of our research is to establish the optimal mode of disruption of yeast *Phaffia rhodozyma* KNH1 by a vibration-resonant low-frequency cavitator.

2. Experimental

2.1. Materials

The carotenoids-overproducing strain KNH1 was selected from the wild-type *P. rhodozyma* NRRL Y-17268 and generated in our laboratory by ultraviolet- and nitrosoguanidine-induced mutagenesis. [16] Erlenmeyer flasks (0.5 L) filled up to 20-24 % with culture medium (0.9 g/L $(NH_4)_2SO_4$; 1.0 g/L KH_2PO_4; 0.5 g/L $MgSO_4 \times 5H_2O$; 0.1 g/L $CaCl_2 \times H_2O$; 2.0 g/L yeast extract (Reanal); 20 g/L glucose; 50 mL of mash concentrate (from Lviv Brewery)) were inoculated with 0.2 g yeast cells per liter of medium. The flasks were incubated on a shaker (200 rev/min) at 293 K for 4, 5, and 6 days. For titration cells were seeded on a medium supplemented with 2 % agar.

2.2. Methods

Disruption of the *P. rhodozyma* KNH1 was carried out for different time periods in a VLC (800 W) with water cooling in the reaction medium saturated with nitrogen at resonant frequencies 30 Hz, 35 Hz, 37 Hz, 37.8 Hz, 39 Hz, or 50 Hz. Nitrogen was supplied throughout the process of cavitation at a speed of 0.2 cm^3/s. Analysis of survival (%) and destruction (%) of cells was performed by sowing 5 % mash-agar medium of titers of intact and processed cells for different duration of ultrasonic cavitation. Each experiment was repeated three times.

Scanning electron microscopy and X-ray microanalysis based on the raster microscope electronic – the REMMA-102-02 microanalyzer (SELMI, Ukraine). Scanning of the sample surface was carried out with the use of a beam with a diameter of several nanometers and electron energy of 0.2-40 keV.[17] We used a computer based on Intel Pentium 1.6 GHz with Windows operating system ("Microsoft", USA). Plotting graphs and statistical analysis of data were performed using the program OriginPro 8.5 and Excel according to generally accepted algorithms.

3. Results and Discussion

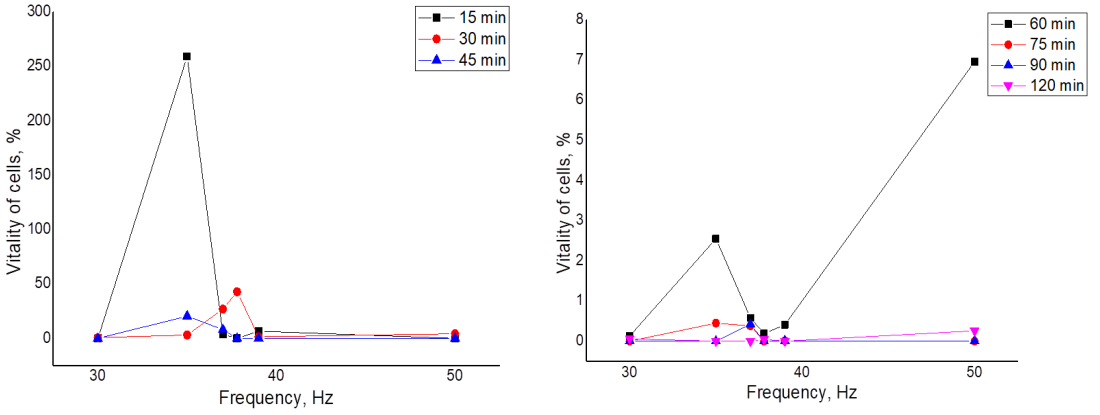
We found that treatment of the six-day culture of *P. rhodozyma* KNH1 for 15 min at a resonant frequency of 35 Hz caused 2.6 times increased growth when compared to the control untreated cells (Fig. 1a). It is possible that a 35 Hz resonant frequency destroys clusters of the cells while the viability of individual cells is still preserved. Cell survival was strongly decreased (from 259.1 % to 2.55 %) after 1 h of the culture treatment at the same frequency of 35 Hz (Fig. 1b).

Similar tendencies we observed in the case of short-term treatment of five-day *P.rhodozyma* KNH1 culture at resonant frequencies 37.0–37.8 Hz (Fig. 1). Such treatment also activated biomass growth, but this was less pronounced (Fig. 1). The growth-stimulating effect of short-term (up to 30 min) ultrasonic treatment can be explained by dispersion phenomena.[18] The hydrophobic zone between cells in clusters is destroyed, which leads to greater penetration of water and nutrients into the cell. Under these conditions, the increased number of vegetative cells dominates the destructive processes of destruction and active biomass growth takes place.

Thus, a comparison of the durations of *P. rhodozyma* KNH1 treatment in VLC revealed the efficient cell damage at the frequencies of 30–35 Hz and 37–50 Hz, while at the frequency range of 35–37 Hz cell growth was enhanced after 30 min of treatment (Fig. 2).

The destruction of the cells by treatment with VLC at the 30–39 Hz resonant frequency range averaged 99 %. In contrast, treatment of six-day culture in VLC at 50 Hz damaged only 93 % of cells as was estimated by the loss of their ability to grow on the 5 % mush-agar medium.

In addition, the role of culture age in the sensitivity of yeast cells to the treatment in VLC was studied. We found that in the four-day culture 99.7 % of yeast cells were disrupted after 30 min treatment at 30 Hz. Prolongation of the treatment for 1 h increased the number of damaged cells to 99.9 % (Fig. 3).



a b

Fig. 1. Survival analysis (%) of yeast *P. rhodozyma* KNH1 for short-term (a) and long – term (b) treatment in VLC at frequencies 30-50 Hz

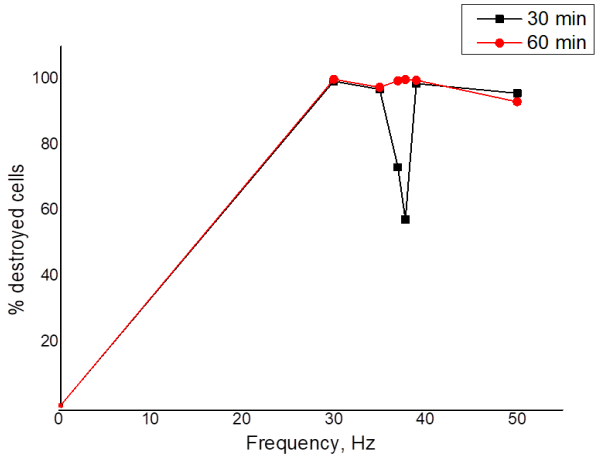


Fig. 2. Disruption (%) of five-day *P. rhodozyma* KNH1 after 30 minutes and 1 h of treatment in VLC at different frequencies

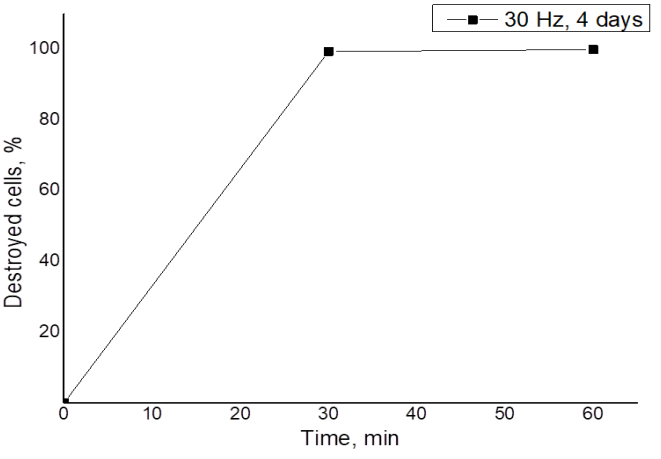


Fig. 3. Cell disruption dynamics of *P. rhodozyma* KNH1 culture in VLC at 30 Hz

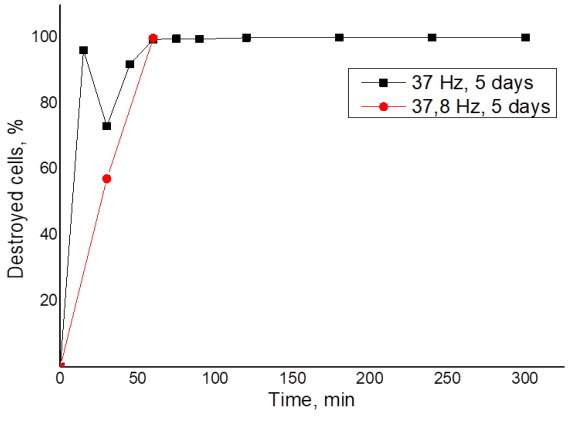


Fig. 4. The dynamics of *P. rhodozyma* KNH1 culture in VLC at 37 Hz and 37.8 Hz

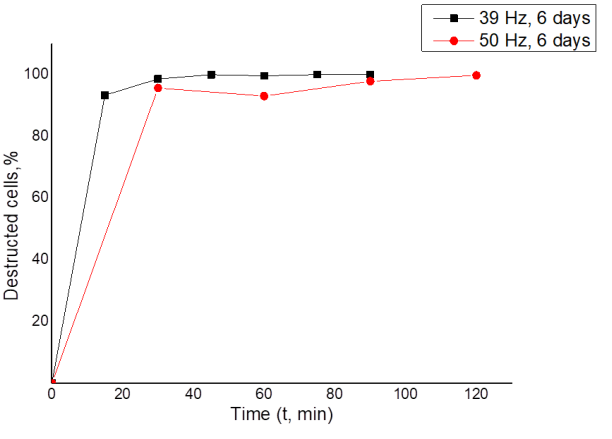


Fig. 5. The dynamics of (%) of *P. rhodozyma* KNH1 culture in VLC at 39 Hz and 50 Hz

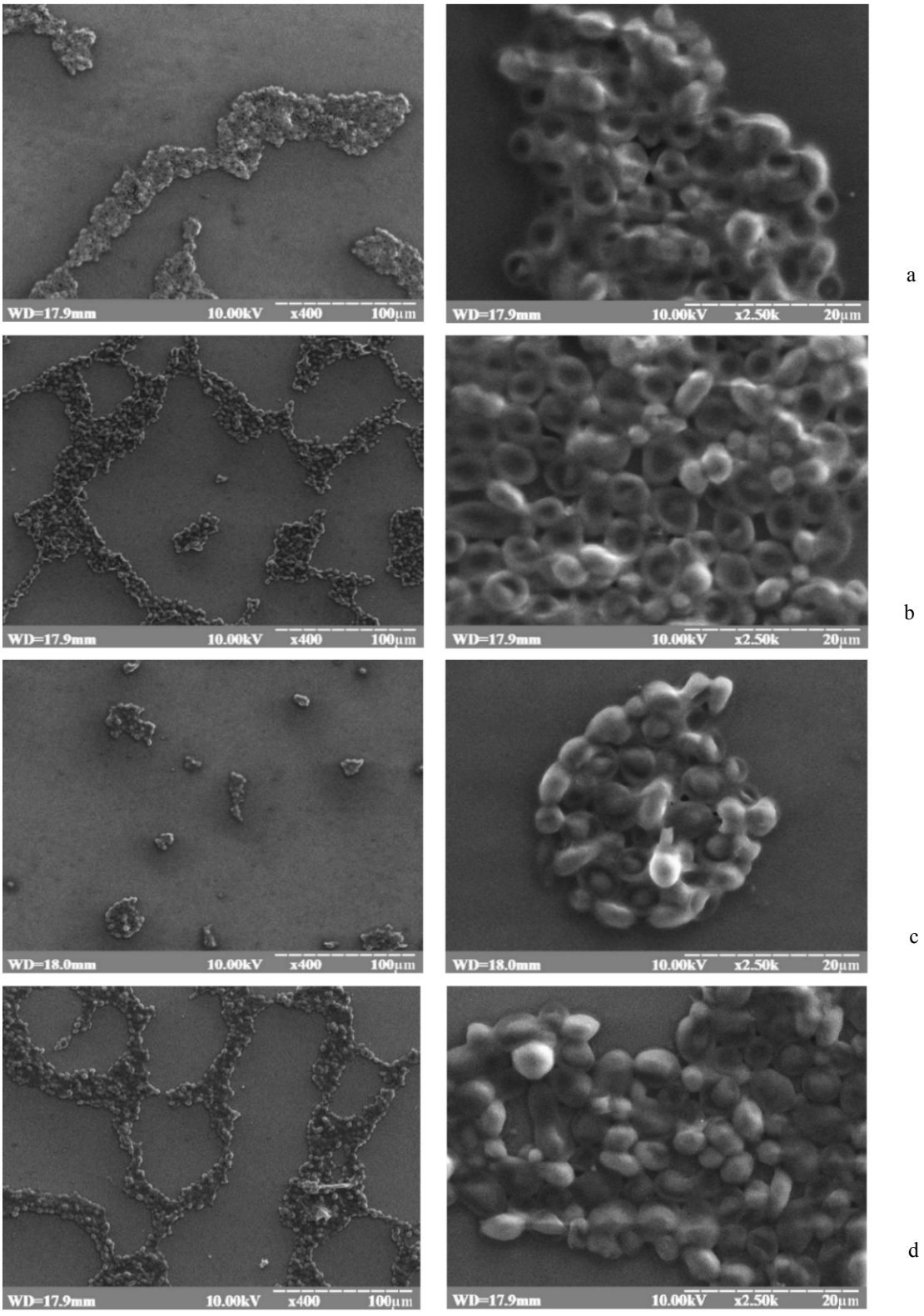


Fig. 6. Scanning electron microscopy the 6-day *P. rhodozyma* KNG1 cells:
(a) control – intact cells; and treated cells at 39 Hz; (b) 30 min; (c) 60 min; (d) 90 minutes.
The range of change is a multiple of 10–300,000, the resolution is about 5.0 nm

Treatment of the five-day culture in VLC for 15 min at 37 Hz resonant frequency led to 96 % destruction of the cells (Fig. 4). However, after an additional 15 min of treatment at frequencies 37 Hz and 37.8 Hz, the efficiency of cell disruption decreased by 57 and 73 %, respectively, and the cell survival rate hovered around 27 and 43 % (Fig. 4).

Treatment of the six-day *P. rhodozyma* KNH1 culture in VLC at 39 Hz resonance frequency for 15 min disrupted 93 % of the cells and reached 98-99.99 % of cell disruption after a longer time (Fig. 5). At higher frequency (50 Hz), treatment of this culture for 30 min and 1 h decreased the number of damaged cells to 96 % and 93 %, respectively (Fig. 5). The cell disruption reached 98-99 % level after 1.5-2 h of treatment (Fig. 5).

The breaking of the cell clusters under 39 Hz was confirmed by scanning electron microscopy and X-ray analysis (especially noticeable after 60 min in VLC) (Fig. 6).

The combination of ultrasound and nitrogen gas, bubbling through the reaction medium, provided a synergistic effect enhancing cell disruption and reducing processing time.[18-20]

Cell survival was less than 0.27% after 5 min treatment of five-day *P. rhodozyma* KNH1 culture in VLC at 37 Hz resonance frequency with nitrogen gas, bubbling through the reaction medium, and after 1 h of treatment, it was further lowered by 2 orders of magnitude (up to 0.0027 %) (Fig. 7). Treatment of the six-day culture under the same conditions resulted in multidirectional dynamics. Thus, after 15 min of treatment in VLC cell disruption reached 99.6 %, whereas after treatment of the yeast culture for 30 min and 45 min we observed a noticeable cell growth from 3.6 % to 6.3 %. Prolongation of the ultrasonic cavitation in the presence of nitrogen to 60 min and 75 min reduces again cell survival to 0.36 % and 0.13 % respectively. After treatment of the culture for 1.5 h yeast cell survival was 1.38 %.

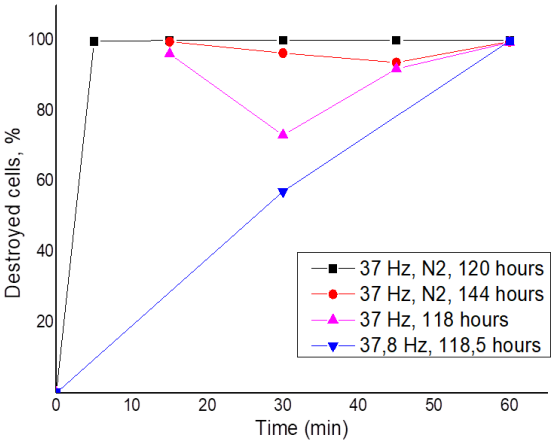


Fig. 7. Cell disruption dynamics (%) of *P. rhodozyma* KNH1 in the presence and absence of nitrogen

Differences in the levels of cell disruption during the ultrasonic treatment in the presence of bubbling nitrogen, which we observed between five-day and six-day yeast cultures, can be explained by different resonant properties of the oscillating system due to technical issues and/or by the changes in the cell wall of the yeast at different ages.

We treated yeast *P. rhodozyma* KNH1 cultures with VLC at various modes of cavitation and analyzed the level of cell disruption (Table).

During 30 min of treatment at 50 Hz we observed only cavitation-induced disturbance in the reaction medium without foam formation. Treatment at 37-37.8 Hz created foam, which remained stable even at the end of treatment (after 1.5 h). Foaming was accompanied by the increase in biomass of cell culture in the bioreactor. The efficiency of cell disruption was higher at 37.8 Hz resonant frequency, as it generates the same yield of processed biomass (1.7 g × L^{-1} × h^{-1}) as the treatment at 50 Hz but for a twice shorter time.

Table. Productivity (g × L^{-1} × h^{-1}) and destruction (%) cell after treatment of yeast cultures of P. *rhodozyma* KNH1 strain for the resonant frequencies of 30-50 Hz in VLC

VLC treatment mode, time	Age of culture, h	Dry mass yield, g × L^{-1} of medium	Productivity, g × L^{-1} × h^{-1}	% of destruction
30 Hz, 2 h	96	2.9	1.45	99.9
35 Hz, 1,25 h	144	2.5	2.0	99.6
37 Hz, 1,5 h	118	1.6	1.1	99.6
37. 8 Hz, 5 h	120	0.18	0.18	99.9
37.8 Hz, 1 h	118.5	1.7	1.7	99.8
37 Hz + N_2, 1.5 h	120	0.3	0.2	99.9
37 Hz + N_2, 1.5 h	144	2.05	1.37	98.6
39 Hz, 1.5 h	144	1.5	1.0	99.9
50 Hz, 2 h	144	3.4	1.7	99.8

4. Conclusions

The process of biomass disruption of *P. rhodozyma* KNH1 in the field of acoustic low-frequency cavitation has been studied. It has been shown that this method is economically advantageous and environmentally friendly. Our results suggest that the yield of the processed biomass by the treatment of yeast culture in VLC depends on the culture age and mode of the treatment. The highest yield from the five-day culture we got after its treatment in VLC for 1 h at 37-37.8 Hz. The lowest yield of disrupted yeast cells was observed after 5-hour treatment in VLC at 37.8 Hz. The results of our experiments show that for such a level of disruption economically profitable is to treat five-day culture of *P. rhodozyma* in VLC at 37 Hz resonance frequency with nitrogen gas, bubbling through the reaction medium.

In addition, it is important to study further the effect of cultivation time prolongation on the level of yeast cell disruption at 37 Hz frequency in the presence of nitrogen gas. For example, the six-day culture gave 7 times higher yield of processed biomass than the five-day one under the same conditions. Another issue, which also needs to be clarified is the growth stimulation of the six-day *P. rhodozyma* KNH1 culture by 15 min treatment in VLC at 35 Hz.

These results can be used both for biotechnology processes and for the disinfection of liquid effluents (organic matter) in industry.

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РУЙНУВАННЯ КЛІТИН ДРІЖДЖІВ *Xanthophyllomyces Dendrorhous* (*Phaffia Rhodozyma*) ЗА ДІЇ ВІБРОРЕЗОНАНСНОГО НИЗЬКОЧАСТОТНОГО КАВІТАТОРА

Анотація. *Метою дослідження було встановити оптимальний режим руйнування клітин дріжджів P. rhodozyta штаму KNH 1 з водяним охолодженням, потужністю 800 Вт та резонансними частотами за дії віброрезонансного низькочастотного кавітатора (ВНК). Руйнування клітинної біомаси культури дріжджів P. rhodozyta штаму KNH 1 здійснено у віброрезонансному низькочастотному кавітаторі (ВНК) з водяним охолодженням, потужністю 800 Вт та резонансними частотами коливань 30 Гц, 35 Гц, 37 Гц, 37,8 Гц, 39 Гц, 50 Гц та за наявності азоту в реакційному середовищі. Наші дані свідчать про те, що вихід біомаси, переробленої дріжджової культури залежить від віку культури та режиму обробки. Для шестиденної культури найбільший вихід зруйнованих клітин ми отримали, обробляючи її в ВНК за допомогою 35 Гц протягом 75 хв. Найвищий вихід з п'ятиденної культури отримано після обробки в ВНК протягом 1 години за 37–37,8 Гц. Найнижчий вихід зруйнованих дріжджових клітин одержано після 5 годин обробки в ВНК за 37,8 Гц. Високий рівень руйнування дріжджових клітин може сприяти полегшеному вивільненню каротиноїдів із біомаси та, одночасно, утворенню пор у структурі бета-глюканового шару клітин. Наші дані показують, що для такого рівня пошкодження економічно вигідна обробка п'ятиденної культури P. rhodozyta в ВНК на частоті резонансу 37 Гц газоподібним азотом, барботажем через реакційне середовище. Це дослідження вперше демонструє встановлений оптимальний режим руйнування дріжджових клітин P. rhodozyta штаму KNH 1 за дії вібраційно-резонансного низькочастотного кавітатора. Аналіз представлених даних вказує, що заявлений спосіб є зручним, ефективним та технологічно виправданим.*

Ключові слова: *дріжджі, Phaffia rhodozyma, вібраційний резонансний низькочастотний кавітатор.*