UDC 543.429.23:544.162:691.175.5/.8 doi: 10.15407/hftp12.01.058

T.V. Krupska [1], V.V. Turov [1], M.D. Tsapko [2], J. Skubishevska-Zieba [3], R. Leboda [3]

ENCAPSULATION OF CELLULAR SUSPENSIONS OF LACTIC BACTERIA WITH SILICA

[1] Chuiko Institute of Surface Chemistry of National Academy of Sciences of Ukraine
17 General Naumov Str., Kyiv, 03164, Ukraine, E-mail: krupska@ukr.net
[2] Faculty of Chemistry, Taras Shevchenko University
60 Volodymyrska Str., Kyiv, 01033, Ukraine
[3] Faculty of Chemistry, Maria Curie-Skłodowska University
3 Maria Curie-Skłodowska pl., Lublin, 20031, Poland

A promising direction for long-term storage of cells at relatively high temperatures may be their encapsulation with nanoscale biologically inert materials capable of creating a shell around microdroplets of a cell suspension, which, on the one hand, provides the possibility of gas exchange between the suspension and the external environment, and on the other hand, inhibits the processes of cell life, so transferring them to a state close to suspended animation. The method of low-temperature ^{1}H NMR spectroscopy was used to study the process of hydration of lactobacilli, the effect of a weakly polar organic medium on it and the encapsulation of cells with silica. The aim of this work was to study the hydration of cell suspensions and the viability of lactic acid bacteria cells encapsulated with silica and the penetration possibility of such an active substance as trifluoroacetic acid into them. As a result of the studies carried out, it has been shown that the spectral parameters of water in concentrated cell suspensions of lactic acid bacteria strongly depend on the concentration of the suspension, which is probably associated with the possibility of the formation of a stable cell gel, which can be encapsulated by silica particles without its destruction in both air and a chloroform medium with addition of trifluoroacetic acid. The radial distribution curves of non-freezing water clusters have two maxima corresponding to R = 2 and 20–100 nm. The contribution to the distribution of the second maximum increases with increasing water concentration.

Keywords: *hydrophilic silica, encapsulation, ^{1}H NMR spectroscopy, lactic acid bacteria*

INTRODUCTION

Currently, for long-term storage of cell suspensions, the method of freezing them with a cryoprotectant (glycerol or DMSO) at the temperature of –196 °C (liquid dinitrogen) is widely used [1, 2]. Its main disadvantage is the low percentage of preservation of viable cells (55–60 %).

In the practice of cryopreservation of cell suspensions, multistage freezing modes are usually used with different values of the transition temperature from stage to stage, dependent on the type of cells [3–5]. As a result, most of the intracellular water is removed from the cells. This is due to the fact that at temperatures down to –30 °C such trans-membrane transfer of intracellular water can occur, which is practically not accompanied by the formation of intracellular ice. At this stage, the cooling rate is limited by the possibility of transmembrane diffusion (different for different types of cells). This is followed by the deep freezing stage, which also leads to damage to frozen cells [6].

A promising direction for long-term storage of cells at relatively high temperatures may be their encapsulation with nanoscale biologically inert materials capable of creating a shell around microdroplets of a cell suspension, which, on the one hand, provides a possibility of gas exchange between the suspension and the external environment, and on the other hand, inhibits the processes of cell life transferring them to a state close to suspended animation [7]. At present, technological schemes have been developed for encapsulating many types of cells, in particular, bone marrow, cancer and other cells [8–13], which allow not only keeping cells in a viable state, but also growing cell cultures.

The aim of this work was to study the hydration of cell suspensions and the viability of lactic acid bacteria cells encapsulated with silica and the penetration possibility of such an active substance as trifluoroacetic acid (TFAA) through the hydrophilic barrier into cells.

Low-temperature ^{1}H NMR spectroscopy was chosen as the main research method, with the help of which, by the change in the intensity of the water signal during the freezing-thawing of the sample, it is possible to determine the temperature dependence of the concentration of non-freezing water, and by the value of the chemical shift of water – the average number of hydrogen bonds formed by each water molecule [14–17].

EXPERIMENTAL PART

Materials. A mixture of lactic acid bacteria of the genus *Lactococcus* was used for the study. During the preparation of the samples, a weighed portion of the dry cell mass was suspended in an equal amount of water. Encapsulation was carried out by adding silica to the cell suspension in an amount equal to 40 % by weight of dry cells. The resulting mixture was gently mixed until a homogeneous material was formed, which was then placed in a 5 mm measuring NMR ampoule. Used amorphous silica, with a specific surface area of 300 m^2/g, TS-100 (Cabot Corporation, USA). Deuterochloroform, deuterodimethyl sulfoxide and deutero-trifluoroacetic acid were used as organic additives and a weakly polar medium. This prevented the appearance of an additional intense signal from solvents in the spectra, and in the case of TFAA, it made it possible to maintain unchanged the signal intensity of protons related to easily exchangeable protons of substances – H_2O and TFAA. The absence of a significant amount of foreign substances in the cell suspension made it possible to attribute the proton signals recorded in the ^{1}H NMR spectra exclusively to water, since the spectra of biopolymer products that form the basis of the cellular material are not recorded in the "liquid" NMR spectra due to the small relaxation times comparable with the relaxation time of protons in solids [15].

Microphotography of powders and emulsions was carried out using a Primo Star microscope (Zeiss, Germany) at a magnification of ×400 and ×1000 using immersion.

^{1}H NMR Spectroscopy. The ^{1}H NMR spectra were recorded using a Varian 400 Mercury spectrometer of high resolution with an operating frequency of 400 MHz. Eight probing 60° impulses of 1 μs duration were used with a bandwidth of 20 kHz. The temperature was controlled by means of a Bruker VT-1000 device with an accuracy of ± 1 deg. The signal intensities were determined by measuring the area of the peaks using the procedure for decomposing the signal into its components under the assumption of a Gaussian form of signal and optimizing the zero line and phase with an accuracy that was not less than 5 % for well-resolved signals and ± 10 % for overlapping signals. To prevent supercooling of the systems studied, the measurements of the amounts of unfrozen water (at $T < 273$ K) were carried out on heating the samples preliminarily cooled to 210 K. The temperature dependences of the ^{1}H NMR signals intensity were recorded in an automated cycle, when the holding time of the sample at a certain constant temperature was 9 min, and the measurement time was 1 min. NMR measurements were carried out in the air medium.

The chemical shift of protons (δ_H) was used as the main parameter that determines the structure of the network of hydrogen bonds in water. It was assumed that water, in which each molecule participates in the formation of four hydrogen bonds (two due to protons and two due to lone electron pairs of oxygen atoms), has a chemical shift $\delta_H = 7$ ppm (realized for hexagonal ice), and weakly associated water (not participating in the formation of hydrogen bonds as a proton donor) – a chemical shift $\delta_H = 1$–1.5 ppm [14–17]. To determine the geometrical dimensions of nanoscale aggregates of liquid limited by solid surface, the Gibbs–Thomson equation can be used, connecting radius of spherical or cylindrical pores (R) with the value of the depression of freezing temperature [18, 19]:

$$\Delta T_m = T_m(R) - T_{m,\infty} = \frac{2\sigma_{sl}T_{m,\infty}}{\Delta H_f \rho R}, \qquad (1)$$

where $T_m(R)$ is melting temperature of ice in the pores (voids) of radius R, $T_{m,\infty}$ – melting temperature of bulk ice, ρ – density of the solid phase, σ_{sl} – the energy of the solid-liquid interaction (for example, via hydrogen bonds), ΔH_f – the bulk enthalpy of melting. For practical use, formula (1) can be applied in the form $\Delta T_m = (k/R)$, in which the constant k for many heterogeneous systems containing water is close to 50 deg·nm [17]. The NMR measurement technique and methods for determining the radii

of interfacial water clusters are described in detail in [14–17]. In this case, clusters can be considered polyassociates with a radius $R < 2$ nm, and larger polyassociates – domains or nanodroplets, since they contain several thousand water molecules [16].

The process of freezing (melting) of bound water corresponds to changes in the Gibbs free energy caused by the effects of confined space and the natural interface. The difference from the process in volume is the less, the further from the surface is the water layer. The process of freezing (melting) of interfacial water, localized in a solid porous matrix, takes place in accordance with changes in the Gibbs free energy due to the influence of the surface. The farther the studing layer of water is from the surface, the less the influence is at $T = 273$ K water freezes, the properties of which do not differ from ones of bulk water, and as the temperature decreasing (without taking into account the effect of supercooling), the water layers, that are located closer to the surface, freeze. Thus, for interface water the ratio is valid:

$$\Delta G_{\text{ice}} = -0.036(273.15 - T), \qquad (2)$$

where the numerical coefficient is a parameter, associated with the temperature coefficient of variation of the Gibbs free energy for ice [20]. Determining the concentration of unfrozen water as a function of temperature $C_{uw}(T)$ by the signal intensity, in accordance with the technique detailed in [14–17], the amount of strongly and weakly bound water (SBW and WBW, respectively) and the thermodynamic characteristics of these layers can be calculated.

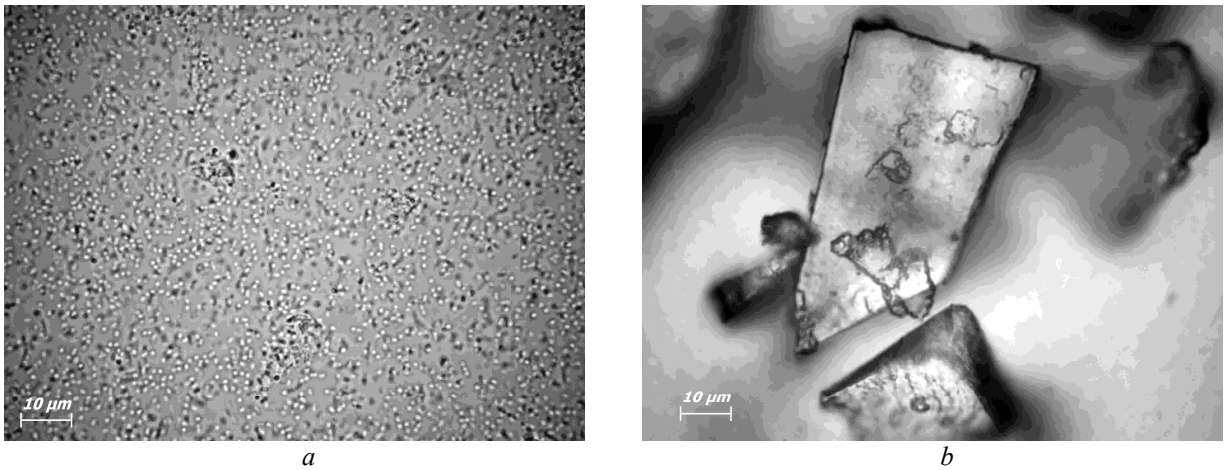
The interphase energy of water at the interface with solid particles or in its aqueous solutions was determined as a modulus of the total decrease in the free energy of absorbed water, due to the presence of an internal water-polymer [14–17] interface by the formula:

$$\gamma_S = -K \int_0^{C_{\text{uw}}^{\max}} \Delta G(C_{\text{uw}}) dC_{\text{uw}}, \qquad (3)$$

where $C_{\text{uw}}^{\max}$ – total amount of non-freezing water at $T = 273$ K.

RESULTS AND DISCUSSION

Micrographs of a cell suspension of lactic acid bacteria (*a*) and a suspension encapsulated with silica (*b*), taken at a magnification of ×1000, are shown in Fig. 1.



a *b*

Fig. 1. Micrographs of the cell suspension (*a*) and encapsulated cell material (*b*) of lactic acid bacteria

In suspensions, lactic acid bacteria are well distinguished (Fig. 1 *a*), which can form aggregates up to 10 μm in size. In the field of view of the microscope, a disorderly movement of cells is observed, which indicates their high viability. In a dry state (Fig. 1 *b*), the cells form crystal-like structures of various sizes. Individual cells in them are not visually identified. Probably, the cells or their aggregates are enclosed in micron-sized silica capsules, where, due to the high viscosity of the system, they are immobilized. However, if a certain amount of the composite is placed in an aqueous medium, then the microorganisms easily move into the

liquid phase and exhibit high mobility, which is retained even if the sample was preliminarily cooled to 210 K for 1.5 hours.

The spectra of water taken at different temperatures in an aqueous 1:1 cell suspension are shown in Fig. 2 *a*, where a broad signal of water is seen, which is due to the high viscosity of the system. The spectra show a water signal, which shifts from $\delta_H = 4$ ppm at 280 K up to $\delta_H = 5.5$ ppm at $T = 240$ K. With a decrease in temperature, the intensity of the signals decreases due to the partial freezing of water. At the same time, against the background of a much more intense water signal, sugar signals are not observed in the spectra.

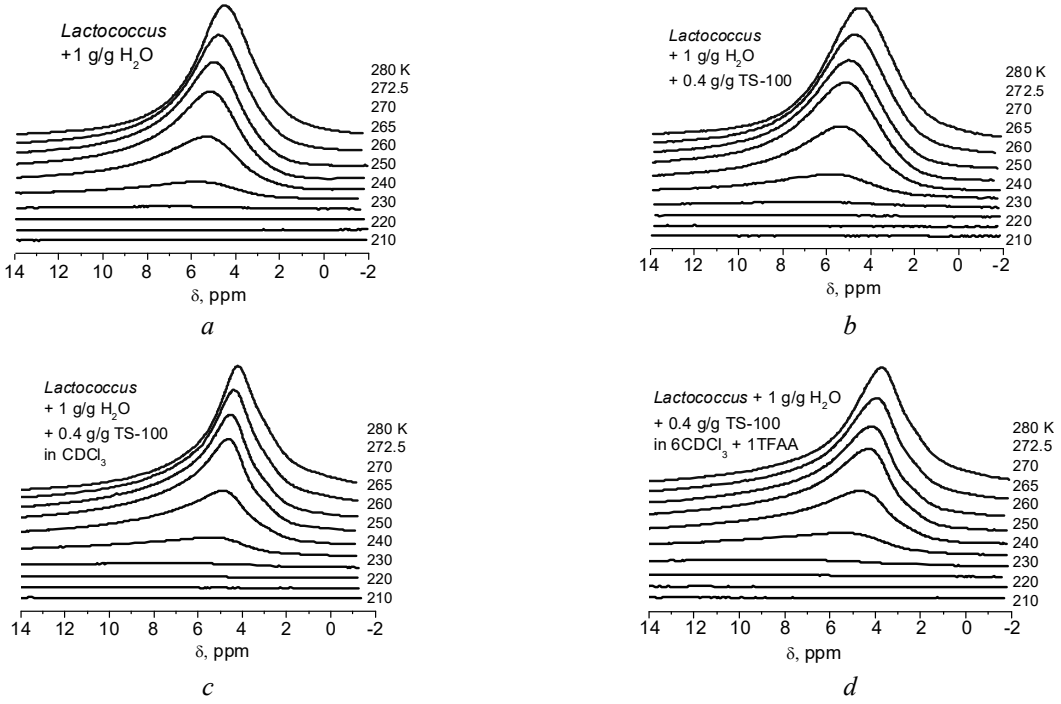


Fig. 2. ^{1}H NMR spectra of an aqueous suspension of lactic acid bacteria 1:1 (*a*) taken at different temperatures; suspension of bacteria encapsulated with TS-100 silica in air (*b*) in $CDCl_3$ medium (*c*) and $6CDCl_3$/1TFAAD1 mixture (*d*)

If silica (TS-100) was added to an aqueous cell suspension, then as a result of low-intensity mechanical stirring, the mixture easily formed a compact, very viscous mass. Microscopic examination data (Fig. 1) show that silica particles are located on the periphery of microdroplets (10–20 μm) of the cell suspension, forming a barrier separating the homogeneous cell suspension into a system of loosely connected compartments. The spectra of water in such a system (Fig. 2 *b*) are represented by a single signal, the spectral characteristics of which are close to the spectra of the initial cell suspension at $h = 1$ g/g (Fig. 2 *a*).

The possibility of weakly polar ($CDCl_3$) and polar (TFAA) substances penetrating through silica was studied by adding chloroform to a composite system containing a cell suspension and silica in an amount sufficient to fill all internal cavities, to which TFAAD1 was then added. For both systems, the ^{1}H NMR spectra recorded at different temperatures had a similar shape and are shown in Fig. 2 *c*, *d*. The chemical shift of the water signal is 4–5.5 ppm, which allows us to conclude that TFAAD1 is absent in the cell suspension.

This is also confirmed by the absence of a signal from TFAAD1 protons in the dispersion medium. Due to the fast proton exchange that occurs during the formation of hydrogen-bonded TFAAD1-H_2O complexes in the case of direct contact of the $CDCl_3$/TFAAD1 mixture with water, one should expect the replacement of a significant part of the deuterons by protons and the appearance of the TFAAD1 signal in the region $\delta_H = 11.5$ ppm [21].

The addition of 0.2 g/g TFAAD1 to the suspension (Fig. 3 *a*) results in the separation of the signal into two signals (signals 1 and 2) differing in the magnitude of the chemical shift. In this case, due to the excess of water and the rapid exchange of protons between TFAAD1 and water, most of the deuterons in the acid molecules are replaced by protons, and the chemical shift of the less intense signal weakly depends on temperature and is $\delta_H \approx 4$ ppm. It can be attributed to the signal of the hydroxyl groups of sugars present in the cell material in the form of a nutrient medium. The second signal has a significantly larger chemical shift, which increases as the temperature decreases (water freezing) from 6 to 8 ppm. Taking into account that the chemical shift of trifluoroacetic acid is $\delta_H = 11.5$ ppm, it can be concluded that signal 1 refers to the part of water that easily exchanges protons with TFA molecules, which leads to an increase in δ_H. Probably, TFAA easily penetrates into cells through cell membranes and its concentration inside and outside cells is close, which explain the presence of only one signal for intra- and extracellular water.

The results of studying the possibility of penetration of organic substances into cells for their concentrated aqueous suspension are shown in Fig. 3 *b*, *c*. Although chloroform in aqueous media forms a separate (heavier) phase localized in the lower part of the vessel (in our case, the NMR measuring ampoule), due to the interphase transfer, one can expect the binding of a certain amount of chloroform to the hydrophobic centers of the cell surface, and, possibly, its dissolution in bilipid layer of cell membranes. An even greater increase in the permeability of cell membranes can be expected upon the introduction of DMSO into the dispersion medium, which dissolves equally well in both water and chloroform. In this case, it is possible to increase the permeability of cell membranes for TFAA molecules and the formation of adducts inside cells containing different amounts of it.

According to the data in Fig. 3 *b*, *c*, several low-intensity signals with larger values of the chemical shift appear in the spectra than those in the TFAA-H_2O mixture. Fig. 4 shows the spectra of the sample shown in Fig. 3 c at a high sensitivity of the spectrometer at 240 K (Fig. 4 *a*) and 280 K (Fig. 4 *b*).

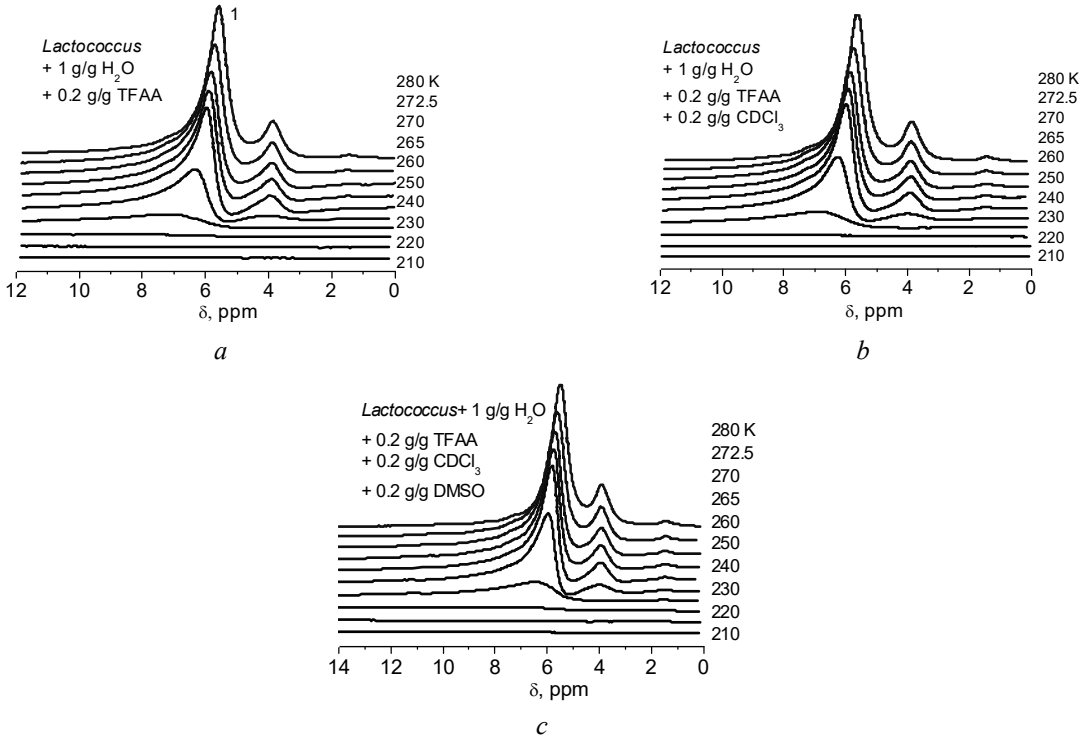


Fig. 3. ^{1}H NMR spectra taken at different temperatures of an aqueous suspension of lactic acid bacteria 1:1, with the addition of TFAA (*a*, *b*, *c*), $CDCl_3$ (*b*) and $CDCl_3$+DMSO (*c*) to the aqueous medium

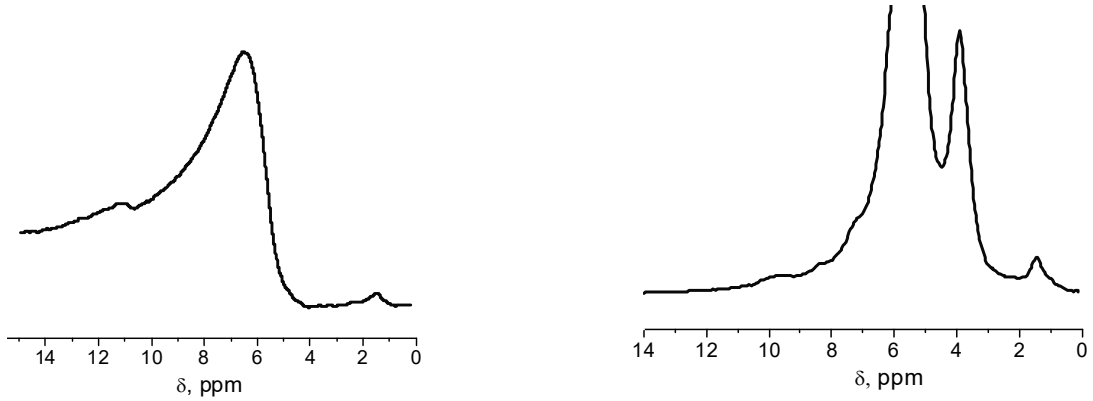


Fig. 4. Highly amplified ^{1}H NMR spectra of an aqueous suspension of lactic acid bacteria with additions of $CDCl_3$ and DMSO at the temperature of 240 (*a*) and 280 K (*b*)

At 240 K, most of the water and sugars present in the sample freeze (Fig. 3 *c*). In the spectra, in addition to the general TFAA-H_2O signal, a signal is recorded at $\delta_H = 11.5$ ppm, which corresponds to the signal of practically undiluted TFAA-water. With increasing temperature, this signal disappears due to the acceleration of proton exchange with water molecules. At $T = 280$ K, several signals are observed with chemical shifts in the range $\delta_H = 7–10$ ppm. They can also be attributed to signals associated with TFAA-H_2O, with a different ratio of ingredient concentrations. The most likely location for these associates should be considered nanosized cavities inside microorganisms or gaps between cells in contact with each other.

Fig. 5 shows the radii distributions of non-freezing water and sugar clusters calculated in accordance with the Gibbs-Thomson formula for the initial cell suspension and its composites with silica (Fig. 5 *a*), as well as cell suspensions containing the addition of TFAA (Fig. 5 *b*, *c*). In accordance with the principles detailed in [6–9], the part of water that freezes at $T > 260$ K ($\Delta G > –0.5$ kJ/mol) can be attributed to weakly bound (WBW) water, and the rest – to strongly bound water (SBW). The characteristics of layers of different types of water are given in Table 1.

Table 1. Characteristics of non-freezing water layers in composite systems based on suspensions of lactic acid bacteria, TS-100 silica and TFAA

Medium	*h*, g/g	C_{uw}^s, g/g	C_{uw}^w, g/g	ΔG_{max}, kJ/mol	γ_S, J/g
-	1	0.5	0.5	-2	31.2
TS-100	1	0.5	0.5	-2	30.2
TS-100/$CDCl_3$	1	0.6	0.45	-2	33.9
TS-100/$CDCl_3$/TFAA	1	0.53	0.47	-2	32.2
TFAA	1	0.85	0.15	-2	41.9
TFAA/$CDCl_3$	1	0.72	0.28	-1.8	35.5
TFAA/$CDCl_3$/DMSO	1	0.75	0.25	-1.8	40.5

The possibility of using the same methods for calculating the parameters of bound water for aqueous solutions of TFAA as for the initial aqueous suspension is due to the fact that the freezing of solution components, as a rule, occurs through their joint crystallization in the form of individual crystals of ice and TFAA. Then, in the process of heating the sample, ice melting occurs at a temperature corresponding to the crystallite radius in accordance with the Gibbs-Thomson formula. Only then water mixes with the melted TFAA and form a solution.

Methods for determining distributions along the radii of clusters formally described in

[8, 13–15] can also be attributed to substances dissolved in water (in our case, sugars). Thus, in [19] it was shown that the constants in the Gibbs-Thomson formula for substances forming molecular complexes of the same type are quite close. Considering that both water and sugars form a crystal lattice during freezing, in which hydroxyl groups participate in the formation of hydrogen bonds with neighboring molecules, and their crystallization occurs in the same temperature range, then we can assume that for them the constants in formula 1 are close.

As follows from the data in Table 1, dilution of the suspension leads to a significant increase in the fraction of weakly bound water. Despite the greater amount of water in the system, the interfacial energy (which determines the total decrease in the Gibbs free energy of water due to the presence of phase boundaries with the cell surface and intracellular structures) decreases with dilution. This may be due to the formation of a colligative cell structure in concentrated suspensions, in which the cells and the surrounding water form a viscous gel that is sensitive to the total amount of water. When the cell suspension is encapsulated with hydrophobic silica, the effect of chloroform or a mixture with TFAA on the parameters of non-freezing water is minimal. The change in the interfacial energy varies within ± 3.5 J/g, which is close to the measurement error of the γ_S value. At the same time, close values of the amount of strongly bound and weakly bound water are recorded. The value of ΔG_{max}, which shows a decrease in free energy in the layer of adsorbed water closest to the interface, does not exceed –2 kJ/mol.

In the case when TFAA is present in water, which serves as a dispersion medium for cells, the parameters of water bound by cells also weakly depend on the presence of organic additives. The growth of the interphase energy of water bound by cells in the presence of TFAA may be due to the contribution from the energy of water solvation by TFAA molecules. In this case, for the crystallization of water from the solution in the form of pure ice, the energy required for the separation of the solution components must be expended.

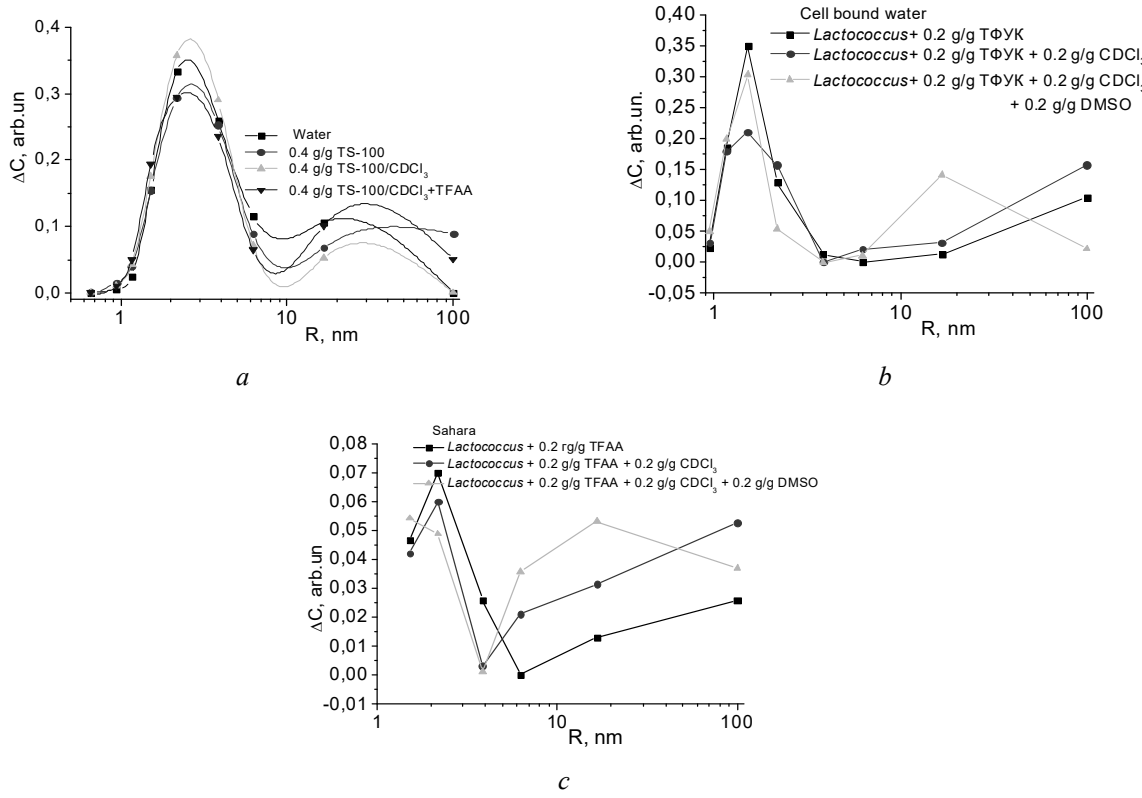


Fig. 5. Radial distributions of non-freezing water clusters for aqueous suspensions of lactic acid bacteria (*a*) and suspensions containing TFAA (*b*, *c*)

For a cell suspension encapsulated with silica (Fig. 5 *a*), the radial distributions exhibit two maxima at $R = 2$ and 20 nm. Dilution of the suspension is accompanied by a significant increase in large and a decrease in small clusters of bound water. This also indicates the possibility of destruction, upon dilution, of the cell gel. Organic additives have little effect on the size of bound water clusters, which is probably due to the difficulty of their penetration into cells.

For cell suspensions containing the addition of TFAA (Fig. 5 *b*, *c*), the form of distributions changes significantly. Cell-bound water is characterized by a maximum at $R = 2$ nm. A significant part of water is also included in bulk clusters with $R > 10$ nm. The presence of common features in the radial distributions of water and sugar clusters suggests that the conditions for the formation of both cluster systems are similar. Probably, as water freezes and nano-sized ice crystals are formed near them, the formation of sugar crystals of similar size also occurs.

CONCLUSIONS

It has been shown that the spectral parameters of water in concentrated cell suspensions of lactic acid bacteria strongly depend on the concentration of the suspension, which is probably associated with the possibility of the formation of a stable cell gel, which can be encapsulated by TS-100 silica particles in both air and chloroform medium with the addition of trifluoroacetic acid. In this case, organic components do not penetrate well into cells. This is probably due to the formation of a capsule around microorganisms or their aggregates, which makes it difficult for the molecules of the medium to penetrate into the aqueous cell suspension.

In relatively dilute aqueous suspensions and in concentrated suspensions containing TFAA and silica-free, cell-bound water and sugars present in the solution are observed in ^{1}H NMR spectra as two separate signals, with chemical shifts $\delta_H = 4$ and 6–7 ppm respectively, which makes it possible to determine the thermodynamic parameters of water and the cluster structure of water and sugars. It is shown that the curves of the radial distributions of non-freezing water clusters have two maxima corresponding to $R = 2$ and 20–100 nm.

Інкапсулювання клітинних суспензій молочнокислих бактерій кремнеземом

Т.В. Крупська, В.В. Туров, М.Д. Цапко, Я. Скубішевська-Зієба, Р. Лебода

Інститут хімії поверхні ім. О.О. Чуйка Національної академії наук України
вул. Генерала Наумова, 17, Київ, 03164, Україна, krupska@ukr.net
Хімічний факультет, Київський національний університет імені Тараса Шевченка
вул. Володимирська, 60, Київ, 01033, Україна
Хімічний факультет, Університет імені Марії Кюрі-Склодовської
пл. Марії Кюрі-Склодовської, 3, Люблін, 20031, Польща

Перспективним напрямком тривалого зберігання клітин при відносно високих температурах може стати їхнє інкапсулювання нанорозмірними біологічно інертними матеріалами, здатними створювати навколо мікрокрапель клітинної суспензії оболонку, яка, з одного боку, забезпечує можливість газового обміну між суспензією і зовнішнім середовищем, а з іншого - гальмує процеси життєдіяльності клітин, переводячи їх в стан, близький до анабіозу. Методом низькотемпературної ^{1}H ЯМР-спектроскопії вивчено процес гідратації лактобактерій, вплив на нього слабкополярного органічного середовища та інкапсулювання клітин кремнеземом. Метою цієї роботи було вивчення гідратації клітинних суспензій та життєздатності клітин молочнокислих бактерій, інкапсульованих кремнеземом, і можливості проникнення в них такої активної речовини, як трифтороцтова кислота. В результаті проведених досліджень показано, що спектральні параметри води в концентрованих клітинних суспензіях молочнокислих бактерій сильно залежать від концентрації суспензії, що, ймовірно, пов'язано з можливістю формування стабільного клітинного гелю, який без його руйнування може бути інкапсульований частинками кремнезему як в повітряному середовищі, так і в середовищі хлороформу з добавкою трифтороцтової кислоти. На кривих

розподілу за радіусами кластерів незамерзаючої води присутні два максимуми, що відповідають R = 2 і 20–100 нм. Внесок у розподіл другого максимуму зростає з ростом концентрації води.

***Ключові слова**: гідрофільний кремнезем, інкапсулювання, ^{1}H ЯМР-спектроскопія, молочнокислі бактерії*

REFERENCES

1. Franks F. *Biophysics and biochemistry at low temperature.* (Cambridge: University Press, 1985).
2. Kuleshova L. G., Kovalenko I.F. Theoretical prediction of optimal cooling rates of cell suspensions. *Bulletin of the Kharkov National University. Biophysical Bulletin*. 2008. **20**(1): 56. [in Russian].
3. Osetskii A.I., Kirilyuk A.L., Gurina T.M. On the possible mechanism of damage to cryopreserved biological objects due to plastic pressure relaxation in closed liquid-phase inclusions. *Problems of Cryobiology*. 2007. **17**(3): 272. [in Russian].
4. Krivokharchenko A.S., Serobyan G.A., Sadovnikov V.B. Cryoembryobanks – promising technology for the conservation of laboratory, domestic and wild animal genetic resources for practical use. In: *IV International Conference and Discussion Scientific Club. Ukraine, Crimea, Yalta-Gurzuf: "IT+ME'98"*, 1998. P. 380. [in Russian].
5. Kirilenko M.A., Kuznetsov O.Yu., Dmitrieva Zh.M. The effect of cryopreservation on the survival of a complex of lactic acid bacterial autostrains during storage and biotechnological scaling processes. *Yu.A. Ovchinnikova Herald of biotechnology and physical and chemical biology*. 2019. **15**(2): 5. [in Russian].
6. Hofmann N., Bernemann I., Pogozhi D., Glasmaher B. Development of system optimization of cryopreservation protocols for cell suspensions. *Problems of Cryobiology*. 2011. **21**(4): 353. [in Russian].
7. Zvyagintsev D.G. *Interaction of microorganisms with solid surfaces.* (Moscow: Publishing house of the MSU, 1973). [in Russian].
8. Tsyrenov V.Zh. *The basics of biotechnology: the cultivation of human and animal cells.* (SSSTU Ulan-Ude, 2005). [in Russian].
9. Lapage S.P., Shelton J.E., Mitchell T.G., Mackenzie A.R. *Culture collections and the preservation of bacteria. In Methods in Microbiology*. (London: Academic Press, 1970). P. 135.
10. Malik K.A., Claus D. Bacterial culture collection: Their importance to biotechnology and microbiology. *Biotechnology and Genetic Engineering Reviews*. 1987. **5**: 137.
11. Uzunova-Doneva T., Donev T. Anabiosis and conservation of microorganisms. *Journal of Culture Collections*. 2005. **4**: 17.
12. Sidyakina T.M. *Preservation of microorganisms.* (Pushchino: ONTI NCBI, 1985). [in Russian].
13. Fakhrullin R.F., Minullina R.T. Hybrid Cellular−Inorganic Core−Shell Microparticles: Encapsulation of Individual Living Cells in Calcium Carbonate Microshells. *Langmuir*. 2009. **25**(12): 6617.
14. Gun'ko V.M., Turov V.V., Gorbik P.P. *Water at the interface*. (Kyiv: Naukova Dumka, 2009). [in Russian].
15. Gun'ko V.M., Turov V.V. *Nuclear Magnetic Resonance Studies of Interfacial Phenomena.* (New York: Taylor & Francis, 2013).
16. Turov V.V., Gun'ko V.M. *Clustered water and ways to use it.* (Kyiv: Naukova dumka, 2011). [in Russian]
17. Gun'ko V.M., Turov V.V., Bogatyrev V.M., Zarko V.I., Leboda R., Goncharuk E.V., Novza A.A., Turov A.V., Chuiko A.A. Unusual Properties of Water at Hydrophilic/Hydrophobic Interfaces. *Adv. Colloid Interface Sci.* 2005. **118**(1–3): 125.
18. Aksnes D.W., Kimtys L. Characterization of mesoporous solids by ^{1}H NMR. *Magnetic Resonance in Colloid and Interface Science.* 2004. **25**: 146.
19. Petrov O.V., Furo I. NMR cryoporometry: Principles, application and potential. *Progress in Nuclear Magnetic Resonance Spectroscopy.* 2009. **54**(2): 97.
20. Frolov Yu.G. *The course of colloid chemistry. Surface phenomena and disperse systems*. (Moscow: Chemistry, 1982). [in Russian].
21. Pople J.A., Schneider W.G., Bernstein H.J. *High-Resolution Nuclear Magnetic Resonance*. (New York-Toronto-London: McGraw-Hill Book Company, JNC, 1959).

Received 28.07.2020, accepted 01.03.2021