engineering proceedings

MDPI

Proceeding Paper

Evaluation of the Safety of Immobilized Microorganisms *Lysobacter* sp. on Inorganic Media †

Galina Shaidorova *, Alexander Vesentsev, Ulyana Krut, Elena Kuzubova , Alexandra Radchenko and Marina Potapova

Institute of Pharmacy, Chemistry and Biology, Belgorod State University, 85 Pobedy Street, 308015 Belgorod, Russia; vesentsev@bsu.edu.ru (A.V.); krut@bsu.edu.ru (U.K.); 1015artek1015@mail.ru (E.K.); 1198157@bsu.edu.ru (A.R.); 1250152@bsu.edu.ru (M.P.)

* Correspondence: shaydorova@bsu.edu.ru; Tel.: +7-915-528-73-50

† Presented at the 2nd International Electronic Conference on Processes: Process Engineering—Current State and Future Trends (ECP 2023), 17–31 May 2023; Available online: https://ecp2023.sciforum.net/.

Abstract: It is known that the immobilization of microorganisms on carriers of various natures increases their safety. The inorganic matrices used were sodium carboxymethyl cellulose, technical brand "KMC 85/500;" colloidal silicon dioxide in the form of a commercial preparation, "Polysorb;" and the sodium form of montmorillonite from the Podgorenskoye deposit in the Voronezh region. Bacterial cells were immobilized by adding *Lysobacter* sp. solid sterile carrier with constant mechanical stirring in a "carrier/biomass" ratio equal to 1: (2–4). During the experiment, it was found that the mineral montmorillonite is a promising material for the immobilization of bacterial cells in order to obtain biocompositions based on them, since a positive trend in the preservation of bacterial cells was revealed.

Keywords: inorganic matrices; immobilization; microorganisms; cell safety

Citation: Shaidorova, G.; Vesentsev, A.; Krut, U.; Kuzubova, E.; Radchenko, A.; Potapova, M. Evaluation of the Safety of Immobilized Microorganisms *Lysobacter* sp. on Inorganic Media. *Eng. Proc.* **2023**, *37*, 71. https://doi.org/10.3390/ECP2023-14706

Academic Editor: Gade Pandu Rangaiah

Published: 18 May 2023

1. Introduction

Currently, a promising direction in biomedicine is the creation of biofilms and compositions based on microorganisms as antagonists of pathogenic microflora on carriers of various natures [1–3].

The aim of the study was to identify a rational inorganic carrier for the immobilization of *Lysobacter* sp.

2. Materials and Methods

As a model bacterial culture for immobilization, a *Lysobacter* culture isolated from the soil of the city of Belgorod using classical biotechnology methods with confirmation of generic affiliation by 16S rRNA sequencing (1484 nucleotides) was used, as a result of which a unique nucleotide sequence of the strain among those presented in GenBank was revealed: the maximum percentage of similarity observed with strain *L-43* (MT229166.1) and *Lysobacter enzymogenes* M497-1 (AP014940.1) was 99.7% each.

As a growth substrate, a liquid nutrient medium containing 0.2 wt% casein and 0.1 wt% yeast extract was used; T = 30 °C; log phase—24 h. The metabolic products were chitosan, beta-1,4-glucanase, and protease.

To study the effect of immobilization of microorganisms and their enzymes, the following solid carriers were used:

1. Sodium carboxymethyl cellulose (NaCMC) technical brand "KMC 85/500" produced by LLC "Davos-Trading". TU 2231-001-53535770-2010 (with change No. 1.2): degree of substitution for carboxymethyl groups 80–90; degree of polymerization 500–550; pH value (pH) of an aqueous solution with a mass fraction of CMC 1% in the range

of 8–12; dynamic viscosity of a 2% CMC solution at a temperature of 25 °C mPa*s according to the Brookfield method, not less than 100.
2. Colloidal silicon dioxide in the form of a commercial preparation called "Polysorb," produced by JSC "Polysorb." Polysorb MP (medical oral) is an inorganic, non-selective, multifunctional enterosorbent based on highly dispersed silica with particle sizes up to 0.09 mm and the chemical formula SiO_2. The sorption capacity of the drug for internal use is 300 m^2/g.
3. Sodium form of montmorillonite (NaMMT) from the Podgorenskoye deposit, Voronezh region [4,5], obtained by introducing soda ash (4 wt%) into a native rock suspension (5 wt%), followed by sedimentation enrichment and drying (t = 95 ± 3 °C). The quantitative content of montmorillonite, determined according to GOST 28177-89—79.35 ± 0.14 wt.%. As a result of the modification, the crystal lattice parameters changed: for the native form of Ca-montmorillonite, a = 5.16 Å, b = 8.94 Å, c = 15.02 Å; for modified montmorillonite, a = 5.22 Å, b = 9.04 Å, c = 13.82 Å. Specific surface, 60 m^2/g, specific pore volume, 0.083 cm^3/g; average pore size, 55.5 Å.

The immobilization of bacterial cells was carried out by adding *Lysobacter* sp. in the logarithmic phase of growth to a solid sterile carrier with constant mechanical stirring in the ratio "carrier/biomass," equal to 1:(2–4), at a temperature of 30 °C; the mixture was thoroughly mixed for at least 40 min, frozen at minus 40 °C, and then freeze-dried at minus 40–45 °C for 24 h to a level of 3–7% moisture content of the composition. The dry compositions obtained were then stored in sterile flacons at room temperature.

Survival after immobilization of microorganisms *Lysobacter sp.* on solid carriers was determined by the Pour Plate method, in which the samples were suspended in a Petri dish using molten agar cooled to about 40–45 °C (just above the solidification point to minimize heat-induced cell death). After the nutrient agar solidified, the plates were incubated for 24 h, and the number of colony-forming units (CFU) was determined by the serial dilution method.

The degree of preservation (α, %) was determined by the following formula:

$$\alpha = 1 - \left(\frac{CFU_{ref} - CFU_n}{CFU_{ref}} \right) \times 100\% \tag{1}$$

where CFU_{ref} is the number of colony-forming units in the biocomposition immediately after immobilization and CFU_n is the number of colony-forming units in the biocomposition after storage on the n-th day.

3. Results

The results of assessing the viability of immobilized *Lysobacter sp.* are presented in Table 1, and Figure 1 shows the dynamics of the preservation of bacterial culture.

Table 1. Viability of immobilized *Lysobacter* sp. cells.

Biomass/Carrier Ratio	CFU * (g/L) after Storage			
	Day 2	Day 15	Day 31	Day 92
Freeze culture	$2.4 \pm 0.04 \times 10^5$	$2.3 \pm 0.06 \times 10^5$	$2.0 \pm 0.04 \times 10^5$	$0.9 \pm 0.05 \times 10^5$
NaCMC 1:2	$3.4 \pm 0.06 \times 10^5$	$3.7 \pm 0.02 \times 10^5$	$3.9 \pm 0.05 \times 10^5$	$2.1 \pm 0.02 \times 10^5$
NaCMC 1:3	$3.5 \pm 0.04 \times 10^5$	$3.8 \pm 0.03 \times 10^5$	$4.1 \pm 0.07 \times 10^5$	$2.7 \pm 0.07 \times 10^5$
NaCMC 1:4	$3.3 \pm 0.07 \times 10^5$	$3.5 \pm 0.03 \times 10^5$	$3.6 \pm 0.05 \times 10^5$	$1.9 \pm 0.03 \times 10^5$
Polysorb 1:2	$3.5 \pm 0.02 \times 10^5$	$3.2 \pm 0.02 \times 10^5$	$2.7 \pm 0.01 \times 10^5$	$1.8 \pm 0.02 \times 10^5$
Polysorb 1:3	$3.2 \pm 0.03 \times 10^5$	$3.1 \pm 0.01 \times 10^5$	$2.5 \pm 0.05 \times 10^5$	$1.7 \pm 0.01 \times 10^5$
Polysorb 1:4	$3.3 \pm 0.03 \times 10^5$	$3.1 \pm 0.05 \times 10^5$	$2.4 \pm 0.07 \times 10^5$	$1.5 \pm 0.04 \times 10^5$
NaMMT 1:2	$3.6 \pm 0.03 \times 10^5$	$3.8 \pm 0.02 \times 10^5$	$4.0 \pm 0.04 \times 10^5$	$4.2 \pm 0.06 \times 10^5$
NaMMT 1:3	$3.5 \pm 0.05 \times 10^5$	$3.7 \pm 0.06 \times 10^5$	$3.9 \pm 0.07 \times 10^5$	$4.1 \pm 0.03 \times 10^5$
NaMMT 1:4	$3.4 \pm 0.01 \times 10^5$	$3.8 \pm 0.04 \times 10^5$	$4.0 \pm 0.06 \times 10^5$	$4.1 \pm 0.03 \times 10^5$

* Std. Deviation.

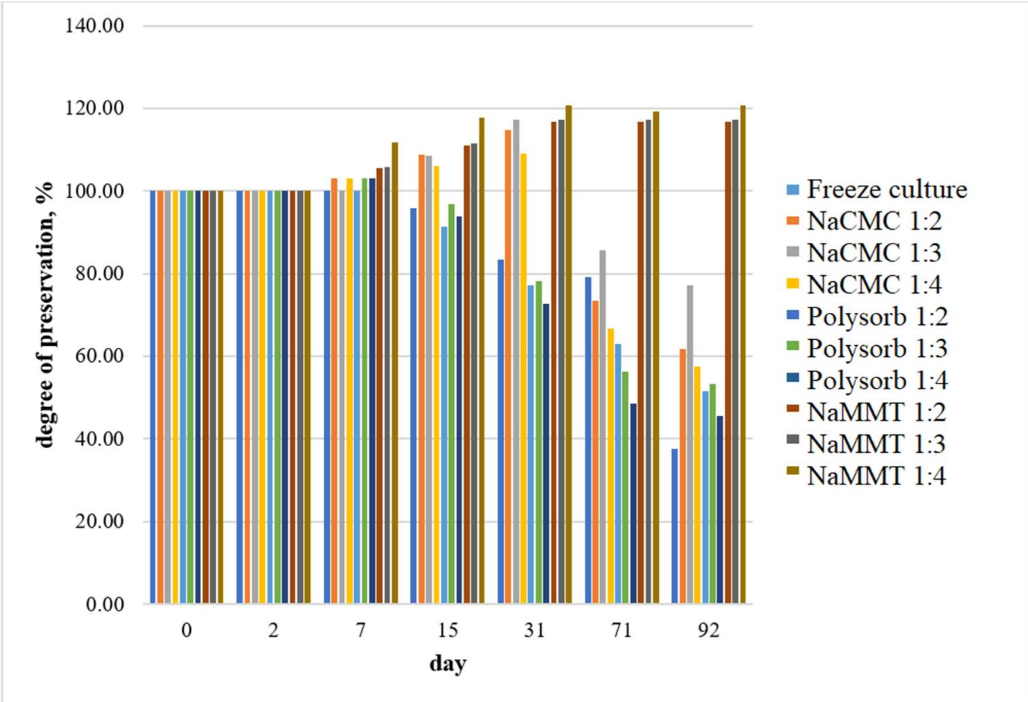

Figure 1. Dynamics of the preservation of the bacterial culture *Lysobacter* sp.

After three months (92 days) of storage of lyophilizates, the following results were obtained:

1. Lyophilization of the bacterial culture of *Lysobacter* sp. without immobilization on the matrix leads to a 37.5% decrease in safety;
2. Cell immobilization on sodium carboxymethyl cellulose allows safety to increase up to 65%, and on colloidal silicon dioxide (Polysorb), it increases up to 50%;
3. When immobilized on the mineral montmorillonite, not only is the preservation of microorganisms manifested, but there is also an 18% increase in the number of cells.

4. Conclusions

In summary, a positive trend in the preservation of bacterial cells during immobilization on solid carriers was revealed. It has been established that the most effective matrix for immobilizing *Lysobacter* sp. is the sodium form of montmorillonite. The obtained research results can be used to create biocompositions based on bacterial cultures for various purposes.

Author Contributions: Conceptualization, U.K., G.S.; methodology and visualization, A.V.; formal analysis, G.S.; investigation, M.P.; writing—review and editing, E.K. and A.R. All authors have read and agreed to the published version of the manuscript.

Funding: The work was carried out within the framework of the state task FZWG-2023-0007. Adaptive reactions of microorganisms: theoretical and applied aspects.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: https://drive.google.com/drive/folders/1EHtDgne05s2bov0CiTfVWb4hBZVl0mhA?usp=drive_link (accessed on 1 May 2023).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Andryushina, V.A.; Balabanova, T.V.; Beklemishev, A.B.; Varfolomeev, S.D.; Vodyakova, M.A.; Demakov, V.A.; Ditchenko, T.I.; Dzhavahiya, V.V.; Drozdova, M.G.; Efremenko, E.N.; et al. *Immobilized Cells: Biocatalysts and Processes*; Publishing Center RIOR: Moscow, Russia, 2018; 500p.
2. Milivojevic, M.; Pajic-Lijakovic, I.; Bugarski, B.; Levic, S.; Nedovic, V. Alginic acid: Sources, modifications and main applications. In *Alginic Acid Chemical Structure, Uses and Health Benefits*; Nova Science Publishers: Hauppauge, NY, USA, 2015; pp. 45–88.
3. Niyazbekova, Z.T.; Nagmetova, G.Z.; Kurmanbayev, A.A. An overview of bacterial cellulose applications. *Eurasian J. Appl. Biotechnol.* **2018**, *13*, 17–25. [CrossRef]
4. Shaidorova, G.M.; Vezentsev, A.I.; Trufanov, D.A. Obtaining the sodium form of clays from the Podgorenskoe deposit of the Voronezh region. *Bull. Technol. Univ.* **2022**, *25*, 101–105. [CrossRef]
5. Shaidorova, G.M.; Vezentsev, A.I.; Trufanov, D.A.; Sokolovsky, P.V. Sorption activity of sodium-modified bentonite-like clays of the Podgorensky deposit of the Voronezh region. Actual physical and chemical problems of adsorption and synthesis of nanoporous materials. In *All-Russian Symposium with International Participation, Dedicated to the Memory of corr. RAS V.A. Avramenko. Collection of Proceedings of the Symposium*; IFCHE RAN: Moscow, Russia, 2022; pp. 177–179.