

## Ultrasonic intensification of sorption and desorption processes during the isolation of virginiamycin from the cultural broth of *Streptomyces* sp. S 15-30

D.A. Durnikin<sup>1</sup>, E.S. Yacenko<sup>1</sup>, I.Yu. Evdokimov<sup>1</sup>, V.B. Akopyan<sup>1</sup>,  
V.V. Dzhavakhiya<sup>2</sup>, V.A. Savushkin<sup>2</sup>, V.I. Glagolev<sup>2</sup>, A.I. Ovchinnikov<sup>2</sup>

<sup>1</sup>Altai State University

Lenin Ave., 61, Barnaul, 656049, Russia

E-mail: [durnikin@list.ru](mailto:durnikin@list.ru), Tel.: (3852) 363-077, E-mail: [mlprx@mail.ru](mailto:mlprx@mail.ru), Tel.: 8-903-948-60-38,

E-mail: [ivan.evdokimov.92@mail.ru](mailto:ivan.evdokimov.92@mail.ru), Tel.: 8-960-940-64-59, E-mail: [akopyan1941@mail.ru](mailto:akopyan1941@mail.ru), Tel.: 8-903-506-06-33

<sup>2</sup>Federal Research Centre "Fundamentals of Biotechnology", Russian Academy of Sciences

pr. 60-letiya Oktyabrya, 7/1, Moscow, 117312 Russia

E-mail: [engbio@rambler.ru](mailto:engbio@rambler.ru), Tel.: 8-499-135-90-75, E-mail: [vanzan113@rambler.ru](mailto:vanzan113@rambler.ru), Tel.: 8-926-371-17-97,

E-mail: [dwait2812@gmail.com](mailto:dwait2812@gmail.com), Tel.: 8-910-458-32-56, Email: [centr-biotech@mail.ru](mailto:centr-biotech@mail.ru), Tel.: 8-499-135-90-01

Submitted: 06.08.2017. Accepted: 29.09.2017

Supplementation of fermentation media with synthetic resins is used in the process of biotechnological production of antibiotics and other biologically active substances to prevent the auto-inhibition of the biosynthesis processes due to the binding of secreted target metabolites to a sorbent and their removal from the fermentation volume. The efficiency of a sorbent application may be improved via the ultrasonic stimulation of the antibiotic sorption from cultural broth during fermentation, and the similar stimulation of the reverse process (desorption) during its isolation and purification. In this study, the possibility of the use of ultrasound to improve the processes of sorption and desorption of virginiamycin, a feed antibiotic produced by the highly active strain *Streptomyces* sp. S 15-30, to/from an Amberlite XAD-16 synthetic resin selectively binding this antibiotic, has been evaluated. According to the obtained results, the ultrasonic treatment with frequency of 22 kHz and acoustic energy density up to 0.05 W·s/cm<sup>3</sup> does not disrupt cells membranes, i.e., does not violate biotechnological processes. At the same time, such treatment increases the sorption capacity of the sorbent in ~1.4 times. The similar ultrasonic treatment of a sorbent at the acoustic field with energy density of 0.5 W·s/cm<sup>3</sup> almost tenfold accelerates desorption of virginiamycin from the resin but does not destroy the sorbent and provides a possibility to re-use it after regeneration. The revealed effects of the ultrasonic treatment may be integrated into the technology of the virginiamycin biosynthesis, isolation, and purification to improve the efficiency of its industrial production.

**Key words:** virginiamycin, sorption/desorption, intensification, ultrasound

---

## Introduction

Target metabolites produced by industrial microbial strains usually inhibit the biosynthetic activity of their producers as they are accumulated in the cultural broth (CB). Therefore, the prevention of their accumulation, for example, via the removal of these metabolites from CB provides a possibility to control the process of fermentation and significantly improve its efficiency (Sushkova et al., 2012).

There are a number of various approaches to remove target metabolites from CB that provides a wide range of possible solutions for arising biotechnological problems. For example, it was shown that during the biosynthesis of a feed antibiotic virginiamycin by a *Streptomyces* sp. S 15-30 strain, developed from the *Streptomyces* sp. DSM 40559 strain by a multi-step UV mutagenesis and characterized by the productivity of ~2.6 g/L (that is almost tenfold exceeds the productivity of the initial strain), an increase in the antibiotic concentration in CB up to 5.0 g/L caused a significant auto-inhibiting effect (Savushkin et al., 2016). One of the ways to reduce or prevent the effect of auto-inhibition by accumulating metabolites is fermentation in the presence of sorbents able to selectively bind the target product and, therefore, reduce its concentration in CB (Phillips, 2013). The preferable sorbents should be resistant to the action of technological media used during fermentation and should be regenerable to provide their re-use without significant losses in the selectivity and sorption capacity.

Earlier it was shown that the use of an Amberlite XAD-16 synthetic resin, characterized by a high specific surface area, provided a significant reduction of the auto-inhibition effect during the fermentation of a virginiamycin-producing *Streptomyces* sp. S 15-30 strain (Savushkin et al., 2016). At the same time, the efficiency of the sorbent use may be additionally increased via the intensification of sorption/desorption processes occurring on a sorbent. Such improvement may be achieved by the ultrasonic treatment causing the appearance of acoustic streaming in liquid media including micro streaming with high velocity gradients, which provide intensive agitation of a medium, destroy near-surface layers, and reduce diffusion limitations (Akopyan, Ershov, 2016).

The purposes of this work were the study of the possibility to apply ultrasonic treatment for the improvement of virginiamycin sorption/desorption processes, occurring on a hydrophobic Amberlite XAD-16 sorbent, and the evaluation of a principal possibility of the introduction of such treatment into the technological process of production of this feed antibiotic.

## Materials and methods

The biosynthesis of virginiamycin was carried out using a highly-active *Streptomyces* sp. S 15-30 strain. The strain was maintained and stored as described earlier (Savushkin et al., 2016). The fermentation medium contained the following components (g/L): glucose, 50.0; soybean flour, 15.0; malt extract, 10.0; corn gluten, 5.0; meat peptone, 2.5; yeast extract, 1.0; CaCO<sub>3</sub>, 5.0; KH<sub>2</sub>PO<sub>4</sub>, 1.6; Na<sub>2</sub>HPO<sub>4</sub>, 1.0; NH<sub>4</sub>NO<sub>3</sub>, 1.0; MgCl<sub>2</sub>, 0.5; (pH 7.0–7.2). Medium preparation and fermentation were carried out as described earlier (Savushkin et al., 2016).

The virginiamycin sorption from CB was provided by the addition of the Amberlite XAD-16 resin (ROHM & HASS, USA) to the fermentation medium prior beginning of fermentation; the amount of the added resin was 5 g per a liter of fermentation medium.

The treatment of a sorbent with ultrasound (22 kHz) was carried out using an UZG-13-01/22 ultrasound generator (UZT Ltd., Russia). The acoustic energy density in a medium was measured by a calorimetric method (Akopyan et al., 2009).

Relative changes in the sorption capacity and desorption rate caused by the ultrasonic treatment were evaluated via the measurement of a virginiamycin concentration in a fixed volume of a liquid phase containing a certain amount of a sorbent. The antibiotic concentration was determined by HPLC using an Agilent 1200 chromatographic system (Agilent Technologies, USA) equipped with a Zorbax SB-C18 column (250×4.6 mm, Agilent Technologies, USA) as described earlier (Dzhavakhiya et al., 2016). The threshold of the acoustic energy density, which limited cell viability, was determined by the changes in the CB optical density at  $\lambda = 600$  nm which reflected changes in the cell concentration in CB caused by the ultrasonic treatment.

Virginiamycin was extracted from the sorbent in the presence or absence the action of ultrasound (22 kHz, acoustic energy density 0.5 W·s/cm<sup>3</sup>) by adding the equal volume of ethyl acetate followed by a 2-h incubation under constant stirring. After the 1-h waiting for the separation of water and ethyl acetate layers, the ethyl acetate layer was separated, centrifuged at 12000 rpm for 3 min, and the concentration of virginiamycin was determined.

## Results and discussions

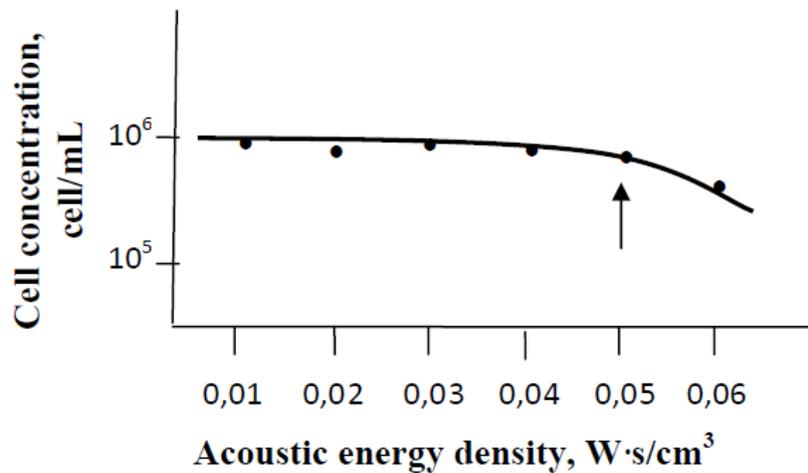
A diffusion layer located at the interphase boundary resists to mass exchange process occurring between the virginiamycin-containing liquid phase (CB) and the solid phase sorbent. The reason is that the mass transfer process in this layer occurs very slowly via molecular diffusion. The thickness and properties of the diffusion layer significantly influence on the rate of heterogenic processes. Turbulent acoustic streaming, arising during intensive agitation in close proximity to the interphase boundary, reduce the effective thickness of the layer resisting to the mass transfer.

This process is significantly accelerated under the action of ultrasound due to the appearance of large- and small-scale vortex streaming, which intensify mass exchange processes. If the density of acoustic energy exceeds the threshold level, it causes ultrasonic cavitation accompanied by shock waves, microstreaming with high rate gradients, sonoluminescence, and sonochemical reactions (Sirotyuk, 2008). Therefore, ultrasonic impact intensifies sorption processes, significantly reduces the sorption time and sometimes increases a sorption capacity (Srivastava et al., 2014). For example, ultrasonic treatment of titanium dioxide significantly increased its sorption capacity (Smirnova, Nazarenko, 2012). The ultrasonic treatment (880 kHz, 1–2 W/cm<sup>2</sup>) in a dynamic mode intensified molecular sorption from water solutions and increased the sorption capacity of carbon sorbents and the sorption rate in 1.2–1.5 and 1.3–1.5 times, respectively (Verbanov et al., 1992).

However, the exceeding of the cavitation threshold results in the arising of hydrodynamic shock waves and intensive turbulent streaming able to destroy both living cells and solid phase sorbents.

At the first stage of the study, we tested the resistance of the Amberlite XAD-16 resin to the ultrasonic treatment. For this, sorbent-containing CB was supplemented with the equal volume of ethyl acetate and then treated for 5 min by ultrasound at

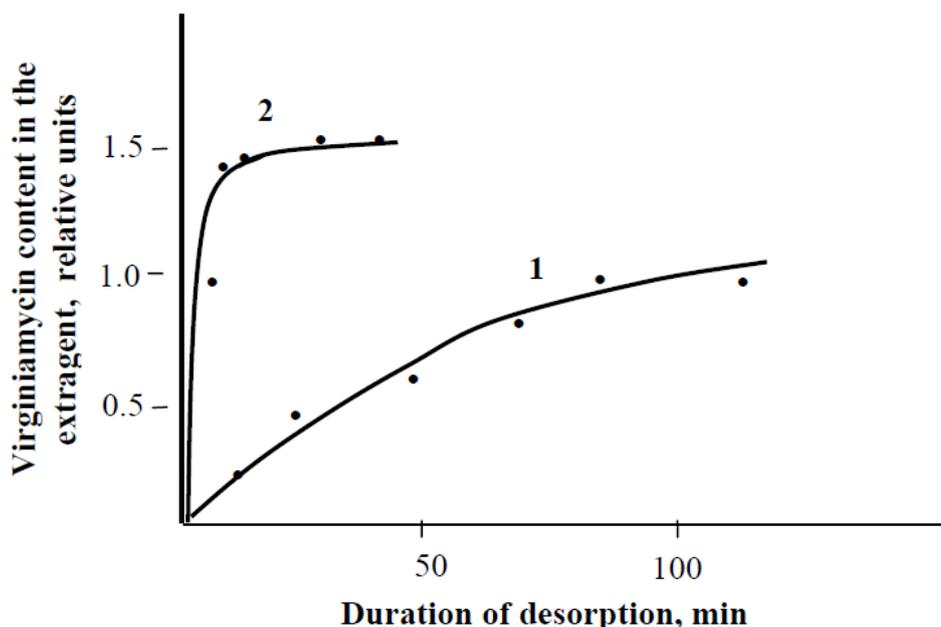
different values of the acoustic energy density. According to the obtained results (data not shown), optical properties of the mixture remained unchanged within the density range of 0.1–0.5 W·s/cm<sup>3</sup>, whereas values exceeding 0.6–0.7 W·s/cm<sup>3</sup> caused the turbidity of the solution that was probably connected with the beginning of the sorbent destruction. The similar results were obtained in the case of the use of water instead of CB. Thus, the permissible range of density of acoustic energy, which does not influence on the sorbent structure, was 0.1–0.5 W·s/cm<sup>3</sup>. To determine the threshold of cell destruction, the cell suspension of *Streptomyces* sp. S-15-30 in CB (10<sup>6</sup> cell/mL) was treated by ultrasound for 10 min at 28°C using different densities of acoustic energy. The obtained results are shown in Fig. 1.



**Fig. 1.** Effect of the ultrasonic treatment with different acoustic energy density on the viability of cells of *Streptomyces* sp. S-15-30 suspended in the culture broth. The threshold level is indicated with the arrow.

If the acoustic energy density did not exceed 0.05 W·s/cm<sup>3</sup>, the treatment did not disrupt cell membranes of the virginiamycin-producing microorganism. Therefore, in our further experiment performed using the fermentation equipment, we applied this level of acoustic energy density, which provided a quite intensive agitation in a near-surface layer of fermentation medium, reduction of diffusion resistance, and acceleration of the target metabolite sorption.

At this level (0.05 W·s/cm<sup>3</sup>), cell destruction does not occur, whereas the gradient concentration of various compounds near cell surface decreases, and the processes of the removal of metabolites and intracellular uptake of nutrients are facilitated (Akopyan, Ershov, 2016).



**Fig. 2.** Dynamics of changes of the virginiamycin content in the extragent during its desorption from the Amberlite CAD-16 resin without (1) and under the action (2) of ultrasound (22 kHz, acoustic energy density 0.5 W·s/cm<sup>3</sup>). One relative unit of the Y axis corresponds to the maximum amount of virginiamycin sorbed by the resin sample from a culture broth in the absence of the ultrasonic treatment.

To study the effect of ultrasound on the virginiamycin sorption on the Amberlite XAD-16 resin, the sorbent was saturated with the antibiotic by a multiple submersion of the same sorbent sample into equal volumes of fresh CB portions, containing the known concentration of virginiamycin, followed by the measurement of the residual antibiotic content in the liquid fraction after the removal of the sorbent. The sorbent was considered to be saturated when the virginiamycin concentration in CB remained unchanged. The same procedure was also used for the preparation of a resin sample saturated by the antibiotic in the presence of ultrasound with the acoustic energy density equal to  $0.05 \text{ W}\cdot\text{s}/\text{cm}^3$ . A comparison of the obtained data showed that the ultrasonic treatment increased the sorption capacity of the sorbent in  $1.4 \pm 0.15$  times.

To study the effect of ultrasound on the process of virginiamycin desorption, we used sorbent samples, which were preliminarily saturated with the antibiotic under the treatment by low-intensity ultrasound ( $0.05 \text{ W}\cdot\text{s}/\text{cm}^3$ ). The desorption of the antibiotic was carried out under the action of ultrasound ( $0.5 \text{ W}\cdot\text{s}/\text{cm}^3$ ) or without it as described in the "Materials and Methods" section. The obtained results are shown in Fig. 2. A comparison of the desorption curves showed that the ultrasonic treatment almost tenfold accelerated virginiamycin desorption from the resin.

We suggested that the ultrasonic treatment at the levels of the acoustic energy density, which did not destroy living cells ( $0.05 \text{ W}\cdot\text{s}/\text{cm}^3$ ) and the sorbent itself ( $0.5 \text{ W}\cdot\text{s}/\text{cm}^3$ ) resulted in the  $1.4\times$  increase of the virginiamycin sorption capacity of the Amberlite XAD-16 resin and in almost tenfold acceleration of the virginiamycin desorption from the sorbent, respectively.

Obviously, the revealed effects of the ultrasonic treatment may be used in the technological processes of the virginiamycin biosynthesis, isolation, and purification to improve the efficiency of its industrial production.

## Acknowledgments

The study was financially supported by the Ministry of Education and Science of the Russian Federation within the framework of the Federal Targeted Program for Research and Development in Priority Areas of Advancement of the Russian Scientific and Technological Complex for 2014-2020 (grant agreement no. 14.580.21.0006 from 15.10.2015, code RFMEFI58015X0006).

## References

- Akopyan, V.B., Bambura, M.V., Rukhman, A.A., Nongayar, B. (2009). Device for measurement of the ultrasonic radiation power [Ustroistvo dlya izmereniya moshchnosti ultrazvukovogo izlucheniya]. Patent RU 2421694 (in Russian).
- Akopyan, V.B., Ershov, Yu.A. (2016). Fundamentals of the ultrasound interaction with biological objects [Osnovy vzaimodeistviya ultrazvuka s biologicheskimi ob'ektami]. Moscow: YURAIT (in Russian).
- Verbanov, V.S., Pogadaev, V.I., Verbanov, A.V. (1992). Method for intensification of molecular sorption from water solutions [Sposob intensivatsii molekulyarnoi sorptsii iz vodnyh rastvorov]. Patent SU 1775167 (in Russian).
- Sushkova, V.I., Berezina O.V., Yarotskiy, S.V. (2012). Approaches to the intensification of the butyric acid biosynthesis. *Khimija Rastitel'nogo Syr'ja*, 1, 171-180 (in Russian).
- Savushkin, V.A., Dzhavakhiya, V.V., Glagoleva, E.V., Savel'eva, V.V., Popova, E.D., Ovchinnikov, A.I., Glagolev, V.I., Novak, N.V., Durnikin, D.A. (2016). Development of highly active virginiamycin-producing strain and improvement of its productivity using synthetic adsorbing resins. *Biological Bulletin of Bogdan Chmelniyskiy Melitopol State Pedagogical University*, 6 (3), 195–208 (in Russian).
- Sirotyuk, M.G. (2008). Acoustic cavitation [Akusticheskaya kavitatsiya]. Moscow: Nauka (in Russian).
- Smirnova, V.V., Nazarenko, O.B. (2012). Effect of preparation conditions and ultrasonic treatment on the sorption activity of titanium dioxide. *Proceedings of IX Intern. Conf. of Students and Young Scientists "Prospects of the development of fundamental sciences"*, Tomsk, Russia, 484-486 (in Russian).
- Dzhavakhiya, V., Savushkin, V., Ovchinnikov, A., Glagolev, V., Savelyeva, V., Popova, E., Novak, N., Glagoleva, E. (2016). Scaling up a virginiamycin production by a high-yield *Streptomyces virginiae* VKM Ac-2738D strain using adsorbing resin addition and fed-batch fermentation under controlled conditions. *3Biotech*, 6, Article ID 240.
- Phillips, T., Chase, M., Wagner, S., Renzi, C., Powell, M., DeAngelo, J., Michels, P. (2013). Use of *in situ* solid-phase adsorption in microbial natural product fermentation development. *Journal of Industrial Microbiology and Biotechnology*, 40, 411–425.
- Srivastava, P., Goyal, S., Tayade, R. (2014) Ultrasound-assisted adsorption of reactive blue 21 dye on  $\text{TiO}_2$  in the presence of some rare earths (La, Ce, Pr&Gd). *Canadian Journal of Chemical Engineering*, 92(1), 41-51.

---

### Citation:

Durnikin, D.A., Yacenko, E.S., Evdokimov, I.Yu., Akopyan, V.B., Dzhavakhiya, V.V., Savushkin, V.A., Glagolev, V.I., Ovchinnikov, A.I. (2017). Ultrasonic intensification of sorption and desorption processes during the isolation of virginiamycin from the cultural broth of *Streptomyces* sp. S 15-30. *Ukrainian Journal of Ecology*, 7(3), 221–224.



This work is licensed under a Creative Commons Attribution 4.0. License