Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2017, 7(4), 187–191, doi: 10.15421/2017_104

ORIGINAL ARTICLE

Preconditioning of a virginiamycin solution for crystallization

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Submitted: 28.09.2017. Accepted: 13.11.2017

Crystallization of antibiotics and other biologically active substances from water solutions represents an important stage of their biotechnological production. The process is based on a sharp reduction of a target compound solubility caused by either temperature decrease, or supersaturation of a solution. A preconditioning of a solution, i.e., its evaporation with a simultaneous temperature decrease seems to be an optimal technical solution, especially advantageous for the treatment of thermolabile substances. This paper describes the technology and equipment for the pre-crystallization treatment of solutions of various substances produced by the biotechnological and chemical industries. The proposed preconditioning technology includes ultrasonic dispersion of a solution and formation of an aerosol with a large integral evaporation surface followed by condensation. Comparing to common tubular evaporators used in various industrial processes, this technology provides about equal productivity and, at the same time, lower energy consumption, since it does not require the heating and the further cooling of a solution needed to evaporate and condensate the solvent, respectively, that prevents undesirable effect of high temperature on thermolabile compounds. In addition, the technology prevents the damage of thermolabile compounds, improves the efficiency of the further crystallization process due to the ultrasound-stimulated formation of crystallizing nuclei, and provides a solvent distillate suitable for the further re-use. The designed device for preconditioning has been successfully tested using culture broth of *Streptomyces* sp. containing a feed antibiotic virginiamycin; such treatment with the further crystallization in standard crystallizers has resulted in the efficient formation of equal-sized antibiotic crystals.
Key words*: c*rystallization; antibiotics; supersaturated solution; ultrasonic dispersion; virginiamycin

Introduction

Today the efficient production of dairy and meat products requires an active use of veterinary antibiotics, which production volume makes almost 2/3 of the total antibiotic production in the world (Van Boeckel et al., 2014). Among the most popular veterinary antibiotics, one can mention streptogramins, which are nontoxic, non-mutagenic, and do not accumulate in animal tissues (Cocito, 1979). Virginiamycin, an antibiotic produced by *Streptomyces virginiae*, belongs to the streptogramin group and has a bacteriostatic and, at high concentrations, bactericidal activity towards the majority of gram-positive and some gram-negative bacteria (Grozina et al., 2014). Since this antibiotic also improves the state of a small intestine epithelium, optimizes the absorption and metabolism of nutrients, and inhibits the synthesis of harmful toxins and metabolites by gut microorganisms, it is also used as a growth stimulator (Cervantes et al., 2002). Virginiamycin is used in the industrial production of ethanol (Arshad et al., 2011) and as a feed antibiotic for cattle and poultry (Shojadoost et al., 2013). To 2020, the predicted increase in the consumption of this antibiotic in the North America, Europe, and Asia will make 6% (Chem-Report, 2015).
To provide a sufficient volume of virginiamycin production, the efficient technologies of its microbial synthesis, isolation, and purification are required. The isolation of antibiotics from culture broth (CB) after the completion of the microbiological biosynthesis and their further conditioning represent important stages of their industrial production. There are a lot of various methods of antibiotic isolation from CB, each having its own features. Among the technological approaches traditionally used in these methods, there are extraction, extragent evaporation until the saturation or supersaturation of a solution, and the further crystallization providing both isolation of the target product into a solid phase and, in some cases, its partial purification

from associated admixtures, since crystallization is one of the most common and efficient methods used to obtain pure substances.

The effect of crystallization of antibiotics or other biologically active compounds is based on a sharp reduction of their solubility caused by either temperature decrease, or supersaturation of a solution. In both cases, the driving force of crystallization is determined by the concentration excess of the solution above the equilibrium (saturation) level. To obtain a supersaturated state of a solution, it should be either evaporated to increase the concentration of a target compound, or cooled to decrease the solubility of the compound.

Obviously, the preconditioning of a solution, i.e., its evaporation with a simultaneous temperature decrease, which is especially important for thermolabile organic compounds, seems to be an optimal technical solution.

There are many approaches for the preconditioning of liquids for crystallization of solved compounds and also for the control of processes occurring in crystallizers (Ignatovich, 2007; Akhmedshina, Bazyutov, 2012; Mersmann, 2001). This fact reflects both the need in crystallizers, which would meet various technical requirements, and the necessity to adapt the crystallization equipment to different technological processes.

Some of the devices used to precondition a solution and to control crystallization processes are based on the use of temperature effects, provided by either regulated coolant supply (Chefonov et al., 1981), or vacuum evaporation (Barata et al., 2007), to increase the concentration of the solution to the metastable state with the further crystallization. Such devices consume a large amount of energy, since processes connected with the medium temperature changes require high energy costs, and the cooling is usually more energy-consuming than the heating.

Ultrasound is often used to initiate crystallization processes in supersaturated solutions (Ruecroft et al., 2005; Kelly et al., 1993; Anderson et al., 1994; Louhi-Kultanen et al., 2006; Akopyan, Ershov, 2016). At the same time, such approach has some disadvantages, since, depending on the acoustic energy density in the medium and the nature and state of the treated solution, such ultrasonic treatment not always results in the appearance of crystallization nuclei and acceleration of the process of their multiplication.

Ultrasound may significantly change crystallization dynamics depending on such parameters as the acoustic energy density, frequency, mode of action (pulse or continuous), and also temperature, saturation pressure of solvent vapor, medium viscosity, and level of solution supersaturation (Kelly et al., 1993). An intensive dissolution of small crystals increasing the concentration of a solution and a fragmentation of large crystals into smaller pieces serving as crystallization nuclei accelerates the crystallization process. In some cases, the formation of crystallization nuclei occurring without ultrasonic treatment is detected after several hours, whereas in the presence of ultrasound it starts after several seconds. The effect of ultrasound is clearly manifested at relatively low frequencies, where the cavitation threshold is rather low. Obviously, intensive microstreams and shock waves accompanying cavitation are responsible for the multiplication of crystallization nuclei and acceleration of the process. In addition, the effect of ultrasound results in the disappearance of a liquid supercooling that often delays the crystallization process.

In general, ultrasound reduces diffusion limitations (Akopyan, Ershov, 2016; Savushkin et al., 2016) and accelerates the growth of crystals in a solution; at the same time, too high acoustic energy density may destroy already formed crystals and delay the basic technological process.

The purpose of this study was the development of a new approach to the preconditioning of target compound solutions, which would improve the efficiency of the virginiamycin crystallization from ethyl acetate used as a liquid sorbent of this feed antibiotic from the culture broth of its producer, *Streptomyces* sp. S 15-30 strain.

Materials and methods

A high-yield *S. virginiae* S 15-30 strain was used as a virginiamycin producer; the composition of fermentation medium and cultivation conditions corresponded to the earlier described procedures (Savushkin et al., 2016).

A preliminary sorption of the antibiotic from fermentation medium was carried out using an Amberlite XAD-16 resin added to the fermentation medium prior sterilization (20 g/L); the further purification of the antibiotic was carried out as described earlier (Dzhavakhiya et al., 2016). The virginiamycin concentration was determined by HPLC (Borisov, 1980).

The problem of a simultaneous combining of evaporation and cooling processes with the ultrasonic treatment (Doubochinski, Tennenbaum, 2014; Doubochinsky, 2006; Hassoun, 2003), resulting in the reduction of a virginiamycin solubility and formation of crystallization nuclei, was solved by the designing of a simple device (Fig. 1), in which the solution of a target compound is sprayed by an aerodynamic ultrasonic atomizer and forms microdrops, which total surface significantly exceeds the surface area of initial liquid. In such case, the evaporation rate of the solution proportionally increases, while the temperature of the solution in microdrops decreases. Then the aerosol is condensed into a supersaturated solution preconditioned for the crystallization.

The principle of operation of the device is based on the known effect of the concentration and cooling of a solution during its evaporation. The preconditioning of a solution for crystallization is realized in the following way. Compressed air is fed to the ultrasonic aerodynamic atomizer (*1*) via the nipple (*6*) (Savushkin et al., 2016; Doubochinski, Tennenbaum, 2014) that results in the sucking of a conditioned solution via the nipple (*7*) and its dispersion with the aerosol formation. This results in a significant increase of the evaporation surface. The flow of the aerosol-air mixture in a working volume (*2*) is directed by guides (*3*); during this process, the solvent is evaporated from the surface of aerosol particles that results in the concentration of the content of aerosol particles with their simultaneous cooling. The produced air-vapor mixture is evacuated via the outlet nipple (*4*) and may be then concentrated into a solvent distillate. Cooled and supersaturated aerosol microdrops are settled on the surface of

guides, trickle down to the outlet nipple (*5*), and then are collected and fed to a crystallizer for the further crystallization under standard conditions.

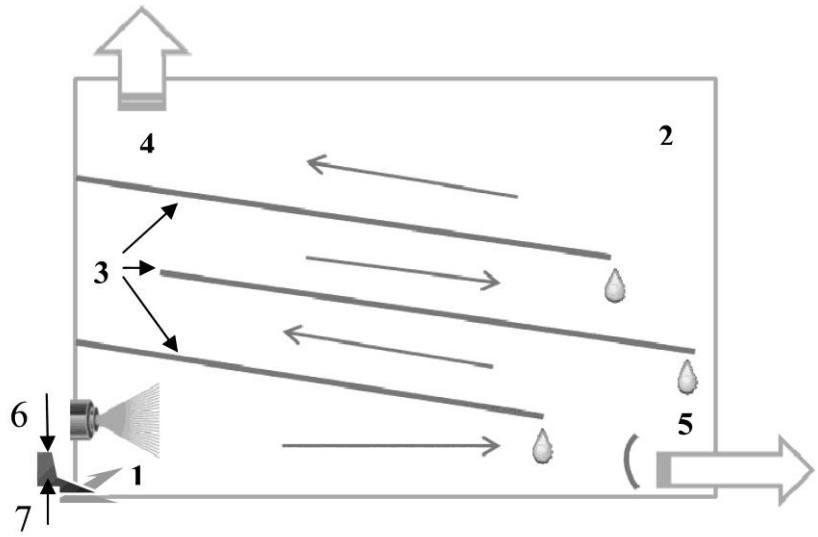

Figure 1. Device for the preconditioning of solutions for crystallization.
1, ultrasonic aerodynamic atomizer; *2*, working volume; *3*, guides; *4*, outlet for the air-vapor mixture; *5*, outlet for the supersaturated solution; *6*, inlet for compressed air; *7*, inlet for the initial solution.

Results and discussion

Obviously, the principle of action and the technical realization of the described device provide reduced energy consumption during the concentration of a solution, since the aerosol formation does not consume energy required for the breaking of almost all intermolecular bonds (as it occurs during evaporation) (Doubochinski, Tennenbaum, 2014; Doubochinsky, 2006). The evaporated solvent may be easily condensed from the air-vapor mixture to obtain a distillate suitable for the further re-use. In addition, such approach does not require any inoculation of a supersaturated solution with crystallization nuclei to stimulate crystallization, since such nuclei are spontaneously generated by the ultrasonic treatment (Hassoun, 2003).
The proposed technical solution provides a required diversity of possibilities to make an optimal choice of tools for the isolation of products of chemical or biological synthesis from solutions via crystallization.
A long thermal treatment of the vast majority of biosynthetic products results in the loss of some of their valuable qualities. Therefore, the evaporation of solutions or suspensions of thermolabile compounds require a special approach.

Table 1. Comparison of performance characteristics of tubular evaporators and the proposed pre-crystallizer device

Device	Rotary-film evaporator (Sirius, Ukraine)	Pre-crystallizer
Method to increase the evaporation surface	Formation of a solution layer on a heated surface	Atomizing of a solution (working pressure: 3 atm.)
Evaporation surface:		
Total, cm^2	12000	2500 cm^2
Per a mass unit	0.33 sm^2/g	8.3 sm^2/g
Productivity	160–200 kg/hour	180 kg/hour
Heat application	Use of an electric heater or heating steam	Not required
Evaporated medium temperature	60–180°C;	20–35°C
Product temperature at the outlet	40–60°C	8–10°C
Concentration of a solution at the inlet	~12 %	≤ 0.5%
Concentration of a solution at the outlet	≤ 35% (dry matter)	≤ 10% (saturated solution)
Cooling	Cold water (20°C) consumption – 2 m^3/hour	Not required
Electric power consumption	0.35-0.75 kW	0.30 kW

For example, during the isolation of virginiamycin from culture broth of *Streptomyces* sp. (Dzhavakhiya et al., 2016), this antibiotic is preliminarily sorbed by an Amberlite XAD-16 resin, then washed with ethyl acetate or any other appropriate solvent and concentrated prior crystallization using the above-described method. As a result, during the whole preconditioning process, virginiamycin is not heated, and ethyl acetate evaporated from the surface of microdrops is condensed into a liquid phase and may be used repeatedly for the virginiamycin isolation. Under these conditions, virginiamycin crystallization occurs efficiently with the formation of crystals of the same size.

In the case of chemical and biotechnological industries, tubular evaporators are usually used because of their compact size. However, the comparison of their characteristics with those of the proposed device shows the last one has some advantages (Table 1). The additional productivity increase and reduction of the energy consumption are provided by the use of ethyl acetate, which saturated vapor pressure at 30°C is ~120 mm Hg, whereas the same parameter of water is only 32 mm Hg. The higher pressure, the higher the volatility of a solvent that, in turn, results in the accelerated concentrating of the virginiamycin solution to the saturated state. Obviously, the time required for the supersaturation of the antibiotic solution in an organic solvent strongly depends on both evaporation surface and volatility of a solvent, which, in turn, is determined by the vapor pressure at the given temperature, latent heat of vaporization, surface tension, and other factors (Table 2).

Table 2. Dependence of the time required to supersaturate 1 l of an antibiotic solution on the mass volatility of a solvent from a smooth surface

Solvent	Volatility, mg/l	Time to oversaturation, s
Acetone	9.33	10.0
Ethyl acetate	6.08	17.5
Methanol	3.24	32.2
Isopropyl alcohol	2.11	48.0
Methyl-isobutylketone	1.52	67.0

Conclusion

The proposed approach to the preconditioning of solutions for crystallization, which includes an ultrasonic atomization of a solution with the formation of an aerosol, characterized by a high integral evaporation surface, and its further condensation to a liquid phase, provides a simultaneous concentration and cooling of solutions. This technology prevents the destruction of thermolabile compounds, improves the efficiency of their further crystallization due to the ultrasound-initiated formation of crystallization nuclei, and provides the possibility to re-use the obtained sorbent distillate.

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).

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Citation:

Durnikin, D.A., Yacenko, E.S., Evdokimov, I.Yu., Akopyan, V.B., Dzhavakhiya, V.V., Glagoleva, E.V., Savushkin, V.A., Savelyeva, V.V. (2017). Preconditioning of a virginiamycin solution for crystallization. *Ukrainian Journal of Ecology, 7*(4), 187–191.