Intensification of probiotics drying. Spray-drying of Bifidobacteria biosuspension

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Abstract

Spray drying has been offered as an alternative method for dry B.bifidum biomass obtaining for production of solid dosage forms. To create the generalized mathematical model according to the proposed strategy the complex of experimental and analytical researches of B.bifidum biosuspension has been carried out. These researches have been directed to study drying kinetics, kinetics of cells death because of heat shock and growth of osmotic pressure, to define the influence of process parameters to the dry product quality and their limitations during drying. On the basis of experimental data and modeling results, the recommendations have been given for the industrial process organization.

Keywords: process intensification, mathematic modelling, spray-dying, Bifidobacteria biosuspention

1. Introduction

The innovation spray-drying of probiotics considered by the example of Bifidobacteria biosuspension drying has been proposed. The spray-drying can be used as an alternative for vacuum freeze-drying because it allows to intensify heat and mass transfer, to organize continuous process, to produce fine powders with high quality, to delete the powdering stage and as result to increase the sterility of product and reduce operation costs [*Kafarov V.V. et al., 1998*].
For scientific foundation and validation of spray drying operation parameters it is necessary to get the clear understanding of the physical basis of phenomena which have place in apparatus, their interconnections and degree of interference between them; to take into account the specificity of biomaterial (it s the biosuspension with live cells) and to considerate the limitations. Development of new probiotics drying technology is a multiparametric task because all parameters have the internal relations. Using of mathematical modeling, modern computer engineering and computer tools makes possible to solve this task.

2. Strategy of generalized mathematical model creation of biosuspension spray drying

Nowadays there is no the generalized mathematical model of biosuspension spray drying which allows don't only to investigate hydrodynamics, heat and mass transfer in apparatus and but to estimate the changes of cells survival during dehydration as a result of exposure to heat and osmotic pressure growth.

The mathematical model for description of hydrodynamics, heat and mass transfer was created on the base of the equations of nonequilibrium thermodynamics and heterogeneous media mechanics [*Kafarov V.V. et al., 1998*], namely the system of conservation equation of mass, energy and momentum has been written in cylindrical coordinates for drying agent and disperse phase, and includes the initial and boundary conditions and additional correlations.

Because the properties of biosuspention of bifidobacteria is not fully described in the scientific literature for the generalized mathematical model creation the complex of analytical and experimental investigations was carried out (Figure 1):

- Investigation of Bifidobacteria biosuspension as drying object which included the following steps: the heat sensitive analysis, drying kinetics, investigations of biosuspension physical and chemical properties;
- Laboratory scale spray-drying of Bifidobacteria biosuspension with the complex analysis of basic dry powder properties in order to indicate influence of the operation parameters on product quality.

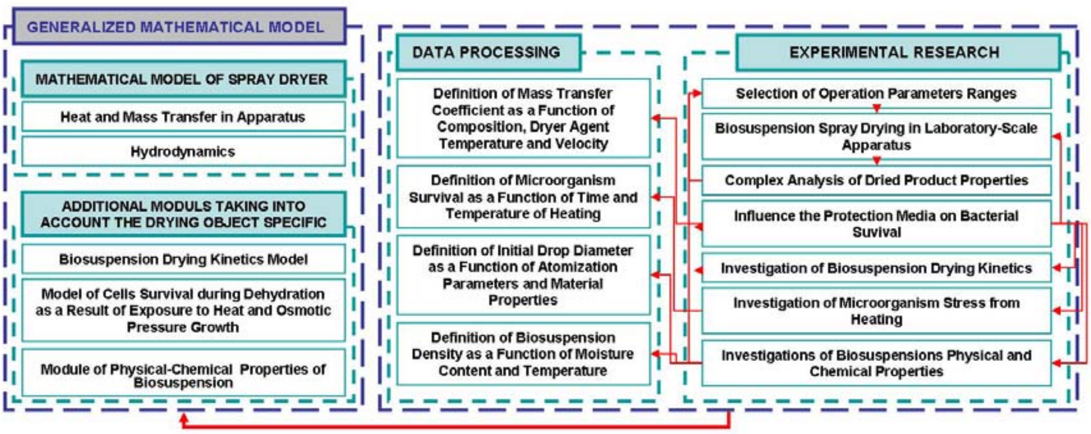

Figure 1. Strategy of the generalized mathematical model development of biosuspension spray drying

3. Experimental research

The biosuspention of Bifidobacterium Bifidum had been chosen as the investigation object. The various protection media have been used. The compositions of samples are presented at Table 1.

Table 1. Biosuspension compositions

Sample	Composition
Sample 1	Concentrated biosuspension of bifidobacteria
Sample 2	Concentrated biosuspension of bifidobacteria with starch (1.0 %)
Sample 3	Concentrated biosuspension of bifidobacteria with milk (2.5 %)
Sample 4	Concentrated biosuspension of bifidobacteria with sugar (10.0 %) and gelatin (1.0 %)

The investigations of biosuspensions physical and chemical properties included the examination of dynamic viscosity to determine nozzle operation parameters and the definition the mathematical relations between density and temperature/moisture content which have been included into the generalized mathematical model.

3.1. Investigation of biosuspension drying kinetics

The investigation of biosuspension drying kinetics with different protection media have been carried out at Lodz Polytechnic University (Poland) by using special drying tunnel which allows to weigh the thin layer of sample during drying. The drying time in this equipment is nearly to one in spray dryer [*Li X., 2004*].

The measuring pan connected with outside high-precision balance with accuracy ±0.0001 g is located inside the drying tunnel. During the experiment the glass plate with thin layer of sample was sunk by special device to the measuring pan after the previous warming-up of the chamber by drying agent. Simultaneously the data on sample weight and air temperature were transferred and recorded to PC each 0.3 s. The inlet air temperatures and velocities were 60, 100, 140 degree of Celsius and 0.73, 0.86 m/s respectively.

Basing of the analysis of the experimental data the drying kinetics curves have been drawn. It was defined that the most part of water from samples was removed during the fist drying period when the moisture content changes versus time has the linear dependence.

The mass transfer coefficients as a function of air temperature and velocity were found for each sample. The biggest mass transfer coefficient was established for the drying of Sample 2 what could be explained by the formation of good porous inner structure. Sample’s 4 mass transfer coefficient didn’t depend on the dryer agent velocity. This effect is explained by formation of thin film on the sample surface at definite temperatures which limits the moisture removal.

The dried product residual moisture content strongly influences on microorganisms survival during drying and storage [*Kudra T. et al., 1991*]. During drying while the moisture content is below some definite critical value the water is removed not only from material and from cells too. At the comfort living conditions the water and feed substances come into the cell. It could be described by the follow equation:

$$J = L_p \Delta p + L_{pD} \Delta \Pi \tag{1}$$

$$\Delta p = \frac{4\sigma}{d} \cos\Theta \tag{2}$$

$$\Delta \Pi = \frac{RT}{\overline{V}_M} \ln \frac{p_{w2}}{p_{w1}} \tag{3}$$

At critical residual moisture content the water and feed substances flows are stopped. This is the anabiosis state which could be described by next equation:

$$-L_p \Delta p = L_{pD} \Delta \Pi \qquad (4)$$

The following removal of water from the system brings to the water removal from the cell. Then the osmotic pressure increases resulting in the cell membrane disruption, the derangement of bilipidic layer structure and metabolisms and finally the cell death. The addition of the protection media allows to increase the degree of microorganism survival due to surface tension diminishing and pores enlargement when the capillary pressure fell and cell wall is strengthened. These regulations could be used in the generalized mathematical model to estimate microorganism osmotic stress.

3.2. Investigation of microorganism stress from heating

The choice of drying temperature regime is caused by necessity of cells preservation. That's why investigation of microorganism stress from heating (investigation of cell thermostability) had been done by equal heating of the samples during various times and temperatures.
It was experimentally found that the protection medium addition into biosuspension compositions promotes the increasing of microorganism survival. The bacterial survival depending on time and heat stress intensity was found for various samples. The following thermal inactivation equation has been used for the description [*Becker M.E. et al., 1987*]:

$$\lg B = \lg B_0 - \frac{A \cdot \tau}{2.3} \cdot e^{-\frac{E_a}{RT}} \qquad (5)$$

$$B = \frac{N_\kappa}{N_{\kappa 0}} \qquad (6)$$

By experimental data processing the unknown coefficient A and E_a have been defined.

3.3. Investigation of biosuspension spray drying (laboratory scale)

All experimental investigations of biosuspension with various protection media have been carried out in laboratory-scale spray-dryer (Universal Spray-Dryer 1, Russia). The inlet air temperature, speed of biosuspension feed flow, atomization air pressure were varied during experiment. The complex analysis of dried product properties (the main of them are colony-forming units per gram, residual moisture content, particle size distribution) has been done.
It was found that spray drying of biosuspension without protection medium (Sample 1) is not possible. The dried product had high residual moisture content and low bacterial survival (10^6 CFU/g) that does not satisfy the qualifying standards of biological active substances.
The best results have been received for spray drying of Sample 4. For example, at inlet air temperature 70 ^{0}C, biosuspension flow rate 0.45 l/h and atomizing air pressure 2.2 atmospheres the dried product had the following properties: the residual

moisture content was 4.5% and 10^{10} CFU/g. The powder photograph and particle size distribution are presented at Figure 2.
Spray drying of Sample 3 gave the good results too (10^{10} CFU/g).

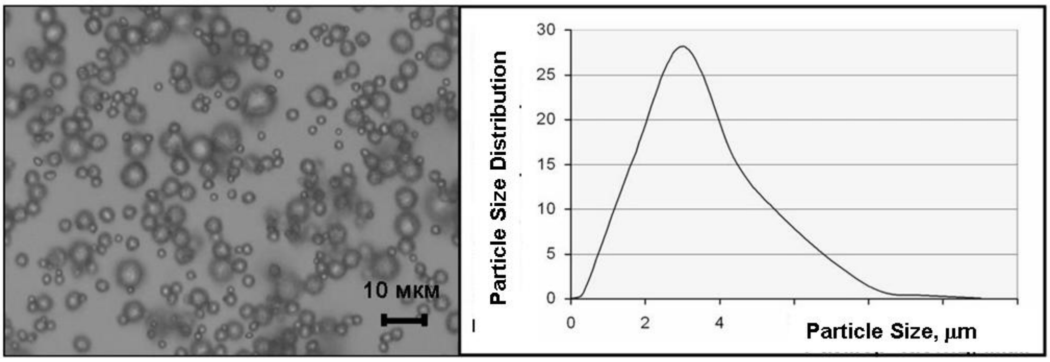

Figure 2. Powder photograph and particle size distribution (Sample 4): inlet air temperature 70^0C, biosuspension flow rate 0.45l/h and atomizing air pressure 2.2 atmospheres

The addition to biosuspension such protection media as milk and starch was found to improve microorganism survival and product flowability, decrease particle agglomeration during non-hermetic storage.
The experimental and analytical investigation allowed to determine the interactions between biosuspension properties, operation parameters and dried material quality and to found the limitations (for inlet air temperature and rate of drying). The experimental data and found mathematical correlations had been used for creation of the generalized mathematical model of biosuspension spray drying.

4. Process Scale-Up

The mathematical model based on heterogeneous media mechanics and non-equilibrium thermodynamics had been developed to choose the optimal operation parameters and to intensify spray-drying. The mathematical model consists of the mass, momentum and energy conservation equations written for gaseous and dispersed phases; equations described drying kinetics of biosuspensions, kinetics of bacteria survival under the high temperatures and osmotic pressure influences; some additional correlations, initial and boundary conditions. The system of equations was written in cylindrical coordinates [*Gordienko M.G., 2006*]. The numerical solution of the system allowed to investigate the drying process and to found the distributions of basic phases parameters in dryer. The mathematical model was verified by using of experimental data (maltodextrin water solution) in the pilot spray-drying [Delag A., 2001]. It was found the model is adequate to the experiment.
The mathematical model has been used to scale-up the spray-dryer for dry biomass production. The main dryer sizes were found by calculation. Figure 3 shows the calculated distribution of basic phases parameters for industry spray-dryer.

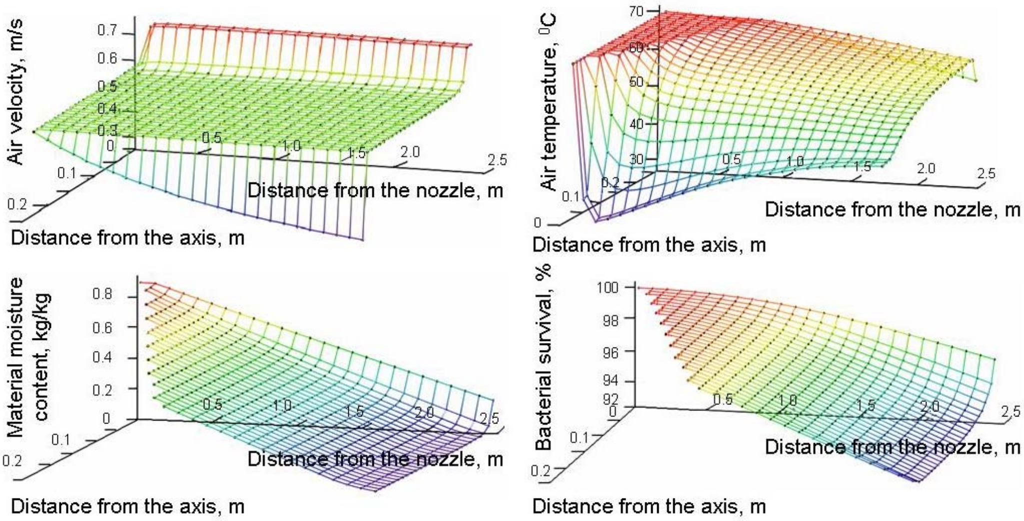

Figure 3. Distribution of basic phases parameters for industry spray-dryer

The energy analysis based on the dynamic coefficients of energy consumption had been done for this apparatus [*Menshutina N.V. et al., 2004*].

The Figure 4 presents the change of energy efficiency criterion versus apparatus height. It is obviously that spray drying process is intensive in whole apparatus volume. The energy efficiency criterion is sharply increased in the drop heating zone achieving the maximum value at the beginning of evaporation. The value of the criterion is remained high practically in a whole working chamber volume and lies between 0.95÷0.80. The decreasing of energy efficiency begins from 2.2 m when the bulk dried (Fig. 4). The value of overall energy efficiency was 0.78.

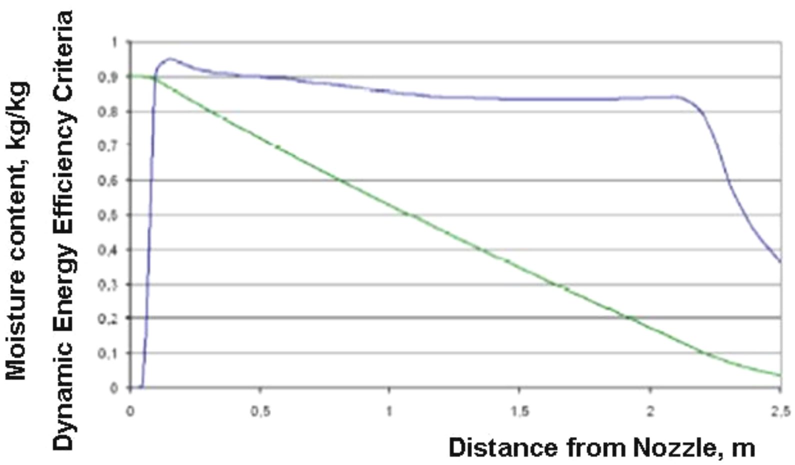

Figure 4. Change of energy efficiency criterion versus apparatus height

It was showed that inlet energy is used in spray-drying more efficiency then in vacuum freeze-dryer.

Thus, the application of spray-drying for probiotics production allowed to intensify the process, to reduce the drying time and to organize continuous process.

Notation

L_p, L_{pD} – coefficients;
$\overline{V}_M$ – molar volume, m^3/mol;
p_{w1}, p_{w2} – internal and external media pressures, Pa;
R – absolute gas constant, 8.31 kJ/(mol·K);
T – temperature, K;
N_{K0}, N_K – number of living cells before and after heating;
B_0, B – microorganism survival before and after heating, %;
τ - heating time, c;
A – numerical coefficient of substance nature characterizing the object (presence of labile sites and bonds and their susceptibility to high temperatures);
E_a – inactivation energy, kJ/mol;

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