USE OF SOME CLAY MATRICES IN BIOTECHNOLOGY OF ACID DAIRY PRODUCTS

Alisa Vasilica ARUS[1], Ana Maria GEORGESCU [1], Iuliana Mihaela LAZAR [1], Ileana Denisa NISTOR [1], Neculai Doru MIRON [1], Azzouz ABDELKRIM [2], Andrea – Eliza FARKAS [1], Ramona Mihaela COJOCARU [1]

[1]Food Engineering Department, „Vasile Alecsandri" University of Bacau, arusalisa@yahoo.com
[2]Département de Chimie, Université du Québec à Montréal

Abstract: *In this paper we analyze the use of some clay matrices in the cultivation process of lactic acid bacteria, in dairy industry. The object of this paper is the use of anionic clays - hydrotalcite type, with food purity grade, that are synthesized in laboratory, and can be conditioned in porous tablets or pellets form. These are introduced into the culture medium and help optimal growth parameters achievement of lactic acid bacteria by temporary retention of lactic acid. After multiplication and cellular growth, the tablet or pellet insoluble clays, can be removed, washed and recovered. The lifetime (using) of such materials and devices is practically unlimited.*

According to this study, the cultivation process of lactic acid bacteria includes the immersion in culture medium of a hydrotalcite clay matrix, with anions exchange role, cultivated through continuous cycle of hydrotalcite matrices immersion, removing and recovering, the lactic anion is continuously fixed, achieving at the same time the proton neutralization of lactic acid, with an aqueous phase, separated from the culture medium, with subsequent extraction and separation of lactic acid.

The advantages of the proposed process are: about four time decrease of achieved period of lactic bacteria cultivation phase; 2 - 3 time decrease of final cultures productivity growth; increase with 50-70% rate of bacteria growth in fermentative process; decrease with 30-60% of lactic bacteria cost for industrial fabrication of acid dairy products.

Keywords: *hydrotalcites, lactic fermentation, lactic cultures, lactic acid*

Introduction

The growing process for the production of lactic acid is, generally, the same for all of these products, based on the classical method, varying only the conditions of control phase fermentation culture medium depending on the desired culture microbiological component to be selected [1].

The disadvantages of these methods of cultivating lactic bacteria are the difficulty of maintaining the culture medium composition optimum and stable. The composition is changing as microorganisms eat nutrients and after metabolic formation of new substances.

The classical growing process of lactic bacteria which serves as the nearest solution consists in sterilization (pasteurization) of milk at the temperature of 63…72ºC, for 0,5 h, cooling milk to the seeding temperature (40...48ºC) for 0,25…0,5 h, adding the milk to seed powder culture, cultivation of lactic bacteria at temperature 36…40ºC and their separation [2]. Technologically the growing of lactic acid bacteria is inhibited by two processes:

1) gradual increase of v pH in the culture

medium, followed by lactose conversion into lactic acid;
2) accumulation of lactic groups.
These two processes combined have a bad influence on cellular growth and development.
In known processes the lactic acid anion is not removed, the principal inhibitor of cellular development. Thus, the classic drawbacks are large losses of bacteria in the fermentative process and low productivity of lactic bacteria, which leads to reduced growth rate of bacteria and growth in the cost of selected final cultures. Therefore, according to statistical laboratory data, growth of lactic bacteria from initial concentration of 5…50 cells/ cm^3 up to final concentration of 20…30 millions cells/ cm^3 takes 8…12h.
We propose to solve this problem, by developing a lactic bacteria cultivation technology, to ensure their growth rate during the fermentative process and to reduce considerable the cost of final selected cultures.

Materials and methods

The problem presented above can be solved by making a cultivating lactic bacteria procedure, which includes the preparation of the culture medium, containing sterilized and cooled milk to the seeding temperature, addition of the culture medium prepared by lactic acid bacteria, cultivation and their separation. Lactic acid bacteria are used for seeding increase on a nutrient medium consisting of yogurt: fresh pasteurized cow milk, volumetrically taken 1:10 and powdered, which is added by agitation in the culture medium at constant temperature; then as the first choice the culture medium is immobilized in the porous matrix. Lactic bacteria cultivation is achieved at the temperature of 36…40ºC for 2…3 hours with the elimination of lactic acid formed by continuous elution of the mobile aqueous phase, which is separated from matrix by the immobilized culture medium and after separation the lactic acid from aqueous phase returns to the culture medium.
The cultivation process of bacteria under the second variant includes the introduction in the culture medium of an alkaline agent with food purity, insoluble, which in cultivation time sets continuously the lactic acid formed in the culture medium to achieve continuous cycle entry, discharge and regeneration of basic agent and extraction and separation of lactic acid.
The cultivating lactic bacteria according to the third variant includes immersion in the culture medium of a solid insoluble matrix of anion exchange resin, filled with groups of OH^- exchangeable, R^+OH^- type, during the cultivation period by continuous immersion cycle, discharge and regeneration of resins into a solution of sodium hydroxide, lactic anion is fixed continuously and with free OH^- group to achieve neutralization of the proton which is made simultaneously with the formation of aqueous phase, which is separated from the culture medium by extraction and subsequent separation of lactic acid.
In all these variants, the fermentation processes lasted for 2…3 hours.
The common element for all the variants of lactic bacteria cultivation processes is milk sterilization (pasteurization) at the temperature of 63…72ºC for 0,5 h, cooling milk to the seeding temperature (40…48ºC) for 0,25…0,5 h, addition in milk of the culture medium seeding powder, their cultivations for 2…3 h by continuous agitation at the temperature of 36…40ºC and lactic bacteria separation.
As initial products we used fresh milk and one of the traditional culture used in milk industry, such as: *Lactobacillus bulgaricus* (for milk fermentation), *Lactobacillus acidophilus* (for acid fermentation of milk), *Lactobacillus longum* (for milk

coagulation), *Lactobacillus casei* (for fermentation and maturation of cheese) etc.
Culture medium composition includes:

- yogurt, buttermilk or another medium in which lactic bacteria were grown – 1 ml;
- pasteurized milk or cow milk fresh pasteurized – 10 ml;
- lactic cultures specific to the group listed above.

For the first variant of proposed processes, as initial products we used fresh milk, water and one of the cultures listed above. As porous matrices we used solid matrixes from organic polymers and mineral categories, for example montmorillonite clay type, or semi porous matrix of cellulose and gels categories.
For the second variant we used as base agent:
a) a disperse aqueous phase, formed by an organic substance from chelates' category, dissolved in comestible liquid fat, from vegetables oil class and animal fat. The continuous fixation of lactic acid is made by organization of a relative movement between disperse phase and culture medium, and before evacuation is made, the separation of disperse phase from culture medium takes place.
b) an insoluble solid matrix, for example a clay from the category used in medicine such as gastric bandages, which is regenerated by washing and impregnation with a sodium hydroxide solution.
The carrying out of the process of cultivating lactic bacteria on the proposed variations is conditioned by three essential factors:
1) Attachment velocity of the lactate group;
2) Elimination and regeneration speed of the lactate group;
3) Attachment capacity of the lactate group.
Lactic acid separation from de regeneration medium or from aqueous solution was achieved by a known process, for example, by treating with calcium hydroxide to obtain calcium lactate. After filling the precipitate, the liquid was decanted and the sediment was passed through the filter. The liquid was treated with sulfuric acid until the reaction became acid for the calcium decomposition. After the filtration of calcium, sulfate was formed; the diluted acid solution concentration was of approximately 50%, thus obtaining a concentrated solution named „ lactic acid for technical use". By keeping the concentration of lactic acid at low pressure until the concentration reaches 50...80% we obtained raw lactic acid, which can be used in industry, being purified by re-crystallization of calcium lactate and subsequent treatment with sulfuric acid, resulting free lactic acid.
Given the complexity of the technical solutions adopted and proposed in this article, we made a statistical study of the process of cultivating lactic bacteria using a factorial program, which took into account the individual effect of all parameters, and their interactions can potentially be synergistic.
Such a study required a small number of experimental attempts made in short time and with negligible costs.
The experimental testing methodology includes the performance of two series of experiments.
In the first of them we carried out a lactic bacteria cultivation process. For seeding the pasteurized milk (producer SC Prolabac SA Bacau, acidity 14…16ºT and 1.8% fat) or fresh pasteurized cow milk we used bacterial cultures grown in yogurt and buttermilk (producer MAMY'S Dairy Sibiu) medium, the volumetric proportion of „yogurt/ pasteurized milk" or "buttermilk/ fresh pasteurized cow milk " was 1:10.
In "buttermilk/fresh pasteurized cow milk" case the composition of milk-culture medium contains:

- buttermilk MAMY'S Lactate, Sibiu

– 1 ml;

- fresh pasteurized cow milk – 10 ml;
- specific lactic cultures listed above.

The fresh pasteurized cow milk composition (energy value 64 kcal/ 100g) was: nutrients – 100 g;
fats – 2 g;
carbohydrates – 3,9 g;
proteins – 3,2 g;
calcium – 125 mg;
dried substances – 11 g.

After some time, the lactic bacteria started to develop, the lactic acid formed in pursuing one of the proposed variants of the process of cultivating lactic bacteria was partially and gradually removed from milk - culture – fermentation I medium.

Under the same conditions of temperature and acidity, we determined the rate of growth and development of the lactic bacteria population that do not depend on the milk-culture medium used, and thus we presented below the results for both milk-culture media.

We studied three alternative ways of achieving continuous elution process of the lactic acid.

1. the culture medium was immobilized in a fix bed, the conditions of the lactic acid were not disturbed by any of the phases, the milk-culture medium maintaining constant its composition
2. the culture medium was continuously dialyzed through a dialysis membrane
3. Immobilization of milk-culture medium in a solid or semi-solid matrix and continuous elution of the lactic acid in the aqueous mobile phase.

We have noticed that the first way raises the problem of excessive consumption of culture medium and the necessity of recycling, which leads to the increasing of technology spending.

The major inconvenience of the second procedure consisted in very small values of efficiency, because of the relatively slow kinetics of the diffusion process of lactic acid during passage through the membrane. The main problem arises from the concern to ensure a good transfer of the lactic acid into the mobile aqueous phase that must be separated.

The third alternative method proved to be the most judicious that was adopted as optimal technical solution for the first variant of the proposed process of formation of lactic acid bacteria. The problem was reduced by creating a porous matrix of organic polymers, cellulose, gels and mineral polymers type montmorillonite, in which the pore size allows the development of bacteria mass.

In the second series of experiments, yeast (grown culture medium), obtained in the fermentation process we used clot pasteurized milk (producer SC Prolabac SA)- fermentation II, to determine the degree of efficiency of the lactic ferment obtained, which is estimated in range of the increase in acidity of inoculated milk in fermentation process II.

In this variant the culture fluid is not fixed, but has a mobile fluid phase. As insoluble alkaline agent we used a liquid dispersed phase, which comes across milk-culture medium, and before discharge it was separated from the culture medium. The testing of various lactic acid binding agents proved that in this purpose the organic substances of chelate type (a complexing agents class) that have a great affinity for acids are better suited. These compounds, before being added to the culture medium, were dissolved in a fat food fluid (vegetable oil or animal fat).

The fermentation period depends on the dose of reducing agent of lactic acid concentration, which in this variant was dosed by varying the amount of insoluble alkaline agent. As a criterion to start the fermentation period, we chose the initial concentration of cells in culture medium –

milk 20 cells/ m^3, and as a finalization criterion of the fermentation period we chose the final concentration of bacterial population of 25 million cells/ m^3.

After selection of lactic bacteria grown for each sample from fermentation I, we used in fermentation II, the efficiency of the culture bacteria grown in fermentation I testing at 36^0C for 7 hours and determining the average speed increase of acidity of milk.

Alternatively, cultivation of lactic bacteria was carried out by stirring at the constant temperature of 37 C. In this variant the technical solution proposed supposes that the culture medium remains fluid and the lactic acid formed is fixed on some movable or immovable matrices (surfaces) with basic character.

Surfaces were chosen to satisfy the following conditions:

- have sufficient physical adsorption capacity for each contact cycle in milk-culture medium
- have no cation exchange properties, otherwise, the lactate group will not be evacuated
- anion exchanger to allow elimination of lactate group
- to have a renewable surface
- to have a long life
- to present inertia in relation to other constituents of culture medium
- to have a low cost.

Such a surface can be achieved relatively easily, for example a classical anion resin type. It was found an advantageous variant of such a varied hydrocalcit similar to that used for the treatment of stomach hyperacidity (clay, used in medicine as gastric bandages).

The surface can be easily made in laboratory according to standard procedure. Hydrocalcits are also much cheaper than ion exchange resins.

The fermentation period depends on the dose reducing agent concentration of lactic acid; which in this variant was assayed by the amount of hydrocalcit. As criteria for stating fermentation period we chose the initial concentration in milk-culture medium 20 cells / m3 and quality criteria for finalization fermentation period, we chose the final concentration of bacteria's population of 25 millions cells/ m^3.

After the selection of grown lactic bacteria for each sample from fermentation I, these ones were used in second fermentation of efficiency testing of growth culture in first fermentation, achieved at 36ºC for 7 h and determining medium growing velocity of milk acidity.

Results and discussion

The results of the three different methods of cultivating lactic acid bacteria consist of:

- reduction of four times the period of implementation of lactic acid bacteria growing phase;
- increase of 2…3 times the growth productivity of the final culture
- increased with 50…70% the growing process rate of bacterial fermentation;
- reduction with 30…60% of lactic bacteria cost for industrial production of lactic-acid products (buttermilk, kefir, sour milk, cheese etc) depending on the procedure used;
- use of cheap, affordable, virtually unlimited lifetime materials, which do not affect the properties of dairy and food products and are not a health risk for consumers either;
- obtain supplementary lactic acid - an additional valuable product for food, chemical, leather industries and medicine.

Conclusion

The result obtained is due to the following issues:

- the lactic acid inhibiting cell growth and proliferation of lactic acid

bacteria, used to obtain cheese and lactic acid-products was removed from the culture medium;

- contrary to known methods, which do not eliminate lactic anion, the main inhibitor of cell growth, the technology proposed expects the fixation of this ion by anion-exchange materials;

- the lactic acid elimination which represents the main reason of the present study, is achieved by using insoluble materials with a high food purity grade and with basic characteristics;

- insoluble material technologies and technological interest, which correspond to the above, can be obtained by ion exchange resins;

- insoluble material of technological interest, corresponding to the above mentioned requirements, can be also obtained on anionic clay of hydrocalcit, having a similarly structure with gastric bandages used in hyperacidity treatment of people;

- anionic clay of hydrocalcit type can be synthesized in laboratory based on calcium, magnesium carbonate and sodium aluminates, after a 6 h of maturation period;

- anionic clays can be conditioned under pills form or as a porous plate that is inserted into the culture medium for lactic acid removal;

- after multiplication and cell growth, the pills or insoluble clay plates can be extracted, washed and regenerated with NaOH;

- life (use) of such materials and devices is virtually unlimited.

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