Հայաստանի Գիտությունների Ազգային Ակադեմիա, Հայաստանի Կենսաբանական Հանդես
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CONTINUOUS PRODUCTION of LACTIC ACID

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Lactic acid was obtained by repeated batch and continuous fermentations using as a producer a rumen bacteria *Streptococcus bovis*, possessing high amylolitic activity. A principal possibility and reasonability of lactic acid production by mentioned methods have been shown.

Կաթնաթթուն ստացվել է պարբերական և անընդմեջ եղանակներով: Որպես արտադրիչ օգտագործվել է գանձակային բակտերիա *Streptococcus bovis*-ը, որն ունի բարձր ամիլոլիտիկ ակտիվություն: Ցույց է տրվել վերոհիշյալ եղանակներով կաթնաթթվի արտադրության հնարավորությունն ու նպատակահարմարությունը:

Молочная кислота получена отъемно-доливным и непрерывным способами. В качестве продуцента использована рубцовая бактерия *Streptococcus bovis*, обладающая высокой амилолитической активностью. Показаны принципиальная возможность и целесообразность получения молочной кислоты упомянутыми способами.

Key words: *lactic acid - continuous fermentation - Streptococcus bovis*

Lactic acid is one of the most important substances produced microbiologically and is widely used in food, pharmaceutical, cosmetic and other branches of industry. During the last decades the problems of its production initiated a significant interest of researchers to look for more efficient microbial producers [1, 6]. Hopes for market expansion are combined with utilization of lactic acid and its derivatives as initial materials to synthesize other substances, and first of all the biodegradable polymeric materials, while relatively high cost of lactic acid is limiting its prospects.

To realize an efficient production of lactic acid a novel method of direct fermentation of starch containing substances into lactate using *Streptococcus bovis* cells was previously suggested [3, 4]. Utilization of this microorganism, which possesses amylolitic activity and is a producer of L(+) lactic acid should exclude an energy consuming stage for precooking of starch, and as a result to decrease the cost of the final product.

However, the continuation of the final stage of batch fermentation was valuably prolonged, due to inhibition of producer's growth and lactate production pro-

cesses by accumulated lactate. Finally, this results significantly decrease of the fermentation productivity and increases its total duration.

To overcome this disadvantage, the possibility of lactic acid production by repeated batch or continuous fermentations using *S. bovis* cells was studied.

Materials and methods. The strain of *S. bovis* isolated from the calf rumen having high digestability for hydrolysis of raw cereal starch and characterized as a homofermentative producer of L(+) lactic acid has been applied. Lactic acid was produced by repeated batch and continuous fermentations using as a producer the culture of *S. bovis*. As a carbon source for both types of fermentation a corn starch was used. For lactic acid production by repeated batch fermentations the medium of following composition was applied (g/l): corn starch – 100,0, yeast extract – 5,0, peptone – 5,0, $CaCO_3$ – 50,0. Medium was srerilized under exsessing pressure of 0,5 atmosphere. When lactic acid was produced continuously, the medium of the same composition was brought into the fermentor, while into the media fed chalk was not added. After transition to continuous fermentation conditions $CaCO_3$ was added in doses directly into fermentator.

Preparation of glucoamylase with the activity of 2000 units/g was added to the sterilized media in a ratio of 0,5 % towards amount of starch.

Repeated batch fermentations have been conducted in a 1 liter fermentator which was filled with 800 ml of media. Inoculate grown on the same medium and containing 10^9 cells of the producer per ml has been added to the fermentation media in a ratio of 5 % only before the first fermentation. After 48 hours of fermentation 80 % of culture broth was removed from the apparatus and substituted with the same amount of new sterile fermentation media containing the necessary amount of glucoamylase. Fermentations have been carried out at 40°C with occasional (every 6 hours) steering.

Feeding fermentation media for the continuous production of lactic acid have been prepared in the separate vessel, where glucoamylase preparation was also added.

Continuous fermentations were carried out at 40° and constant stirring, with the rotation speed of stirrer blades of 40 rotations per minute. Anaerobic conditions in the fermentator were mantained by supplying of carbon dioxide.

Concentration of the produced lactate was determined by enzymatic method [5]. The concentration of starch and digestible carbohydrates, as well as remaining sugars were determined by phenol – sulphuric acid method [2].

Results and discussion. Fermentations using different starch containing substrates, such as low- grade wheat flour, sorghum grains and potato tubers were carried out. For fermentation of starchy materials their sterilization was done by steeping them into a dilute sulfuric acid (0.05 N) for several hours at room temperature. It was found, that steeping of potato mashed tubers, milled sorghum grains and wheat flour into dilute sulfuric acid was effective not only for sterilization of the substrate, but also for causing an evolution of carbon dioxide upon addition of calcium carbonate. The amount of calcium carbonate was slightly more than the equivalent for neutralization of the added sulfuric acid and lactic acid produced.

When potato or its waste products were used for the fermentation, during the sterilization procedure mentioned they were also treated by acid phosphatase, because the phosphates combined with glucose residues of potato starch inhibit the activity of α-amylase of *S.bovis*.

The influence of temperatures, pH values, as well as concentrations of substrates and inoculum on the fermentation processes and on their kinetics were exam-

ined and their optimal values were estimated. The optimal temperature for cell growth was 37°, although the maximum rate of lactate production was obtained at 40°.

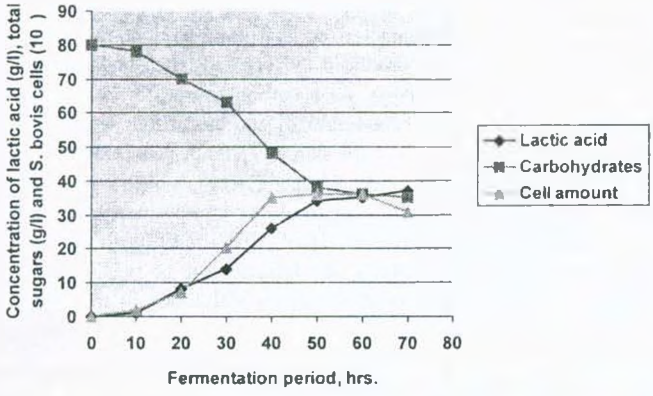

Fig. 1. Lactate fermentation time courses of starch containing media.

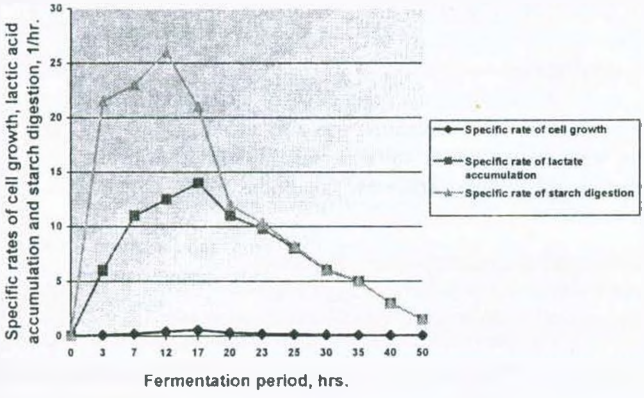

Fig. 2. Specific rates of cell growth, lactate accumulation and starch digestion.

The results presented in Fig.1 indicate, that lactic acid production began and proceeded in parallel with the cell growth. However, even after cell density began to decrease, the lactate formation was continued, but proceeds more slowly. Processes were characterized by relatively continuous lag-phase (about 10 hrs).

Maximums of specific starch (glucose) consumption rate, specific lactate production and cell growth rates were observed at the exponential phase (Fig. 2).

As the fermentation proceeded the specific lactic acid production rate approached the specific glucose consumption rate. This fact indicates that starch (glucose) conversion rate to lactic acid becomes theoretical as the fermentation proceeds to a certain stage.

Results obtained permit to make the conclusion, that investigated starchy material of fermentation media without addition of any other components provides with a complete source of carbon, nitrogen, organic and inorganic compounds for *S. bovis* cell growth and lactic acid production.

The data obtained revealed that only 60-65% of starch was consumed during the fermentation. It was caused due to the specificity of amylolytic activity of *S. bovis* what belongs to the type of sacharogenic α-amylases. This type of amylases provides a higher reducing ability, than the so-called liquefying or dextrinizing amylases. By hydrolysis of α-1,4-glucoside bonds of starch, at first these amylases produce as

an end product from the reaction fermentable sugars glucose, maltose and maltotriose as well as various branched olygosaccharides. Secondly, this type of amylases attacks and hydrolyses maltotriose molecule into glucose and maltose. Thus it can be predicted, that remaining non-converted sugars are represented by branched olygosacharides, which cannot be digested by the α-amylase of *S. bovis*.

To accelerate raw rice starch saccharification stage and to achieve its more complete conversion into lactate, a commercial preparation of glucoamylase - an enzyme capable to digest as α-1,4 and α-1,6-glucoside bonds yielding glucose as the end product of hydrolysis, as an additional saccharification factor was used.

The addition of *Rhyzopus* glucoamylase (up to 0.05% towards starch content) valuably decreases the fermentation period. However, the addition of more amounts of glucoamylase did not result in further acceleration of fermentation.

Promoting the increase of intensity of hydrolysis of the raw starch, glucoamylase jointly with the α-amylase of *S. bovis* actively provide a significant decrease of the period of lactic acid homofermentative production process (about 40%).

Addition of the glucoamylase caused the increase of the values of obtained maximums of specific starch (glucose) consumption rate, specific lactate production and cell growth rates observed at the exponential phase. To find the optimal quantity of inoculate of *S. bovis* for lactic acid fermentation, studies were carried out with various initial ratios of inoculum to the culture media. Glucoamylase preparation in a ratio of 0.05% towards starch content and calcium carbonate were added. Initial pH was 6.5 and temperature was kept at 40°. Initial concentration of starch in the fermentation media was 10%. The obtained results of analyses have shown, that the increase of the amount of inoculate is accompanied by the accelerations of the fermentation process and finally initiates its shortening. The maximal level of lactate production with the ratio of starch conversion 0.9 was achieved in all the experiments tested. The ratios of 10 % of inoculum are to be more economically preferable.

Special series of researches aimed to determine the optimal initial concentrations of starch for fermentation have been conducted. Obtained results have shown that 10-12 % of initial concentrations of starch are more preferable (Table 1).

Table 1. **The influence of the concentration of raw rice starch on the lactic acid fermentation**

Characteristics	Initial concentration of starch, g / l					
	60	80	100	120	140	160
Yield of lactate, g/l	54.5	72.3	89.9	112.1	121.7	160
Fermentation period, hrs.	31	41	60	81	88	106
Productivity, g/l	1.76	1.76	1.50	1.38	1.38	1.31
Coefficient of conversion, g/g	0.91	0.90	0.90	0.93	0.87	0.87.

Based on the obtained results a method for lactic acid production directly

from non-cooked starchy materials has been developed (Fig. 3).

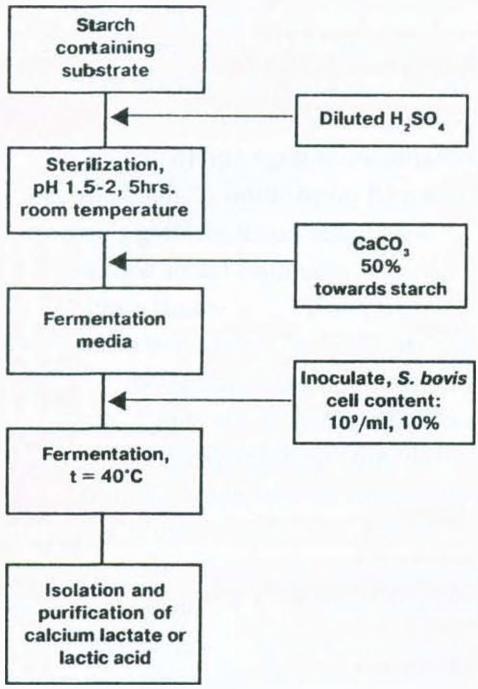

Fig. 3. The principal scheme of the lactate production from starchy substrates.

When lactic acid was produced by repeated batch fermentations, the increase of the amount of production cycles didn't lead to any significant changes in amylolytic activity and lactate producing abilities of the producer (Fig. 4). Total amount of lactic acid produced within five cycles (240 hours) from 320 g of starch (80 + 4 x 60) was about 277 g, while the amount of remained sugars was less than 28 g (around 8,5 %).

More complete fermentation of substrate was achieved due to the prolongation of the duration of the first and the last cycles respectively for 24 and 12 hours. Such a prolongation permits to increase the yeild of lactic acid to 293 g, and to decrease the amount of remaining sugars to 11 g (around 3,5 %). The only disadvantage of such approach was, that total productivity of the process also was decreased from 1,15 g/hour to 1,06 g/hour.

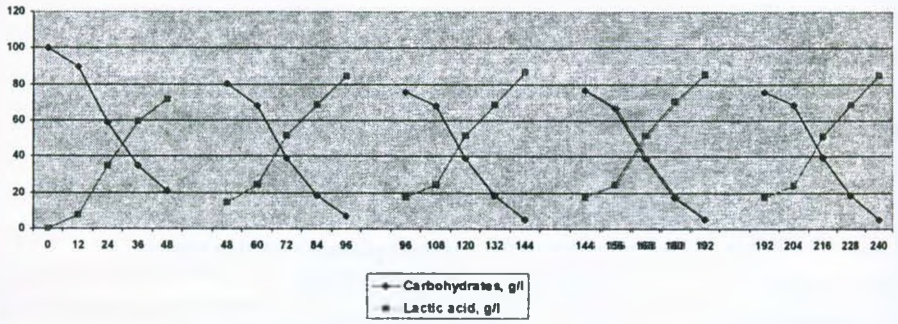

Fig. 4. Lactic acid production by repeated batch fermentations.

Studying the possibilities of continuous production of lactic acid using the cells of *S. bovis* it appears, that it is difficult to achieve steady state at the dilution rates lower than 0,1 h^{-1}. Figure 5 represents the results of continuous lactic acid fermentation at the dilution rate of 0,12 h^{-1}. At the early stage, which was passing as

a batch process, lactic acid was accumulated gradually. The concentration of lactic acid at the end of batch stage reaches to 61,5 g/l.

After the transition from batch to the continuous process, the concentration of lactic acid sharply decreases and reaches the minimum of 38 g/l at 45 – 46 hours of fermentation. Later its concentration begins to increase and was settled at a range of 45 – 50 g/l. On this level lactic acid concentration remains from 60-th to 144 hours of fermentation. On this steady state concentration of lactic acid in limits of 45 – 50 g/l corresponds to the average productivity of about 5,4 – 6,0 g/l/h, what is significantly higher, than in the repeated batch process.

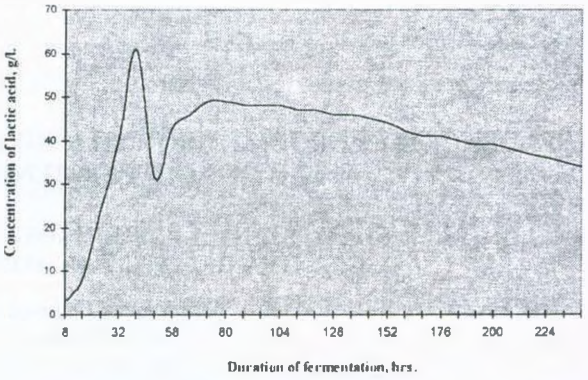

Fig. 5. Continuous production of lactic acid using *S. bovis* cells.

The results obtained evidently indicate on the principial possibility and expediency of the production of L(+) lactic acid by repeated batch and continuous methods, using as a producer a culture of *S. bovis*.

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