Production of lactic acid on enzymatic hydrolysates of cellulose-containing raw materials

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Abstract. The production of lactic acid by the microbiological method using as the basis of the nutrient medium hydrolysates of cellulose-containing raw materials is a promising, but poorly studied area of modern biotechnology. Plant or lignocellulose raw materials are considered to be very useful for the production of a variety of microbiological synthesis products. Lignocellulosic raw materials are recognized as the most used carbohydrate material due to renewability and large volumes of production. The possibility of producing of lactic acid on enzymatic hydrolysates of cellulose-containing raw materials (wheat straw, waste paper, mixed substrate) is shown. It is established that hydrolysates of all types of cellulose-containing raw materials allow to obtain lactic acid and probiotic preparations. The yield of lactic acid depends on the type of raw material used. The highest yield of lactic acid is observed when using an unclarified waste paper hydrolyzate as the basis of the nutrient.

1. Introduction
The global lactic acid market has been growing steadily over the past decade. Lactic acid is used in medicine and in various industries (food, textile, etc.). Global consumption of lactic acid has been steadily increasing in recent years due to its use in such promising industries as cosmetics, biodegradable polymers and food additives [1, 2].

Currently, half the production of lactic acid is produced by microbiological method on sugar-containing and starch-containing substrates. However, these substrates are food raw materials. The production of lactic acid on cellulose-containing raw materials seems to be promising. Cellulose-containing raw materials (wood, straw of various types, husks, waste paper) are easily accessible, renewable and universal for obtaining various bioconversion products.

The aim of the work was to study the processes of lactic acid production on enzymatic hydrolysates of cellulose-containing raw materials.

2. Methods
Commercial enzyme preparation "Cellulux A" (Russia) was used in the research. Preparation contains cellulase - 2000 units/g; β-glucanase - 1500 units/g; and xylanase 8000 units/g.

Wheat straw, waste paper and mixed substrate (waste paper + straw) were used as cellulose-containing raw materials.
2.1. Growth of lactic acid bacteria on clarified hydrolysates of cellulose-containing raw materials

Hydrolysis of cellulose-containing raw materials was carried out at a temperature of 50 °C and pH = 4 for 4 hours. The ratio of solid phase: liquid was 1: 10, the content of the enzyme preparation - 20 mg/cm³. The obtained hydrolysates were separated from the solid residue (unreacted cellulose substrate) on the filter, the precipitate was washed with a small amount of tap water. The obtained clarified hydrolysates of cellulose-containing raw materials were supplemented with dry yeast extract (5g/l), sodium chloride (5 g/l) and cysteine (0.1 mg/l). The nutrient media were sterilized at 0.75 atm 35-40 min. Sterile chalk and inoculum were added to the medium.

Hydrolysates of cellulose-containing raw material were fermented by probiotic preparation "Lactobacterine." The "Lactobacterin" is the living microbial mass of Lactobacillus plantarum 8P-F3 or Lactobacillus fermentum 90T-C4 strain. The amount of living lactobacteria is not less than 2 x 10⁹ CFU/cm³. The fermentation was carried out for 72 hours at 37 ± 1 °C.

Determination of lactic acid concentration was carried out by enzymatic colorimetric method (LACTATE-VITAL, Russia) [3].

Glucose content was determined by glucose oxidase method (GLUCOSE AGAT 400, Russia) [4].

The number of viable cells (CFU/cm³ or titer) was determined by 10-fold dilution of the sample and plating on the agarized MRS medium.

2.2. Cultivation of lactic acid bacteria on unclarified hydrolysates of cellulose-containing raw materials

Prepared on tap water nutrient medium had the following composition: dry yeast extract-5g/l; sodium chloride-5 g/l; cysteine-0.1 mg/l. Chaffed cellulose-containing raw materials-100 g/l - were introduced into the liquid phase. The media was sterilized at 115 °C for 30 minutes. Then, the prehydrolysis of cellulose-containing raw materials in the nutrient media was carried out at a temperature of 50 °C, pH 4 for 2 hours with the enzyme preparation “Cellolux A” added at a rate of 20 mg/cm³. After that nutrient media was cooled to a fermentation temperature (37 °C) and sterile chalk and starter culture (“Lactobacterin” probiotic preparation) were added. Fermentation was carried out for 72 hours at a temperature of 37 ± 1 °C.

The concentration of lactic acid, glucose content and the number of viable cells (CFU/cm³ or titer) were determined in the hydrolysates.

3. Results and Discussion

At the first stage of the work, clarified wheat straw hydrolysates were used. The use of wheat straw as the basis of the nutrient medium has the following advantages: availability, renewability, the presence of not only cellulose components, but also sources of nitrogen, micro and macro elements.
Figure 1. Cultivation of lactic acid bacteria on clarified hydrolysates of wheat straw: a) glucose consumption; b) growth of lactic acid bacteria; с) dynamics of lactic acid accumulation.

The growth of lactic acid bacteria, the dynamics of lactic acid accumulation and glucose consumption by culture of lactic acid bacteria, when grown on clarified hydrolysates of wheat straw are shown in figure 1.

As can be seen from the data presented in figure 1, the accumulation of lactic acid is parallel to the growth of the culture. After 24 hours of cultivation, the yield of lactic acid was 0.15 % and then changed slightly.

Growing on clarified hydrolysates of wheat straw allows to get not only lactic acid, but also concentrate of lactic acid bacteria. The number of viable lactic acid bacteria per 24 hours of growth was $6.61 \times 10^{11}$ CFU/cm$^3$.

At the next stage of work waste paper was used as cellulose-containing raw material. The main characteristics of the process are shown in figure 2.

Figure 2. Cultivation of lactic acid bacteria on clarified hydrolysates of paper waste: a) glucose consumption; b) growth of lactic acid bacteria; с) dynamics of lactic acid accumulation.
As can be seen from figure 2, the glucose concentration of waste paper hydrolysates is higher than that of wheat straw hydrolysates. This makes it possible to significantly increase the yield of lactic acid. At 40 hours of cultivation the lactic acid concentration is about 2.5 times higher than that obtained on straw hydrolysates, due to the high glucose content in the medium.

However, at culture growth on the hydrolysates of the waste paper mass, a longer lag phase is observed. It can be explained by a lower content of nitrogenous compounds, as well as macro- and microelements in the waste paper mass in comparison with straw.

At the second stage of work it was decided to use as the basis of the nutrient medium unclarified hydrolysates of cellulose-containing raw materials. At the case, the nutrient medium contains a catalytically active complex preparation "Cellolux A" and an insoluble cellulose-containing substrate. It was suggested that nutrient media has a significant advantage over media on the base of clarified hydrolysates. So, due to the action of the active cellulase complex constant feeding with glucose and other sugars takes place, and it allows to increase the yield of lactic acid.

Initially, wheat straw hydrolysates were used as the basis of the nutrient medium. As the results show (see figure 3), the initial glucose content is only 5.7 mm/l. During the next 4 hours of cultivation the glucose concentration, due to the hydrolysis of cellulose, first increases, then gradually decreases.

![Figure 3](image)

**Figure 3.** Cultivation of lactic acid bacteria on unclarified hydrolysates of wheat straw: a) glucose consumption; b) growth of lactic acid bacteria; c) dynamics of lactic acid accumulation.

The dynamics of culture growth is ambiguous. There is some growth diauxi. Probably, the initial growth is due to the consumption of glucose, and after 48 hours due to the consumption of other sugars in the medium. The pattern of lactic acid accumulation is similar to the pattern of growth. It means that significantly higher yield of lactic acid in this case is due to the constant inflow of sugars in the nutrient medium than when using clarified hydrolysates.
At the end of cultivation, the culture liquid was separated into liquid and solid (unreacted cellulose substrate and bacteria) phases. The solid residue was dried at a temperature of $50 \pm 1 ^\circ C$ for 4 hours. Received a probiotic additive designed to correct the nutrition of farm animals. The number of viable cells of lactic acid bacteria in the additive was $3 \times 10^8 - 8 \times 10^9$ CFU/g, and it practically did not change during storage for 21 days.

Very interesting results were obtained when using nutrient medium on the basis of the waste paper.

As can be seen from the data presented in figure 4, the concentration of glucose in the initial medium is even lower than in the medium based on wheat straw, but by 4 hours of cultivation due to the action of cellulase, it increases by more than 2 times. And what is interesting is that the glucose content in the process of cultivation changes in waves, remaining constantly at a high level. It happens owing to decreasing of glucose concentration in the medium due to it consumption by lactic bacteria. So, an inhibitory effect of glucose on cellulase is removed, and hydrolysis of cellulose-containing raw materials is increased. That gives rise to glucose concentration in media.

Constant feeding culture with glucose results in a high yield of lactic acid. Already by 48 hours of cultivation the yield of lactic acid reaches 4% and further during cultivation little changes.

4. Conclusion

Thus, it was shown that unclarified hydrolysates of cellulose-containing raw materials (straw, waste paper) containing catalytically active cellulase provide a significantly higher yield of lactic acid in comparison with clarified hydrolysates of these substrates. The highest yield of lactic acid was detected using unclarified waste paper hydrolysates.

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**Figure 4.** Cultivation of lactic acid bacteria on unclarified hydrolysates of paper waste: a) glucose consumption; b) growth of lactic acid bacteria; c) dynamics of lactic acid accumulation.
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