# IMPROVEMENT OF ALCOHOLIC FERMENTATION OF THE PARTIAL DEPROTEINIZED WHEY

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**Abstract:** Experimental investigations of the new technology for biofuel production from whey are discussed in detail. The technological scheme of alcoholic fermentation of serum is described. It was shown that further studies of alcoholic fermentation of whey are necessary in order to increase the quantity and improve the quality of ethanol. The results of experimental investigations of ethanol production from whey using a mixture of cultures of yeast species Saccharomyces cerevisiae and mesophilous culture of lactic acid bacteria Lactobest is presented. The impact of electric current of small density on the whey being fermented for biostimulation of yeast, which leads to an increase in output of ethanol, is considered.

**Key words:** whey, alcoholic fermentation, yeast.

## Introduction

Investigations aimed at the elaboration of technologies and equipment for biofuel production are among the priority tasks.

The main stages of technology for production of ethanol from whey comprise a partial deproteinization of whey, fermentation with a mixture of mesophilic lactic acid bacteria and yeasts, and alcohol distillation [1]. The studies aimed at establishing the optimal method for alcoholic fermentation of whey.

Modern industrial distillation columns for alcohol production have rather large overall dimensions and specific consumption of materials. The costs associated with the manufacturing of these columns are very high. That is why, the elaboration of compact distillation columns with high fractionating ability, small hydraulic resistance, possibility to use liquids containing mechanical particles is an urgent scientific problem.

One of the promising methods for the intensification and control of mass transfer processes in gas-liquid systems is the application of the electric field effect. Several methods for realisation of mass transfer processes in electric field conditions were elaborated and investigated. As a result, a new type of distillation columns was created, and they were named as electrohydrodynamic (EHD) distillation columns [2].

EHD distillation columns have been investigated for the cases of distillation of crude alcohol, wine material, ethanol water mixtures and other liquids. Application of EHD distillation columns for new technology of biofuel production from whey has to be estimated.

## Experimental setup

The technological scheme of alcoholic fermentation of whey includes: the heating of the clarified whey to 93 °C for pasteurisation, cooling, decantation, and fermentation with lactose fermenting yeast at a temperature of 32 - 34 °C. Biochemical properties of the cultures are characterized by the fact that besides the main fermentation products - ethanol and carbon dioxide (figure 1.), fusel oils, aldehydes and esters are also accumulated in liquid media. The first phase of our research was to obtain yeast, which has a high efficiency of ethanol formation at a low percentage of fermentation by-products (aldehydes, fusel oils and esters). In addition, it is necessary to provide a minimum duration of fermentation process at the highest possible content of ethanol. We studied mixtures of yeast species Saccharomyces and mesophilic culture Lactobest DLF-N-86DI10UCadorago (CO) (Italy), freeze dried culture for food industry.

Starter has been prepared in pasteurised clarified whey (OST 10-02-02-3-87) by the introduction of bacteria and lactose fermenting yeast in its 10% suspension and subsequent cultivation at 34°C for 2 days. Thus, the resistance of the culture has gradually increased and selection of improved variants has been performed.

Application of Saccharomyces serevisiae is promising, because Saccharomyces serevisiae can operate at higher ethanol concentrations in the medium. Consequently, a greater amount of lactose can be transformed in ethanol at its pre-splitting by enzyme.

The volumes of the ferment, temperature and fermentation time were varied in a wide range. Analysis of the initial and fermented whey at various stages of fermentation was performed by nuclear magnetic resonance spectroscopy.

$$CH_3-CO-COOH \xrightarrow{CO_2} CH_3-C\genfrac{}{}{0pt}{}{/\!\!/ O}{\backslash H}$$

$$CH_3-C\genfrac{}{}{0pt}{}{/\!\!/ O}{\backslash H} \xrightarrow{H_2} CH_3-CH_2OH$$

***Fig. 1.*** The scheme of ethanol biosynthesis

A vigorous stirring for 24 hours creates favourable conditions for the accumulation of the required quantity of yeast cells and intensification of the alcohol production. This method allows one to ferment lactose completely.

Alongside with improving of the ferment preparation method, we looked for the ways to increase the amount of the yeast.

Various methods of stimulation of the growth of microorganisms are used to increase the productivity [3].

Our objective was to study the effect of electric treatment on fermented whey. The number of microorganisms was determined by counting the colonies on Petri dish.

## Results and discussion

The end of alcoholic fermentation is determined by the residual lactose content (%). In some experiments, the high quantity of lactic acid is obtained, which exceeds the content of ethanol.

The alkalinity of whey increased in the first day of fermentation, since the yeast during its growth consumes the acidic components: lactic acid, citrates, and soluble proteins. The alcoholic fermentation is more intense in the third day of fermentation

(Table 1.). The intensity of alcoholic fermentation reduces due to the depletion of nutrient medium and increasing of the ethanol content in it.

***Table 1.*** Analysis of the samples of the fermented whey

| Time, day | pH | Composition, % | | | |
|---|---|---|---|---|---|
| | | Ethanol | Acetic acid | Lactic acid | Sugar |
| **1** | 4,9 | 19,9 | 5,5 | 34,6 | 36,6 |
| **2** | 4,6 | 16,8 | 5,0 | 28,2 | 46,5 |
| **3** | 4,6 | 22,6 | 5,3 | 27,8 | 41,7 |

In the experiments with yeast and lactic bacteria (Table 2) both alcohol and lactic acid fermentations occur. A combined using of yeast and lactic acid bacteria has the following advantages: acidification of the medium leads to splitting of complex nitrogen-containing compounds, and this favours the nutrition of yeast. However, in order not to inhibit the growth of yeast the amount of lactic acid bacteria should not be great (no more than 1%) (Figure. 2.).

Among the secondary alcoholic fermentation products other than lactic acid there are acetic acid, acetaldehyde, 2,3 butylene glycol, succinic acid, and acetoin. Acetaldehyde is generated from glucose during the alcohol fermentation. It can also be formed by the oxidation of ethanol. In general, in our experiments the contents of acetaldehyde were from 3 to 5% of the fermented whey.

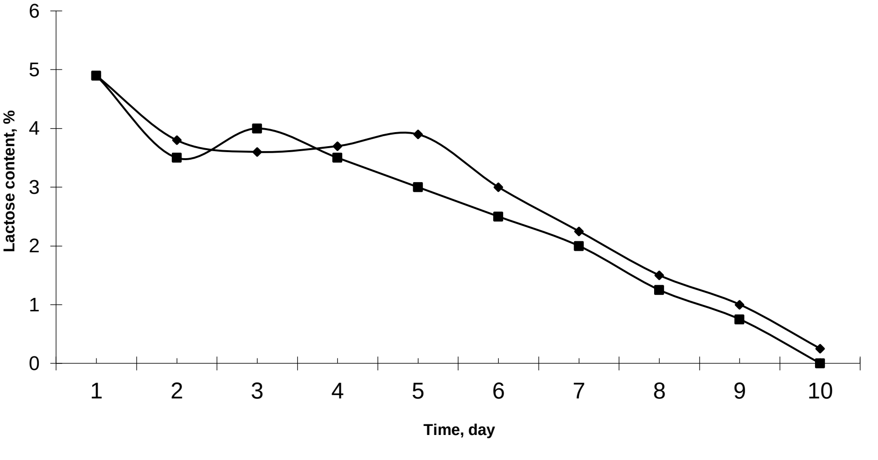


***Fig. 2.*** The results of fermentation of whey by different amount of yeast and lactic acid bacteria:

■ – yeast, 100 g/1 L; ♦ Lactob. 10% + 100 g yeast/1 L.

***Table 2.*** Fermentation products formed during alcoholic fermentation of whey

| Yeast fermentation | | Composition, % | | | |
|---|---|---|---|---|---|
| **Whey** | **pH** | 34,9 | **Ethanol** | **Acetic acid** | **Sugar** |
| **Initial** | 4,75 | 32,9 | 4,5 | 4,7 | 54,8 |
| **1 day** | 4,65 | 33,9 | 3,9 | 5,3 | 56,9 |
| **2** | 4,75 | 34,9 | 3,8 | 5,1 | 55,9 |
| **4** | 4,75 | 39,2 | 4,5 | 4,7 | 54,8 |
| **7** | 4,45 | | 6,4 | 7,1 | 46,3 |
| **Yeast + Lactobest** | | | | | |
| **1 day** | 4 | 32,6 | 11,3 | 5,2 | 48,2 |
| **3** | 4,05 | – | 24,5 | 22,2 | 35,9 |
| **4** | 4,3 | – | 19,9 | 17,9 | 49,3 |
| **6** | 4,6 | 65,1 | – | 20,4 | – |

Formation of acetic acid is due to oxidation of acetaldehyde oxygenated water. The content of secondary fermentation products depends on the aeration, medium pH, temperature, and composition of the initial whey. Concentration of succinic acid increases due to aeration and reduces under anaerobic conditions.

At the mentioned conditions, these analyses were carried out in the second, third, and sixth day of fermentation. The chromatographic peaks were identified for ethanol, lactic acid, acetic acid, and succinic acid. The pH value of 4,0 – 4,6 coincides with the isoelectric point of the lacto-albumin fraction of proteins and provides them a quick coagulation. Whey as secondary product from milk factory was used as raw materials for the investigation.

In separate investigation a preliminary thickening of the initial whey by evaporation was carried out. The thickening makes it possible to increase the concentration of lactose and, hence, the concentration of ethanol.

Osmosis is a more preferable method for thickening of whey than the evaporation in vacuum.

The most economically advantageous is to conduct the process of whey conversion in ethanol employing the separation of protein from whey, its concentration by reverse osmosis and the subsequent fermentation.

The impact of electric current of small density on the whey being fermented for biostimulation of yeast, which leads to an increase in output of ethanol, is considered (Table 3.).

***Table 3.*** Microbiological parameters of the fermented whey during electrotreatment

| Time, h | *I*, A | pH | The number of microorganisms in 1 cm$^3$ | |
|---|---|---|---|---|
| | | | **Colonies of Lactob.** | **Yeast** |
| | 0,2 | 4,25 | 6400 $10^8$ | |
| **1,5** | 0,2 | 4,8 | 1100 $10^6$ | Growth |
| **3,5** | 0,2 | 4,75 | Colonies | 14400 |
| **5,5** | 0,2 | 4,75 | 4800 | Growth |
| **7,5** | 0,2 | 4,7 | 7200 | Solid growth |
| **9,5** | 0,2 | 4,7 | 4000 | Solid growth |

## Conclusions

The results of the investigation allow us to optimise the operational parameters of technology for ethanol production from whey. The following steps for the whey pre-treatment were defined: the separation of protein from whey, concentration of the whey by reverse osmosis, and fermentation.

Further studies of alcoholic fermentation of whey are necessary in order to increase the quantity and improve the quality of ethanol.

The obtained results of the study make it possible to proceed to the elaboration of the next stage of the technology, namely, the EHD distillation of ethanol.

## References

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