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OBTAINING OF PROTEIC BIOMASS BY CULTIVATION OF LACTIC ACID BACTERIA ON GRAPE MARC DIFFUSION SOLUTION

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Abstract

In this article are presented the researches made in order to obtain protein biomass with the aid of lactic bacteria grown on an economically medium, achieved by using secondary products from the winery: marc and wine yeast. Therefore, there were cultivated two strains of Lactobacillus sp. on five different growth medium. The protein biosynthesis and evolution of lactic fermentation were monitored by determining the optical density (OD) of the culture at a wavelength λ = 600 nm and by counting the colony forming units (CFU) by serial dilutions and seeding on plates and by determination of lactic acid obtained. The results showed that the fermentation medium represented by diffusion solution of the marc, enriched with peptone is economically profitable compared to other culture media containing peptone, yeast extract, glucose, minerals, amino acids and vitamins presented in the literature.

INTRODUCTION

Valorization of winery waste products has become an urgent need of the winemaking companies because of the high taxes of the waste disposal. This is why the recovery of by-products of the wine industry is extensively studied in the literature [1], in search of economically viable solutions for valorizing the production residues [2]. This could be also a process that might provide additional sources of income [3].

In the present study was investigated the possibility to obtain protein biomass with the aid of lactic bacteria grown on an economically medium, achieved by using secondary products from the winery industry, namely grape marc and wine yeast.

Lactic acid bacteria are widely used in industrial food fermentations and are receiving increased attention for use as cell factories [4].

Our research focused on the possibility of obtaining protein biomass with the aid of lactic bacteria grown on different cultivation media achieved by exploiting both the solution of marc containing sugars [5] and mineral salts and also the proteic extract from wine yeast which brings the organic nitrogen source [6] and the growth factors.

MATERIALS AND METHODS

Two strains of lactic acid bacteriaWere subjected to study as follows: *Lactobacillus sp GM* isolated from ruminal fluid of cattle and *Lactobacillus sp. A_1. Lactobacillus sp.* is a gram positive, microaerophilic bacteria.

The two strains of *Lactobacillus sp*. were grown statically at 40° C on 5 different culture media at pH 6.0.

We used grape marc originating from processed white wine and wine yeast (yeast sediment from fermentation of white and red wines). The grape marc was obtained from continuous press and was represented only by the skin and seeds, while the clusters were separated and discarded earlier in the production process. The grape marc was collected immediately after pressing, so it was

fresh, unfermented and originating from healthy grapes.
Both wine yeast and the grape marc were distributed immediately in plastic bags and deposited in a freezer at -12 °C.

RESULTS AND DISCUSSIONS

The protein biosynthesis and the evolution of lactic fermentation were monitored by determining the optical density (OD) of the culture at a wavelength λ = 600 nm and by counting the colony forming units (CFU) by serial dilutions and seeding on plates and by determination of lactic acid obtained, also (figure 1 -7).
There were obtained the growth curves of the two strains in parallel with the dynamics of the growth of lactic acid on the 5 different culturing media.
We have experienced the following working variants:

1. MRS - the standard medium (control);
2. Marc washing solution;
3. Marc washing solution with the addition of peptone l g% (corresponding to the concentration of the standard);
4. Marc solution mixed with yeast extract (3:1);
5. Marc solution mixed with yeast extract (1:1)

By analyzing the dynamics of accumulation of lactic acid and biomass formation in standard MRS medium compared with the proposed economic variations can be withdrawn that on the standard MRS medium the fermentations were conducted with *Lactobacillus sp. GM* and *Lactobacillus sp. A_1*.
Regardless of the microorganism used (Figure 1 and Figure 2), the evolution of biomass accumulation was typical for bacteria. It can be observed the logarithmic growth phase for 6 hours, followed by a slower phase of growth of approximately 2 hours, followed eventually by a stationary phase.

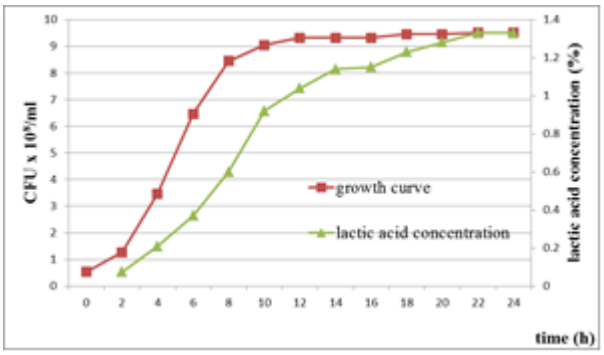

Figure 1. The profile of the growth curve and accumulation of lactic acid for strain *Lactobacillus sp.* GM, on MRS medium

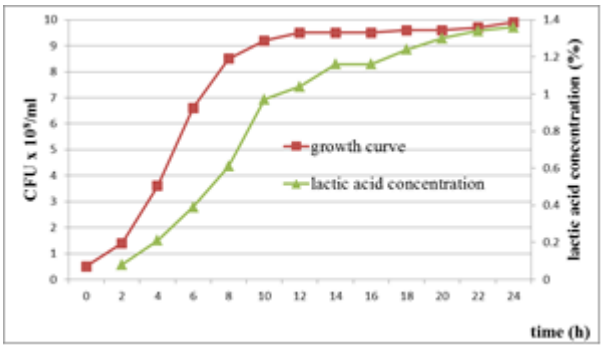

Figure 2. The profile of the growth curve and accumulation of lactic acid for strain *Lactobacillus sp. A_1*, on MRS medium

Lactic acid biosynthesis occurred at a slower pace at the beginning of fermentation, followed by an increase from the middle of the logarithmic phase and continued during the stationary phase of growth. Economically profitable media based on diffusion water of the grape marc, containing 3.6% glucose and 33.6% protein d.m. showed a differential evolution. Thus, the diffusion water of the marc used as it was, represents the control (Figure 3), and encouraging results have been obtained. This growing medium allowed the accumulation of biomass and lactic acid at mean values. The logarithmic growth phase was extended to 18 hours, observing a maximum accumulation of lactic acid by the end of fermentation.
On the medium from marc enriched with peptone solution, when used as biological material the *Lactobacillus sp GM* strain (Figure 4), the biomass accumulation value was 7 times higher than the standard medium.

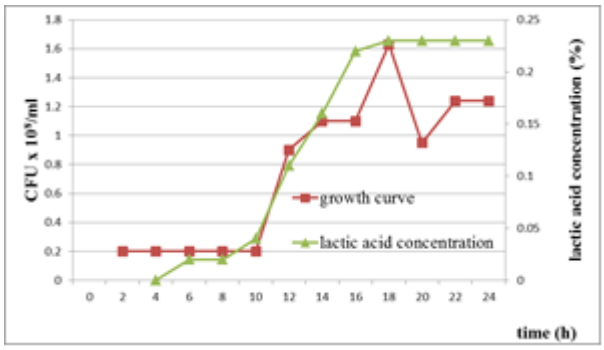

Figure 3. The profile of the growth curve and accumulation of lactic acid for *Lactobacillus sp*. strain GM, on marc washing solution

Logarithmic growth phase was extended to 18 hours, being observed a high accumulation of lactic acid by the end of fermentation period (22-24 hours).

On a medium with the same composition, using as seed material the transformed strain of *Lactobacillus sp. A₁*. (Figure 1), were obtained higher values than in the case of fermentation with the parent strain (Figure 4).

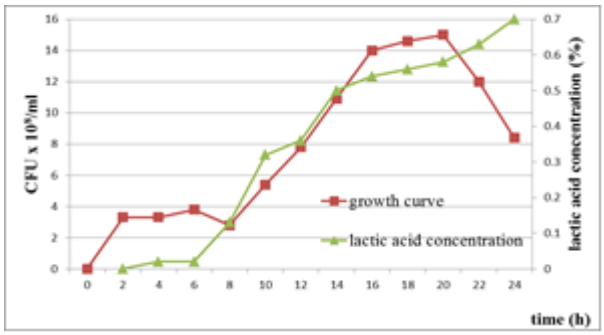

Figure 4. The profile of the growth curve and accumulation of lactic acid for *Lactobacillus sp. GM80* strain, on marc washing solution with the addition of 1 % peptone

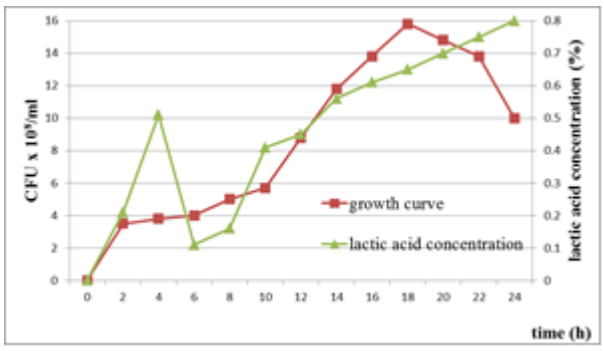

Figure 5. The profile of the growth curve and accumulation of lactic acid for strain *Lactobacillus sp. A₁*, on marc washing solution with the addition of 1 % peptone

By replacing the complex source of nitrogen peptone with yeast autolysis solution, (Figure 6 and 7), regardless of its concentration in the medium (3:1 and 1:1), did not provide the polypeptides necessary for the microorganisms growth. This was reflected in the decrease of the number of cells in the medium and the extension of the phase of logarithmic growth to 20 hours. The accumulation of lactic acid took place was gradually until the end of the fermentation process.

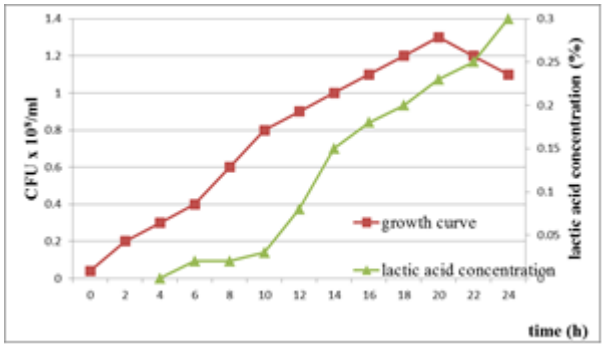

Figure 6. The profile of the growth curve and accumulation of lactic acid for strain *Lactobacillus* sp. GM, on medium from marc solution mixed with yeast extract (3:1)

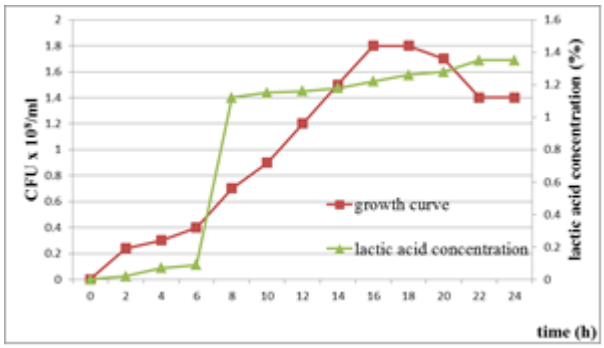

Figure 7. The profile of the growth curve and accumulation of lactic acid for strain *Lactobacillus* sp. GM, on medium from marc solution mixed with yeast extract (1:1)

CONCLUSIONS

By comparing the growth curves of the two strains, in parallel with the dynamics of the accumulation of lactic acid can be seen that the maximum biomass accumulation occurred in the medium originating from the marc solution supplemented with peptone, obtaining much better results than on the

standard medium (MRS). By replacing the complex source of nitrogen peptone with yeast autolysis solution, (Figure 6 and 7), regardless of its concentration in the medium (3:1 and 1:1), did not provide the polypeptides necessary for the microorganisms growth. This was reflected in the decrease of the number of cells in the medium and the extension of the phase of logarithmic growth

The results showed that the fermentation medium used in the experimental scheme, represented by diffusion solution of the grape marc, enriched with peptone is economically profitable compared to other culturing media containing peptone, yeast extract, glucose, minerals, amino acids and vitamins presented in the literature.

Rapid multiplication realized by both strains of lactic acid bacteria on the economic medium diffusion represented by the diffusion solution of the grape marc enriched with peptone allow the application of a continuous or semi-continuous fermentation technology, which would bring back a good efficiency of the bioprocess.

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