THE STIMULATING EFFECT OF VITAMIN B1 ON THE PRODUCTION OF BIOMASS IN *SACCHAROMYCES CEREVISIAE* LOLVIN D 47

Laslo Vasile, Vicaş Simona Ioana, Purcărea Cornelia, Popa Dorin

University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea; Romania, e-mail: vasilelaslo@yahoo.com

Abstract

The current study aims to determine the measure in which vitamin B1, when added to the culture medium of Sacharomyces cerevisiae Lolvin D47 stems stimulates its biomass production. The results we obtained confirm the stimulating effect of vitamin B1 (2,37 mM) when added to the culture medium. Compared to the control culture, the addition in the culture medium of this quantity of vitamin B1 leads to an increase in duration of the logarithmic growth phase by 36% and to an increase in biomass by 32%.

Keywords*:* yeast,vitamin B1,biomass

INTRODUCTION

The demographic explosion that started in the final decades of the XXth century has generated an acute lack of proteins available on the global market. It has become certain fact that the combined production of agroecosystems cannot sustain a continuously increasing population. Recent studies highlight the fact that the minimum necessary global fodder protein production for a healthy human diet (100 KG of fodder protein/year/human) is 500 million Mt yearly. Currently the global production of fodder protein of vegetal or animal origin does not exceed 75 million Mt yearly, which leaves a deficit of about 425 million Mt of fodder protein which needs supplementing (Raicu P. et al., 1990).Because of this it is necessary to use new sources of protein, semi-conventional (soy extracts, fish-based concentrates) and unconventional (SCP – single cell protein: proteins harvested from bacteria, yeasts, molds, algae). The term "single cell protein" refers to dried cells of microorganisms (yeasts, bacteria, fungi and algae) that grow on different sources of carbon (.Anghel, I., et al., 1993, Banu, C., et al., 2000). The term was first used more than forty years ago (1966) by the MIT professor Carol Wilson to offer a more agreeable denomination to replace the old "microbian protein" term.

Obtaining biomass on different growth environments with the aid of microorganisms is and has been an important aspect of the biosynthesis industry. If at first this mass refered to as "single cell protein" was used mainly as a fodder additive meant to enrich animal fodder with protein and vitamins (Jurcoane et al., 2006; Lichtfield, 1968). Today its uses have diversified. Single cell biomass is now used as:

- biocatalyst for obtaining certain optically active products used in the medical industry (Miller and Litsky 1976; Israelidis and Evangelopoulos 1980)
- "alimentation-medication" type products for human use, especially as biological normalizers (probiotic) of saprophyte intestinal flora – human food additives (Dan, 1991).

MATERIALS AND METHODS

Preparation of culture environments

The culture environment that we used is GYEP (10% glucose, 1% yeast extract, 1% peptone). For each experimental variant we prepared 250 ml of culture environment. Bioreactors along with culture environments were autoclaved, sterilized at 121^{o}C for 20 minutes.

Experimental variants

We produced the following experimental variants:

Vm / GYEP medium with no addition of vitaminB1 (control),
V1 / GYEP medium with 0.59 mM vitaminB1,
V2 / GYEP medium with 1.18 mM vitaminB1,
V3 / GYEP medium with 2.37 mM vitaminB1.

Yeast and culture conditions

The yeast we used for this study was a commercially available *Sacharomyces cerevisiae*, Lovlin D47 strain.

Inoculation took place in two stages: i) in the first stage a single colony obtained in a Petri dish was inoculated in a small quantity of GYEP environment (10 ml) and then incubated for 24 hours in a thermostat set at 300; ii) at the end of the exponential growth phase, the culture was transferred (stage 2) in a 250 ml GYEP medium, resulting in 8.7 x 10^{6} cellular density in all experimental variants.

To ensure air circulation of 3.5 l/h we used a type AC-9906 air pump. The air which was introduced to the bioreactors was filtered with textile filters (Fig. 1).

Analytical methods used

Microorganism density in the culture medium was determined with a DEN 1 densiometer and pH was measured using a Crison meter pH 25.

A GL 30 tester was used to determine the sugar content.

1 ml of test material was harvested for the determination of produced biomass, which was centrifuged, washed with distilled water and dried in drying closet until constant weight was achieved, at 110^{o}C for 48 hours.

Fig.1 Bioreactors

RESULTS AND DISCUSSION

Dynamics of cellular density

Microorganism development under normal conditions follows a process which is conventionally divided in four phases:

Lag phase (Fig. 2) takes approximately 6 hours in all experimental variants. This phase is characterized by the fact that multiplication does not occur but metabolic processes intensify. Regeneration of protoplasm from old cells occurs in this phase, and as a result the inoculate receives the characteristics of young protoplasm.

Logarythmic multiplication phase – is characterised by a period of rapid growth in yeast cell numbers, in geometric progression (doubling their number every 20-30 minutes). The time in which this phase takes place varies: it takes 15 hours in the control variant and 11 hours in the three experimental variants.

Stationary phase – at the end of the logarythmic multiplication phase, the number of microorganisms reaches a peak, establishing a ballance between the number of cells being born and cells dieing. The lenght of this phase is influenced by the decrease in nutrient quantity as well as accumulation of autotoxic metabolic compounds in the substrate that the microorganisms develop, as a result of their own metabolism.

The following chart illustrates that this phase lasts between 22 hours in the control variant and 25-27 hours in the experimental variants.

The largest cellular density was recorded 17 hours after the innoculation in the culture medium in V3 (75 $x10^6$), a density which was maintained almost constantly throughout the experiment. This value is distinctly significant compared to the control (65 $x10^6$) and significant when compared to V1 (66$x10^6$) and V2 (67 $x10^6$).

Decline phase – is highlighted by the drastic decrease in living cell numbers, leading to the death of all microorganisms because of the toxic compounds resulted during vital activity. Because the purpose of the experiment was to obtain as much yeast biomass as possible, the experiment came to an end upon observing the decline in cell numbers.

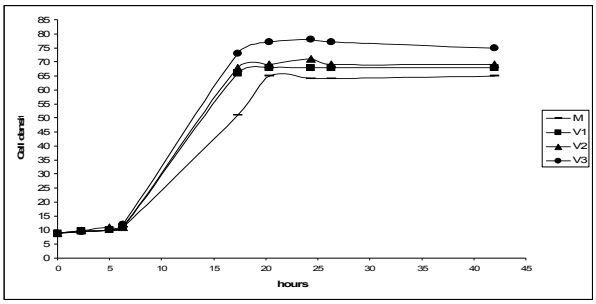

Fig.2. Evolution of cell density in experimental variants

pH dynamics

The initial pH in all experimental variants was 5.24. Within the first 6 hours, corresponding to the lag phase, all experimental variants registered a decrease in pH, with lower values in the control (4.68) and V2 (4.65) and higher values in V1 (4.82) and V3 (4.8) (Fig. 3). Within the exponential growth phase timeframe we measured a slight decrease in pH in V1 and V3 (4.8 and 4.75 respectively) and a hightened decrease in V2 and the witnes (4.58 and 4.52 respectively).

At the beginning of the stationary phase the pH values oscillate significantly in all experimental variants, the lowest value being recorded in the control (4.42) and the highest in V1 (4.85), after which, in all three variants the pH rises, becoming stable at 5.02 in V3 and V2 and 4.95 in V1.

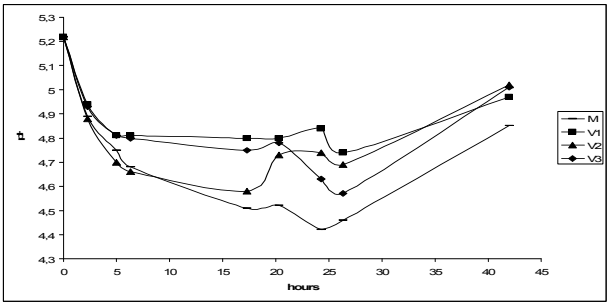

Fig. 3. pH dynamics in experimental variants

Modification of sugar content

According to the culture medium recipe, each experimental variant has a sugar content of 95 g/l at the beginnig of the experiment (Fig. 4). Throughout the recording period (27 hours) the sugar content dropped constantly in all experimental variants, reaching a constant value in the witnes, V1 and V2 of 26 g/l and a lower one in V3 – 8 g/l.

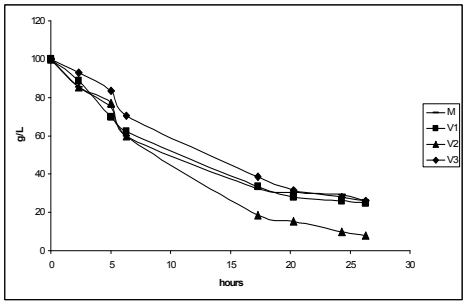

Fig. 4. Dynamics of sugar content in experimental variants

Wet biomass dynamics

Biomass determinations were made at three intervals: 8, 24 and 48 hours. Values obtained were expressed in g/l (Fig. 5).

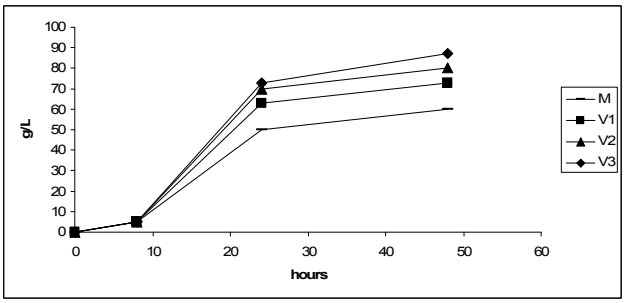

Fig. 5. Chart expressing the biomass dynamics

The first determination, made 8 hours after inoculation, showed that the quantity of wet biomass was 4g/l in all experimental variants. Significant differences between experimental variants were recorded at the end of exponential growth phase (Fig. 2). 24 hours after incoulation, the control registered a wet yeast biomass quantity of 55 g/l, an inferior value when compared to the biomass synthesized in the culture mediums with a supplement of vitamin B1. The experimental variants yeald significant differences between V3, with a value of 81 g/l on one hand, and V1 and V2

with values of 67 g/l and 77 g/l respectively, on the other hand. After 24 hours the differences between the experimental variants and the witnes are significant. After 48 hours, the biomass quantity in experimental variants V1 and V2 is aproximately equal, with a significant difference of 11% compared to the witnes. V3 experimental variant has the largest quantity of biomass with a distincly significant positive difference compared to the control.

CONCLUSIONS

1. The logarythmic multiplication phase is characterised by a period of rapid growth in yeast cell numbers, in geometric progression (doubling their number every 20-30 minutes). Its duration varies, taking place over 15 hours in the control variant and 11 hours in all 3 experimental variants with vitamin B1 added to the culture medium composition.

2. The highest cell density was recorded 17 hours after inoculation in the culture medium, in the V3 experimental variant (75×10^6), a value which remained almost constant throughout the experiment. This value is distinctly significant when compared to the control (65×10^6) and significant compared to V1 (66×10^6) and V2 (67×10^6).

3. Regarding pH levels, thei have a similar dynamic in all experimental variants, stabilizing at the end of the observation period in all experimental variants at 5,02 in V3 and V2 and at 4,95 in V1.

4. Throughout the recording period (27 hours) the sugar content registered a constant decrease in all variants, reaching equal values in the control, V1 and V2 (26 g/l) and a lower value in V3 (8 g/l) after 27 hours. This fact suggests a faster sugar metabolic rate in the experimental variant with the highest content of vitamin B1.

5. After 48 hours, the quantity of biomass present in experimental variants V1 and V2 is approximately equal, with a significant difference of 11% compared to the control. The V3 experimental variant registeres the highest biomass quantity, with a distinctly significant positive difference compared to the control (32%).

REFERENCES

1. Anghel, I., et al., 1993, Biologia şi tehnologia drojdiilor, vol.III, Editura Tehnică, Bucureşti
2. Banu, C., et al., 2000, Biotehnologii în industria alimentară, Editura Tehnică, Bucureşti
3. Dan, Valentina, 1991, *Controlul microbiologic al produselor alimentare*, Universitatea Galaţi, 1991
4. Blackwelder, J. 1977, "Depend on us for more `food` hunger", The Wall Street Journal, Jan. 1977.
5. Brinton M. Miller & Warren Litsky,1976, "Single Cell Protein in Industrial Microbiology". McGrow-Hill Book Comany, pp 408.
6. Brown, L.R. 1968. "World Food Problems, Single-cell Protein", Mateles and Tannebaum, eds. The M.I.T. Press, Cambridge MA.
7. Han, Y.W., C.E. Duhlap and C.D. Callihan (1971). "Single cell protein from cellulosic wastes". Food technology 25: 130-154.
8. Humphrey A.E. (1974). "Cunenet Developments in Fermentation". Chemical Engineering, December 9.
9. Israelidis, C.J. and A.E. Evangelopoulos (1980), "Enzymatic and Microbial conversion of cellulosic agricultural by-products for the production of animal feed, ethanol and chemicals." Procc. Balkan chemistry days, chimica chronica 9(4): 337-352.
10. Israelidis, C.J., and M.I. Coduonis (1982). "Utilisation of agricultural wastes for animal feed and energy". Agricultural research 6: 243-253.
11. Jurcoane S., Săsărman E ş.a. Tratat de biotehnologie. Vol. I. Bucureşti., Editura tehnică. 2004.
12. Jurcoane S., P.Cornea ş.a. Tratat de biotehnologie. Vol. II. Bucureşti., Editura tehnică. 2006. 668 p.
13. Lichtfield, J.H. (1968). "The production of fungi. Single-cell Protein I", Mateles and Tannenbaum, eds. The M.I.T. Press, Cambridge, MA.
14. Raicu P., ş.a., Biotehnologii moderne, Editura Tehnică, Bucureşti, 1990
15. Rotaru, V., Filimon, N., 1976 – Tehnologii în industria alimentară fermentativă, Editura Didactică şi Pedagogică, Bucureşti
16. Solomons G.L. (1984). "Single-cell protein", CRC Critical Reviews in Biotechnology 1(1) 21-58.
17. Ware, S.A. (1977), "Single cell protein and other food recovery technologies from waste". Municipal environmental research laboratory. Office of R. & D. U.S. E.P.A. Cincinnati, Ohio 45268.