https://doi.org/10.34101/actaagrar/74/1674
ACTA AGRARIA DEBRECENIENSIS 2018/74

Influencing the growth kinetics of yeast strains with vitamin supplementation

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SUMMARY

The aim of the current experiment was to optimize the creation process of single cell protein on plant-based substrate solution with the intention to improve end-product turn out by means of adding vitamin solution. Based on the results of the fermentation processes of yeast strains, it was concluded that the vitamin-supplementation produced its greatest effect on the dry matter production, primarily on the K. marxianus DSM 4908 strain, while it was less beneficent when it comes to the figures of wet cell mass. In addition, it can be assumed that vitamin supplementation increased the maximum specific rate of growth (μ_{max}) and decreased the generation time (t_g) significantly. In the case of the K. marxianus yeast strain on corn steep liquor treated with vitamin-supplementation, the highest (μ_{max}) and the lowest (t_g) data were observed [(0.226 h^{-1}) and (4.4 h), respectively]. Based on the results it was found that K. marxianus DSM 4908 is expedient to be applied on corn steep liquor medium in order to determine its suitability to produce additive for feeding.

Keywords: *functional feed, yeast strain, SCP*

INTRODUCTION

As a result of the expansion of fermentation biotechnology, the growth of the significance of creating single cell protein supplementation additives may appear on the market. These protein supplementation additives feature perfect quality indicators and can be exploited in the forage industry. *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* strain of yeast species are applied in increasing numbers to produce these products. In these fermentation processes, the most commonly used substrate of plant origin in molasses, however, corn steep liquor is also a popular yeast medium.

The main purpose of my research was to optimise the process of producing single cell protein on molasses and corn steep liquor medium. As a next step, it was also an objective to improve yeast reproduction, as well as production indicators by adding vitamin solution.

MATERIAL AND METHODS

The experiments were carried out with a batch fermentation system in Sartorius Biostat A Plus type 5 litre reactor chamber fermentor at the Department of Food Science, Faculty of Agricultural and Food Sciences, Széchenyi István University. Following the calibration of the measuring device of the equipment BIO PAT MFCS/DA data collection package was used in order to monitor set values and these data were then recorded.

A *Saccharomyces cerevisiae* NCAIM Y.00200 yeast strain was provided by NCAIM – National Collection of Agricultural and Industrial Microorganisms; Budapest. *Kluyveromyces marxianus* DSM 4908 was provided by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM, Brauschweig, Germany). Following the revival and reproduction of the yeast strains inoculum was made by washing one plate of yeast culture into 50 cm^3 of physiological saline solution (6.5 g NaCl dm^{-3}), which ensured the constant inicial cell concentration also applied by Albertin et al. (2011) on the basis of setting cell concentration (10^6 cells cm^{-3}; Albertin et al. 2011). This cell density was set by Bürker-chamber cell count determination. Molasses serving as a basis for fermentation medium was provided by the Distillery and Refinery Company of Győr and corn steep liquor was provided by Kévés Ltd of Soltvadkert.

According to technical literature sources, molasses was used 11–12% (Kutasi 2007) and 18% (El-Gendy et al. 2013) in the form of solution for fermentation, these concentrations were set in the experiments as the dilution ratio of molasses and corn steep liquor on the basis of the method of Kutasi (2007) and El-Gendy et al. (2013). The plant based mediums (2 dm^3) were heat treated then revolution, airing, pH and temperature values were set and dissolved oxygen concentrations were measured.

In this article, values resulting in the highest cell mass productivity appearing during the optimisation process are revealed. In the case of *Saccharomyces cerevisiae* NCAIM Y.00200 a temperature of 30 °C, 200 min^{-1} revolution, 1.5 VVM (Vessel Volumes per Minute) airing, 5.5 pH and 11–12% substrate dilution was set on molasses medium both with the optimised (control) and the vitamin added fermentation processes. Moreover, on corn steep liquor medium 11–12% dilution, a temperature of 30 °C, 5.5 pH, 400 min^{-1} revolution and 1.5 VVM airing was set in the same fermentation processes. In the case of *Kluyveromyces marxianus* DSM 4908 yeast strain 11–12% dilution, a temperature of 30 °C, pH 4.5, 300 min^{-1} revolution, 1.5 VVM airing was set in the fermentation processes on both mediums. When achieving stable parameters, the inoculum was injected into the solution and registration of data was started. During the 72-hour process, samples were taken, of which dry matter values, wet cell mass and the number of yeast cells were calculated. After the optimisation of the medium, which was then used as control, was enriched with Wickerham vitamin stock solution (Furutani et al.

1953) at a proportion of 1 cm^3 dm^{-3}, the composition of which can be seen in *Table 1*.

Yeast cell count determination was done by means of colony forming yeast cell concentration at 25±1 °C, during 96–120-hour incubation, applying YGC (Yeast-Glucose-Chloramphenicol) medium. For the determination of the wet cell mass value, samples were centrifuged at 5000 rpm for five minutes, after removing supernatant, the residual substrate drops were mopped. In order to measure dry matter values, three grams of sample was used which was dried at 105 °C until reaching constant weight on the basis of the method described by Csapó and Csapóné (2003). In the course of the examination of the characteristics of yeast cell reproduction, the maximum specific growth rates (μ_{max}) and generation times (t_g) were determined.

Table 1

Composition of the vitamin stock solution according to Wickerham

Component	Concentration (mg dm^{-3})
Folic acid	2
Biotin	2
Calcium pantothenate	400
Inositol	2000
Niacin	400
p aminobenzoic acid	200
Pyridoxine hydrochloride	400
Thiamin hydrochloride	400
Riboflavin	200

To assess the impact of adding vitamin solution the method of one-way ANOVA was applied using MicroCal Origin 3.0 programme.

RESULTS AND DISCUSSION

Dry mass, wet cell mass and yeast cell count values were determined by using samples gained from the optimisation fermentation processes. As a next step, the experiment was repeated by adding vitamin solution, but not changing the optimised parameters. The results clearly show that dry matter production of *K. marxianus* increased to two-threefold as a result of adding vitamin solution on both substrates by the end of the third day ($p<0.05$). However, this growth in the case of *S. cerevisiae* NCAIM Y.00200 was moderate, around 50% on molasses. In this experimental setting, not only dry matter production, but also wet cell mass values showed significant growth ($p<0.05$) due to vitamin supplementation. In the course of the experiment, cell count values were determined, which varied between $\log_{10}$ 8.36–8.52 cfu (Colony Forming Unit) cm^{-3} in the case of the control experimental settings and varied between $\log_{10}$ 8.36–8.50 cfu cm^{-3} in the case of vitamin supplementation. From the logarithmic cell count values, the maximum specific growth rate was also determined, as indicated in *Table 2*.

According to the findings of *Table 2*, significantly higher μ_{max} values were gained in the fermentation process with added vitamin solution compared to the control processes. Following the experiment of the maximum specific growth rate of SCP, generation times were also determined in order to describe reproduction charasteristics in a more exact way, as indicated in *Table 3*.

Table 2

Maximum specific growth rates [μ_{max} (h^{-1})] of yeasts in the control fermentation process and in the fermentation process involving vitamin supplementation

Experimental setting*	Control[b]	Vitamin supplementation[a]
A	0.217	0.224
B	0.218	0.221
C	0.211	0.220
D	0.203	0.226

Note: [a,b] subcolumn means within row and experimental setting without a common superscript differ ($P < 0.05$), * *Saccharomyces cerevisiae* NCAIM Y.00200 in molasses (A) and corn steep liquor (B) media, and *Kluyveromyces marxianus* DSM 4908 in molasses (C) and steep liquor (D) media.

Table 3

Generation times [t_g (h)] of yeasts in the control fermentation process and in the fermentation process involving vitamin supplementation

Experimental setting*	Control[a]	Vitamin supplementation[b]
A	4.6	4.5
B	4.5	4.4
C	4.7	4.5
D	4.9	4.4

Note: [a,b] subcolumn means within row and experimental setting without a common superscript differ ($P < 0.05$), * *Saccharomyces cerevisiae* NCAIM Y.00200 in molasses (A) and corn steep liquor (B) media, and *Kluyveromyces marxianus* DSM 4908 in molasses (C) and steep liquor (D) media.

As indicated by findings of *Table 3*, generation times significantly decreased as a result of vitamin supplementation as compared to the control process.

CONCLUSIONS

Vitamin supplementation stimulated cell count values and as a result, dry matter production especially in case of *K. marxianus* DSM 4908 strain, it did not have such good influence on wet cell mass values though. Adding vitamin solution resulted in significant increase of the maximum specific growth rates (μ_{max}) and significant decrease of the generation times (t_g). The highest μ_{max} (0.226 h^{-1}) and the lowest t_g (4.4 h) values were obtained with *K. marxianus* DSM 4908 in vitamin-supplemented corn steep liquor therefore I found this strain the most expedient to produce additive for forage.

ACKNOWLEDGEMENT

I am highly indebted to my thesis supervisor Prof. Dr. László Varga PhD, and Dr. Balázs Ásványi PhD associate professor for their guidance and constant supervision and also for their support in completing this research. The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

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