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# GROWTH OF MICROORGANISMS IN THE PRE-FERMENTATION TANKS IN THE PRODUCTION OF ETHANOL

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## ABSTRACT

Our research was carried out to determine the plate count with a special observation *Saccharomyces cerevisiae* in the pre-fermenters cereal grains using the classical microscopic method. The cell counts were performed in the Bürker chamber. We followed changes in the plate count, number of *Saccharomyces cerevisiae* and changes during the yeast propagation in the mash. The mash would present only cultivated yeast *Saccharomyces cerevisiae* but may occur in a small number of other microorganism's types. Samples were taken during the propagation process in distillery factories. During this period, 30 samples of corn mash were examined. Samples were collected from two tanks during the fifteen generations. The total number of Saccharomyces cerevisiae was reduced and we got a number of unwanted microbiota. The statistical evaluation demonstrated that the growth of unwanted microbiota is directly related to the increase in the propagation of generation in corn mash. The maximum number of yeast cells was found in the twelfth generation 3.052 x $10^8$ mL in the propagation tank. The total number of microorganisms in this generation was 3.149 x $10^8$ mL and yeasts represent 96.92% of the total microbiota. In the sample B, 95.62% were *Saccharomyces cerevisiae* during the fifteenth generation. Our results showed that the optimal exchange of the yeast is in 15th generation. Subsequently, repeat the whole process but now with new yeast. These results confirmed our understanding of the relationship between Saccharomyces cerevisiae and contamination during the ethanol fermentation.

**Keywords:** microbiota; *Saccharomyces cerevisiae*; yeasts; generation

## INTRODUCTION

Many microorganisms produce ethanol and the efficiency amongst them varies greatly and eliminates the practical industrial usage of many **(Akinosho et al., 2015)**. Many microorganisms are able to produce ethanol, but the force between them is very different, and thus eliminating the practical industrial use. For the quality of the final product is therefore crucial to what extent, when and under what physical conditions this process participated **(Furdíková and Malík, 2016)**. Yeasts with guaranteed optimal control of fermentation processes have maximum yield. Final product is very clean with standard quality and from a natural source **(Pelikán and Sáková, 2001)**.

*Saccharomyces cerevisiae* is a yeast that has an extensive history in industrial fermentation and exhibits exceptional ethanol tolerance **(Ginley and Cahen, 2011)**. Clasification of *Saccharomyces cerevisiae*: *Fungi, Ascomycota, Saccharomycotina, Saccharomycetes, Saccharomycetidae, Saccharomycetales, Saccharomycetaceae, Saccharomyces* **(Mycobank Database, 2016)**.

Yeasts form cells of two types – large ones being of 5 to 12 microns and smaller ones of 3 to 10 microns. Cell size increases with age **(Čerňanský and Khun, 2011)**.

Although wild-type *Saccharomyces cerevisiae*, commonly known as baker's yeast, is unable to ferment xylose into ethanol **(Demake et al, 2013)**. But it is able to metabolize certain types of carbohydrates as glucose, fructose and sucrose **(Manikandan and Viruthagiri, 2010)**. It is most commonly used micro-organism in a wide range of processes to higher the recoveries of alcohol and productivity **(Demake et al, 2013)**.

The potential development of the life cycles is largely influenced by the availability of nutrients **(Casalone et al., 2005)**. Yeast can use for building cells only substances which penetrate cell membranes. If the mash does not contain enough nutrients, there is no sufficient multiplication of yeast **(Rob and Hrabě, 2009)**. An increased availability of phosphorus accumulated in phytic acid residues can constitute an additional source of this element which is necessary for the yeast growth **(Mikulski et al., 2014)**. Several researchers found that yeast extract, ammonium and calcium have a protective effect on growth or viability, and fermentation **(Khongsay et al., 2010)**. Calcium ions together with magnesium ions strengthen the yeast cell membrane thereby improving the resistance to

increasing concentrations of ethanol and high osmotic pressure **(Mikulski, 2014)**.

*Saccharomyces cerevisiae* is yeast that has an extensive history in industrial fermentation and exhibits exceptional resistence to ethanol **(Ginley and Cahen, 2011)** and high concentrations of sugar. This yeast is not expensive, produces low levels of by-products, is osmotolerant and presents high viability for recycling **(Muruaga et al, 2016)**. However, they have a high ability to yeast survive. Cell responds and adapts to ethanol, thermal and osmotic stress, by series of defence mechanisms, thereby increasing their flexibility and resilience. *Saccharomyces cerevisiae* has the ability to change their membrane structure that is more resistant to ethanol **(Dong et al., 2015 and Wang et al., 2015)**.

The main factors influencing the activity of the yeast during fermentation process are temperature, concentration of the substrate, pH and the presence of stimulating and inhibitory substances **(Pelikán and Sáková, 2001)**. Reduced availability of minerals in bound phytate can inhibit the metabolic activity of yeast **(Kumar, 2012)**. On the other hand a high level of copper in the mash can inhibit the activity of the yeast. This slows the fermentation and reduces the alcohol production **(Sun, Liu, 2015)**. Variations in temperature also have a significant impact on the viability of yeast. Thermal shock is much more severe than mild temperature fluctuations (**Amillastre et al., 2012)**. Lactic acid bacteria can inhibit the growth of yeast cells, because it competes with yeast nutrient and living space. This is influenced by the kinetics of glucose consumption, consequently reducing the final fermentation and the ethanol yield **(Dong, Lin, Li, 2015)**.

In the microbiological practice is often necessary to evaluate the growth and reproduction of microorganisms, which is very important especially in the fermentation, using fermentation processes for the balance. In the basic research cell count is used to assess the kinetics of yeasts growth, and to set the specific growth rate and the reproduction in the different stages of their development. The control laboratories use the cell count in the control of microbial contamination of different materials **(Veselá, 2004)**.

Our research was focused on the determination of the viability of yeast and changing the microbiota during recirculation of cells.

## MATERIAL AND METHODOLOGY

During our research, we investigated the plate count in two propagation reactors using the direct method. The aim of the study was the process of yeasts propagation and their viability, and observes how microorganisms are developed during the propagation in real conditions of production. The principle of direct method for the determination of cells, consists in direct cell counting by the microscope slide, therefore also known as the microscopic method. The plate count is determined in a unit volume. Promotional tanks contain water, grain stillage and grinding grain. Liquefaction of starch mash was performed in a continual reactor with a propeller stirrer. The liquefactions were done at constant temperature of 90 °C during 2 hour. This mixture is called mash and was liqufied using α-amylase and β–amylase. Then, the saccharification step was carried out by addition of glucoamylase which converted dextrins to glucose syrup obtained in the previous liquefaction step. In our conditions, there is no need for any pH adjustment, because it is provided by an appropriate ratio water and stillage. The pH value varied in the range of 4.7 – 5.0 and this is the optimal pH for used enzymes. Saccharification was carried out at pH 5.0 and temperature 61 °C for 1 hour. This mash is already sweet mash, because it contains only simple sugars with small amounts of unexpanded starch. Sweet mash had 18.8 sacharimeter degrees, and at this stage material was inoculated with yeast. We used dry alcohol yeast genus *Saccharomyces cerevisiae* with a trade name Safdistil C-70 from the company Fermentis. For the 25 $m^3$ volume of promotional tanks were used 9.5 kg of dry alcohol yeast. In order to rehydrate the dry yeasts optimally, 1 kg of yeasts in about 10 liters of mash (10 % dry matter) at about 30 – 33 °C were slowly stirred. After repeated stirring for 15 – 20 minutes significant foam was noticeable. It was a sign of the activity of the yeast. To ensure that the high viable cell count of Safdistil C-70 brings dry yeasts into the fermentation, the warm yeast suspension must not be stirred into a cold fermentation vessel. The temperature drop could cause the yeasts death. When the yeast suspension worked then was added to the propagation reactors while stirring. The pH was adjusted by adding sulphuric acid to 3.30. This partially prevents the proliferation of undesirable lactic microbiota.

Both propagation tanks were tested to the same specific conditions at the temperature 34 °C and pH 3.3. The volume of both tanks was 25 $m^3$ mash with saccharification of 18.8 degrees.

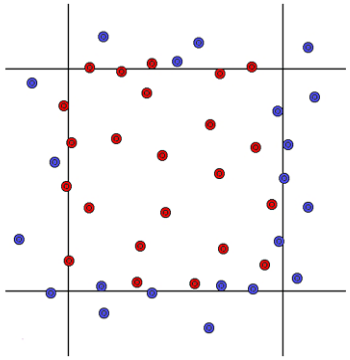
### The process of preparing generations

Sweet mash, which passes from saccharification tank was pumped into a propagation tank about volume 25 $m^3$ – tank A. The function of propagation tank was to multiply yeasts and bring them into an active proces, in which the yeasts are able to convert simple sugars to alcohol. 9.5 kg dried distilling yeasts were dissected in the mash and added to the pre-fermentation tank A. During filling the tank was all the time stirred to ensure a homogeneous mixture. Thus we prepared the first generation.

After 3 hours we took 2 samples from tank A and calculated the average shown in the chart below. The promotion process was checked by counting yeasts and their activity.

After 5 hours of propagation, 80% of the active yeasts were pumped from propagation tank to the fermentation one. At this point, the first generation of promoting is completed. The propagation tank was refilled for the same volume with fresh mash, by which we prepared a second generation of yeast. We again took two samples after 3 hours and the average we stated in the chart. After 5 hours of propagation, 80% was repumped into the fermentation tank. The propagation tank was refilled for the same volume of the fresh sweet mash. We repeated this process until the fifteenth generation. The same process was chosen for the preparation of promotional tank B.

30 samples with a 50 mL volume of mash were taken after three hours of propagation from propagation tank A and 30 samples from tank B, too. From each fifteenth



**Picture 1** Illustration of method for counting in the chamber.

generation, two samples were examined and the average of the measurement was reported in the charts. The same concept was used in the tank B, also. These samples were 70 times diluted (mixture of 0.5 mL sample with 34.5 mL distilled water). All experiments were performed in duplicate. The total numbers of microorganisms were counted in a Bürker chamber with a light biological microscope Bresser 40 – 1000x Researcher Trino, WF 10X, trinocular head, transmitted light.

Normal microscopic image should contain the yeast in the form of globose with part of them being in a budding cells stage. The image should contain a minimum of sticks or small balls of bacterial origin. We counted the cells touching the right and top sides of the circuit area picture 1.

Cells touching on the left and bottom sides were not counted (we counted red cell, not the blue cells).

In the Bürker counting chamber we had the number of cells in 1 $mL^{-1}$ determined using the relationship (1):

$$x = p \cdot r \cdot 2.5 \cdot 10^5 \quad (1)$$

where x is the plate count, p is the average number of cells in a small square, and r is the dilution. From the total number of microorganisms, we deducted the amount of unwanted microbiota and obtained the number of yeast, which we expressed in percentages.

All the data in this study were analyzed using Microsoft Excel 2010.

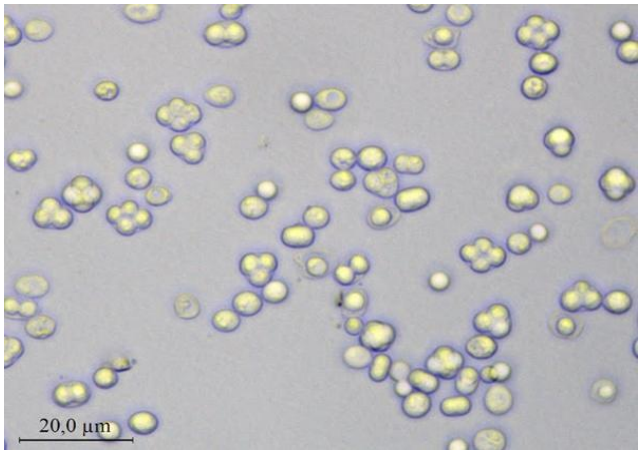
## RESULTS AND DISCUSSION

Fermentation process for the production of ethanol is based on the action of microbial cells (usually of some yeast cells) in a process called ethanol – alcohol fermentation. The pre-treatment of the raw material plays an important role for its concersion to ethanol **(Paschos, Xiros, Christakopoulos, 2015)**. As a general rule, ethanol fermentation starts with the yeast controlled hydrolysis, based on the fermentation of sugars **(Kasavi et al., 2012)**. The rate of fermentation (productivity) can significantly increase the concentration of cells, what is practically used in the recirculation of cells. Advantageously, the generated strains have increased tolerance to stress conditions **(Muruaga et al., 2015)**.

The results of the experiment confirmed that the highest number of yeast was observed in the 12ve and 13th generation. In the propagation tank A, the highest amount of yeast was in the 12th generation with a number 3.052 x $10^8$ mL (Table 1). From this generation on, the results showed only decrease in the count of yeast *Saccharomyces cerevisie.*

The total number of microorganisms and *Saccharomyces cerevisce* was in the first generation the same 0.555 . $10^8$ mL. It means that the propagation mass didn´t contain any unwanted microorganisms Table 1. There was gradual increase of unwanted miroorganisms in the subsequent generations.

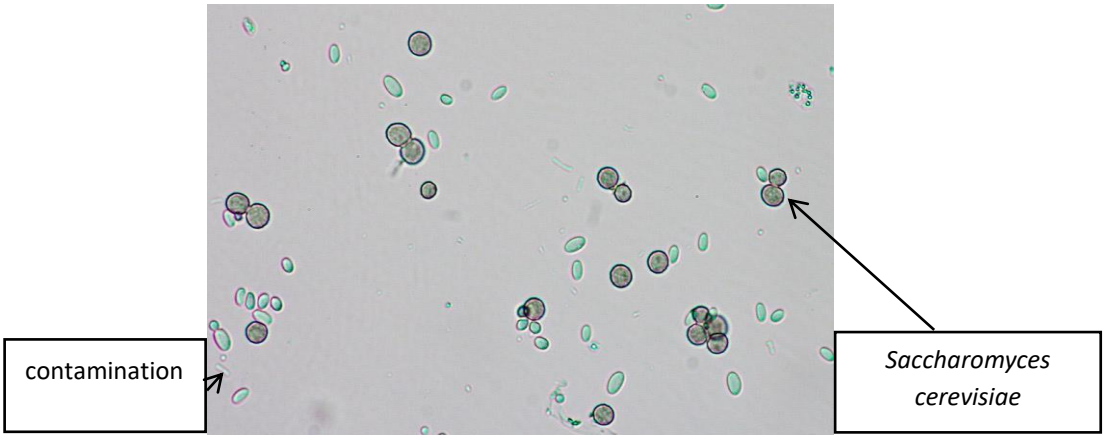
In the 15th generation, the total number of microorganisms was 3.2 x $10^8$ mL, but of these, only 3.043 x $10^8$ mL were already yeasts *Saccharomyces cerevisiae.* Microscopic




**Picture 2** Microbiota – *Saccharomyces cerevisiae* the first generation in propagation tank.

**Table 2** The observed indicators for propagation tank A, total number of microorganisms is the average of two measurements.

| Generation of yeasts | Total number of microorganisms x $10^8$ mL | *Saccharomyces cerevisiae* | |
|---|---|---|---|
| | | x $10^8$ mL | percentage of total number of microorganisms |
| **1.** | 0.623 | 0.622 | 99.84 |
| **2.** | 1.077 | 1.075 | 99.81 |
| **3.** | 1.590 | 1.586 | 99.75 |
| **4.** | 1.692 | 1.686 | 99.65 |
| **5.** | 1.815 | 1.808 | 99.61 |
| **6.** | 2.062 | 2.048 | 98.32 |
| **7.** | 2.220 | 2.200 | 99.10 |
| **8.** | 2.408 | 2.382 | 98.92 |
| **9.** | 3.062 | 3.027 | 98.86 |
| **10.** | 3.115 | 3.058 | 98.17 |
| **11.** | 3.214 | 3.138 | 97.64 |
| **12.** | 3.305 | 3.215 | 97.28 |
| **13.** | 3.347 | 3.250 | 97.10 |
| **14.** | 3.347 | 3.238 | 96.14 |
| **15.** | 3.381 | 3.233 | 95.62 |




**Picture 3** *Saccharomyces cerevisiae* and other microbiota in the last generation of the propagation tank B.

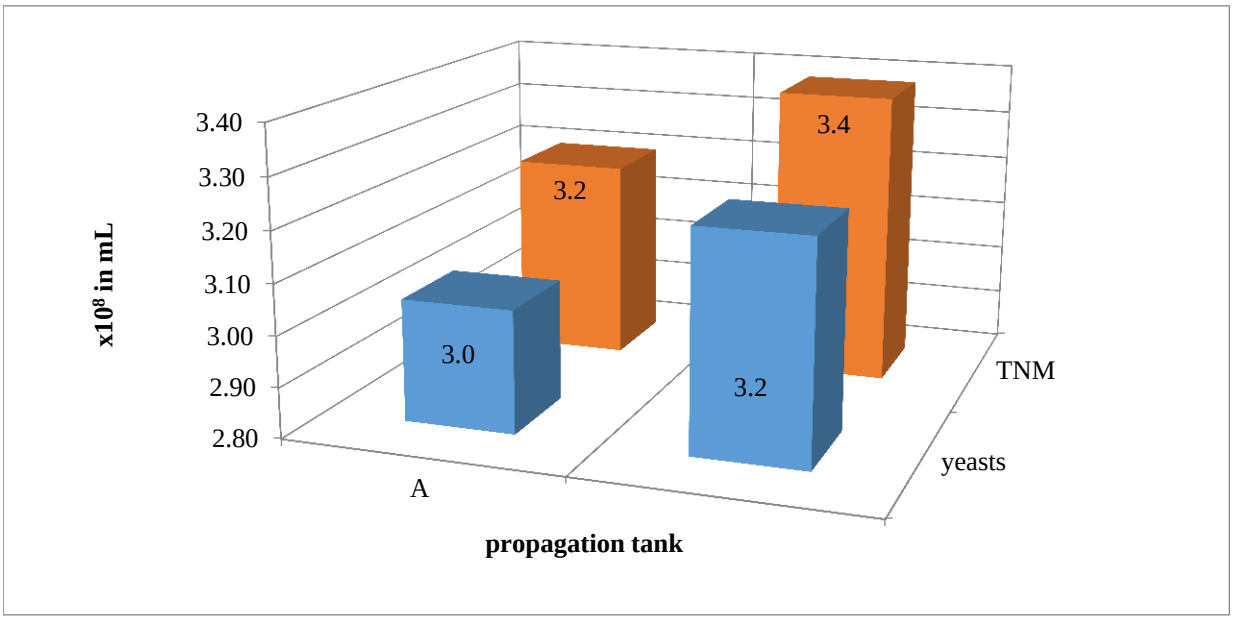



image contained only 95.09% of yeasts and this indicator signalled the need of total replacement of propagation. Undesirable microbiota in the microscopic slide was present in the form of sticks. During the industrial bioethanol fermentation, *Saccharomyces cerevisiae* cells are often stressed by bacterial contaminants, especially lactic acid bacteria. Generally, lactic acid bacteria contamination can inhibit *S. cerevisiae* cell growth through

secreting lactic acid and competing with yeast cells for micronutrients and living space **(Dong et al., 2015b)**. In our research, we did not identify representatives of undesirable microbiota, but only lactobacilli that could contaminate particularly ethanol production during the industrial production.

In the propagation sample B, the highest amount of yeasts was in the 13th generation, the number 3.233 x $10^8$ mL (Table 2). The first generation, in contrast to sample A did not contain 100% *Saccharomyces cerevisiae* yeasts but only 99.84%. It shows that already at this stage were observed unwanted microbiota. On the other hand, the 15th generation of the propagation sample B contained 95.62% of yeasts, which was 0.53% higher than in sample A. From the first to the thirteenth generation we observed the increase of total plate count and also yeasts. But the number of yeasts in next generation declined. The total number of microorganisms has increased but more undesirable microbiota began to multiply at the expense of yeasts.

## CONCLUSION

In spite of the fact that the promotion tanks A and B had the same volume and were prepared in the same way, there were visible slight variations in the results. The entire research process took place at the production factory, where it was influenced by several factors, in contrast to the laboratory conditions. The course of the promotion of both tanks was very similar. In the 13th generation there was a reproduction of yeast *Saccharomyces cerevisiae*, which was the predominant microbiota. Then it began to prevail in the undesirable microbiota. The modification of the microbiota was performed in acid-free reactor with sulphuric acid to prevent the spread of unwanted microbiota. The pH was controlled at 3.3 to 3.4. When the plate count of the microscopic image is less than 95% of yeast, it is necessary to reduce the pH of a propagation mash to pH 0.2. If such action would not improve conditions it is necessary to prepare a new mash with fresh yeasts. Preparation of the promotial tanks from 9.5 kg of dried alcohol yeast is sufficient, because it ensures that production runs for 6 days. The number of yeasts is sufficient for alcoholic fermentation lasting 70 hours. If there was no exchange in the 15th generation of promotion, unwanted microbiota would begin to multiply uncontrollably. This would lead to a reduction of ethanol yield.

It is necessary to constantly monitor the process of propagation as a healthy basis for the alcoholic fermentation. If a sufficient number and vitalality of yeast is ensured, it is possible to say that the fermentation process will proceed standard way. Therefore, it is important to control the constant propagation tanks.

According to the Regulation of the operating company Po-05 it is necessary to perform a full exchange of promotion tanks when the amount of the yeast is under 95%. In both cases, research has confirmed that the fifteenth generation of yeasts is already marginal and therefore it is necessary to perform a full exchange yeasts in promotional tanks. The figure 1 showed us the fifteenth generation of both promotions where we carried out a 100% replacement of yeast, to prevent further spread of unnecessary microbiota. The research was conducted at the factory, where the 100% equivalent conditions cannot be modulated. Therefore, there were observed two promotional tanks A and B. They were identical and the measurements were done in parallel. In the preparation of promotial tanks the same amount of yeast and the same successor saccharification was used. Therefore, the results of both observations were not completely consistent. On the other hand, the promotial tank B was confirmed by the measurements taken in the tank A.

Yeasts are among one of the most expensive components participating in the fermentation process. At the same time, they are the most important, because without the yeast, fermentation would not be possible and yeast is the component that affects the whole process of fermentation. Therefore, it is very important to check the contamination, vitality of yeasts and their count.

## REFERENCES


Akinosho, H., Rydzak, T., Borole, A., Ragauskas, A., Close, D. 2015. Toxicological challenges to microbial bioethanol production and strategies for improved tolerance. *Ecotoxicology*, vol. 24, no. 10, p. 2156-2174. https://doi.org/10.1007/s10646-015-1543-4 PMid:26423392

Amillare, E., Aceves-Lara, C. A., Uribellarrea, J. L., Alfenore, S., Guillouet, S. E. 2012. Dynamic model of temperature impact on cell viability and major product formation during feg-batch and continuous ethanolic fermentation in *Saccharomyces cerevisiae*. *Bioresource Technology*, vol. 117, p. 242-250. https://doi.org/10.1016/j.biortech.2012.04.013 PMid:22617033

Casalone, E., Barberio, C., Cappellini, L., Polsinelli, M. 2005. Characterization of *Saccharomyces cerevisiae* natural populations for pseudohyphal growth and colony morphology. *Research in microbiology*, vol. 156, no. 2, p. 191-200. https://doi.org/10.1016/j.resmic.2004.09.008 PMid:15748984

Čerňanský, S., Khun, M. 2011. *Utilization of technical microbiology in the fermentation and food industry (Využitie technickej mikrobiológie v kvasnom a potravinárskom priemysle)*. 1st ed. Bratislava, Slovakia : Univerzita Komenského. 250 p. ISBN 978-80-223-3135-7.

Demeke, M. M., Dietz, H., Li, Y., Foulquie.Moreno, M. R., Mutturi, S., Deprez, S., Den Abt, T., Bonini, B. M., Liden, G., Dumortier, F., Verplaetse, A., Boles, E., Thevelein, J. M. 2013. Development of a D-xylose fermenting and inhibitor tolerant industrial Saccharomyces cerevisiae strain with high performance in lignocelluloses hydrolysates using metabolic and evolutionary engineering. *Biotechnology for Biofuels*, vol. 6, no. 1, p. 89. https://doi.org/10.1186/1754-6834-6-89 PMid:23800147

Dong, S. J., Lin, X. H., Li, H. 2015. Regulation of *Lactobacillus plantarum* contamination on the carbohydrate and energy related metabolisms of *Saccharomyces cerevisiae* during bioethanol fermentation. *The International Journal of Biochemistry & Cell Biology*, vol. 68, p. 33-41. https://doi.org/10.1016/j.biocel.2015.08.010 PMid:26279142

Dong, S. J., Yi, CH. F., Li, H. 2015. Changes of *Saccharomyces cerevisiae* cell membrane components and promotion to ethanol tolerance during the bioethanol fermentation. *The International Journal of Biochemistry &Cell Biology*, vol. 69, p. 196-203.

https://doi.org/10.1016/j.biocel.2015.10.025
PMid:26515124

Furdíková, K., Malík, F. 2016. Methods of conducting alcohol fermentation (Spôsoby vedenia alkoholovej fermentácie). *Agroporadenstvo*.

Ginley, D. S., Cahen, D. 2012. *Fundamentals of Material for Energy and Environmental Sustainability*. UK : Cambridge University Press. 754 p. ISBN 1139502689, 9781139502689.

Kasavi, C., Finore, I., Lama, L., Nicolaus, B., Oliver, S. G, Oner, E. T., Kirdar, B. 2012. Evaluation of industrial *Saccharomyces cerevisiae* strains for ethanol production from biomass. *Biomass and Bioenergy*, vol. 45, p. 230-238. https://doi.org/10.1016/j.biombioe.2012.06.013

Khongsay, N., Laopaiboon, L., Laopaiboon, P. 2010. Growth and batch ethanol fermentation of *Saccharomyces* on sweet sorghum stem juice under normal and very high gravity conditions. *Biotechnology*, vol. 9, no. 1, p. 9-16. https://doi.org/10.3923/biotech.2010.9.16

Kumar, V., Sinha, A. K., Makkar, H. P. S., Becker, K. 2010. Dietary roles of phytate and phytase in human nutrition : A review. *Food chemistry*, vol. 120, no. 4, p. 945-959. https://doi.org/10.1016/j.foodchem.2009.11.052

Manikandan, K., Viruthagiri, T. 2010. Kinetic and optimization studies on ethanol production from corn flour. *International Journal of Chemical and Biological Engineering*, vol. 3, no. 2, p. 65-69.

Mikulski, D., Kłosowski, G., Rolbiecka, A. 2014. Effect of phytase application during high gravity (HG) maize mashes preparation on the availability of starch and yield of the ethanol fermentation process. *Applied Biochemistry and Biotechnology*, vol. 174, no. 4, p. 1455-1470. https://doi.org/10.1007/s12010-014-1139-0
PMid:25119551

Muruaga, M. L., Carvalho, K. G., Domínquez, J. M., de Souza Oliveira, R. P., Perotti, N. 2016. Isolation and characterization of *Saccharomyces* species for bioethanol production from sugarcane molasses: Studies of scale up in bioreactor. *Renewable Energy*, vol. 85, p. 649-656. https://doi.org/10.1016/j.renene.2015.07.008

MycoBank Database, 2016. Fungal Databases, Nomenclature and Species banks [online] s.a. [cit. 2017-02-01] Available at: http://www.mycobank.org/.

Paschos, T., Xiros, Ch., Christakopoulos, P. 2015. Simultaneous saccharification and fermentation by co-cultures of *Fusarium oxysporum* and *Saccharomyces cerevisiae* enhances ethanol production from liquefied wheat straw at high solid content. *Industrial Crops and Products*, vol. 76, p. 793-802. https://doi.org/10.1016/j.indcrop.2015.07.061

Pelikán, M., Sáková, L. 2001. *Quality and processing of plant products (Jakost a zpracovaní rostlinných produktů)*. České Budějovice, Czech Republic : ZF JČU. 233 p. ISBN 80-7040-502-3.

Rebroš, M., Rosenberg, M., Krištofíková, Ľ., Stloukal, R. 2005. Microbial production of fuel ethanol: bacteria or yeasts? (Mikrobiálay produkcia palivového etanolu: baktérie alebo kvasinky?). *Chemické listy,* vol. 99, no. 402-409.

Rob, O., Hrabě, J. 2009. *Non-alcoholic and alcoholic beverages (Nealkoholické a alkoholické nápoje)*. 1st ed. Zlín, Czech Republic : Univerzita Tomáše Bati. 129 p. ISBN 978-80-7318-748-4.

Sun, X., Liu, L., Zhao, Y., Ma, T., Zhao, F., Huang, W., Zhan, J. 2016. Effect of copper stress on growth characteristics and fermentation properties of *Saccharomyces cerevisiae* and the pathway of copper adsorption during wine fermentation. *Food Chemistry*, vol. 192, p. 43-52. https://doi.org/10.1016/j.foodchem.2015.06.107
PMid:26304318

Veselá, M. 2004. *Practice in general microbiology (Praktikum z obecné mikrobiologie)*. Brno, Czech Republic : FCH VUT. 3rd ed. 99 p. ISBN 80-214-2567-9.

Wang, Y., Zhang, S., Liu, H., Zhang, L., Yi, Ch., Li, H. 2015. Changes and roles of membrane compositions in the adaptation of *Saccharomyces cerevisiae* to ethanol. *Journal of Basic Microbiology*, vol. 55, no. 12, p. 1-10. https://doi.org/10.1002/jobm.201500300
PMid:26265555

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