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INFLUENCE OF LONG-TIME CONTINUOUS WINE FERMENTATION ON YEAST IMMOBILIZED ON FOAM GLASS

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Abstract. The purpose of this work was to compare the influence of long-term continuous wine fermentation on two yeast strains (*Saccharomyces bayanus* S.o./1 and *S. bayanus* S.o./1AD) immobilized on foam glass. Fermentation was carried out in the 4-columns fermentor for 3.5 months, and the must contained approximately 320 $g \cdot dm^{-3}$ total sugar. During the course of the process, the number of cells decreased both with the column number and with every subsequent month of the fermentor's work. The number of cells of the S.o./1AD strain was higher than that of the S.o./1 strain. After the end of fermentation, the number of viable S.o./1AD cells on the surface of carrier was higher than those of the S.o./1 strain. Yeast isolated from the carrier from the first column was characterized by the highest part of viable cells (43%). In the third and fourth columns, the amount of viable cells was similar for both strains (adequately 20% and 10%). Moreover, various shapes of cells isolated from the carrier after the end of fermentation were observed, for example: elongated, in the shape of a "pear", wrinkled and in the form of few connected cells. Yeast cells S.o./1 were more distorted.

Key words: yeast morphology, immobilized yeast, continuous fermentation, wine

INTRODUCTION

In continuous fermentation, technology, as well as choosing the right yeast strain, are both very important. In our previous research we stated that in long-term wine fermentation of high-sugar musts with the yeast immobilized on foam glass, it is more favourable to use the yeast *Saccharomyces bayanus* S.o./1AD. The application of this strain results in higher ethanol content and higher process efficiency in comparison with *S. bayanus* S.o./1 yeast [Bonin and Wzorek 2004]. The strain S.o./1 (previous name *Saccharomyces oviformis* strain Bratislava) is recommended in literature for continuous fermentation [Lipiec 1969].

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We should note however, that in continuous wine fermentation of high-sugar must, yeast is still subjected to high sugar and ethanol concentration, CO_2, fermentation by-products and anaerobic enviroment. The above conditions have an adverse effect on yeast morphology.

The aim of this research was to study the influence of long-term continuous wine fermentation of high-sugar fruit must on the yeast *Saccharomyces bayanus* S.o./1AD and *S. bayanus* S.o./1 immobilized on foam glass.

MATERIALS AND METHODS

Technological methods

The wine yeast *Saccharomyces bayanus* strains S.o./1 and S.o./1AD from the collection of pure cultures at the Department of Food Biotechnology and Microbiology were used. Yeast was immobilized on cubes of foam glass, the length of each side being about 1 cm.

Must was prepared from the apple juice (10°Be) obtained through the dilution of apple concentrate (70°Be). The content of juice in the must was 70%. In order to obtain high-sugar concentration (320 $g \cdot dm^{-3}$), sucrose was added. The medium was enriched with ammonium salt – $(NH_4)_2HPO_4$ in quantity of 0.5 $g \cdot dm^{-3}$. To prevent development of harmful microflora, $K_2S_2O_5$, was added. The amount of added $K_2S_2O_5$ was such so as to obtain an SO_2 content in the must equalling about 80 $mg \cdot dm^{-3}$. Continuous fermentation was carried out in the 4-column glass fermentor (working volume about 2.5 dm^{-3}) at 22 ±1°C. The time of flow through the bioreactor was 5 days, which was adequate for fruit wine fermentation. The total time of continuous fermentor work was 3.5 months.

Determination of the number of yeast cells

During the time of the fermentor's work, the number of viable yeast cells in fermented medium from each column of the bioreactor was determined by plate technique in a malt extract broth, after incubation at 28ºC for 48 hours [Duszkiewicz-Reihard et al. 2002]. After the end of the fermentor's work, the total number of cells and the number of viable cells on the surface of foam glass from different levels of each column (top, midle, bottom) was determined. For the purpose of the isolation of yeast cells from the carrier, 2 cubes of foam glass (in duplicate) from each level of the columns were placed in Erlenmeyer flasks with 100 cm^3 sterile 0.8% NaCl solution. The flasks were shaken at 160 rpm at 28°C for 2 hours to remove yeast from the carrier. The total number of cells was estimated by direct microscopic counting using a haemocytometer. The number of viable cells was determined by methylene blue staining and by plate technique in a malt extract broth after incubation at 28°C for 48 hours. The number of yeast cells was calculated on the surface of 1 cm^2 of foam glass.

Determination of yeast morphology

In order to determine changes of yeast morphology as a result of long-term fermentation, cells before fermentation and cells isolated from the carrier after the end of the

process were examined. After continuous fermentation 10 cubes of the carrier from each column were placed in Erlenmeyer flasks with 100 cm^3 sterile 0.8% NaCl solution. The flasks were shaken at 1600 rpm at 28°C for 2 h to remove yeast from the carrier. The yeast suspension was washed and shaken at 2500 rpm several times. Yeast cells were fixed in a solution of 2% glutaraldehyde, dehydrated in a graded series of ethanol and acetone concentrations and dried to the critical point in CO_2. The samples were sputter-coated with carbon and silver and observed with a Joel JSM-35 microscope.

Statistical methods

In most cases the obtained results were analysed statistically by Statgraphics Plus. Multiple analysis of variance (PI = 0.05) was applied. Tukey's multiple range test was used to determine significant differences (HSD) among the results.

RESULTS AND DISCUSSION

The number of yeast cells

Before beginning the process of continuous fermentation, pure culture of yeast, in the form of "yeast's mother", was added to each column of the bioreactor. The total number of yeast cells in this medium was 1.4-1.8 × 10^8 cells/cm^3. Differences in the number of cells among the columns were not statistically significant. The number of yeast cells in the "yeast's mother" should be between 6.0 × 10^7 and 1.6 × 10^8 cells/cm^3 [Wzorek and Pogorzelski 1998]. Also we can state, that at the beginning of the fermentation process, we used a sufficient amount of yeast cells in each column of the bioreactor.

During the process of continuous fermentation, the total number of yeast cells in the fermenting medium was examined every 30 days (Table 1). The number of yeast cells was decreasing with the column number. In the case of yeast *S. bayanus* S.o./1AD we found that there were an average 2.1 × 10^7 cfu/cm^3 of the medium in the first column and an average of 2.8 × 10^6 cfu/cm^3 in the fourth column during the course of the entire fermentation process. For the S.o./1 yeast strain the difference in the number of yeast cells among the columns was higher: in the medium from the first column numbered 1.4 × 10^7 cfu/cm^3 and only 1.2 × 10^5 cfu/cm^3 in the last column. Moreover, while the fermentor was working, the number of yeast cells in the fermenting medium was decreasing with every 30 day examination. For example in the case of the S.o./1AD strain, after 30 days of continuous fermentation, the amount of yeast cells, depending on column number, was 3.2 × 10^7-6.2 × 10^6 cfu/cm^3 and after 90 days, the number was 9.6 × 10^6--1.9 × 10^5 cfu/cm^3.

Lipiec [1969] and Lipiec and Krawczyk [1971] also observed a decrease of the number of yeast cells in subsequent tanks during continuous fermentation with free cells. Moreover, Juneja at al. [1986] determined, that the amount of cells in a fermenting medium depends on flow rate of the medium. These authors observed that in the case of a slow flow rate, the highest number of cells was in the first fermentor. When the flow rate was higher, the highest number of yeast cells was found in the third – last fermentor and the smallest number in the first.

Table 1. Influence of work time of fermentor on cells number in fermented medium – plates methods, cfu/cm^3
Tabela 1. Wpływ czasu pracy fermentora na liczbę komórek drożdży w fermentującym medium – metoda płytkowa, jtk/cm^3

Number of fermentor columns Klumna fermentora	Work time of fermentor, days – Czas pracy fermentora, dni							
	30		60		90		średnio – average	
	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1
I	3.2×10^7	2.9×10^7	2.2×10^7	7.0×10^6	9.6×10^6	7.2×10^6	2.1×10^7	1.4×10^7
II	1.5×10^7	5.1×10^6	8.2×10^6	1.5×10^6	3.7×10^6	4.8×10^6	8.9×10^6	3.8×10^6
III	1.0×10^7	7.2×10^5	6.7×10^6	6.3×10^5	2.1×10^6	9.0×10^5	6.4×10^6	7.5×10^5
IV	6.2×10^6	1.5×10^5	2.1×10^6	6.5×10^4	1.9×10^5	1.5×10^5	2.8×10^6	1.2×10^5
Average Średnio	1.6×10^7	8.7×10^6	9.7×10^6	2.3×10^6	3.9×10^6	3.3×10^6	–	–

When the fermentation was complete, the number and viability of yeast cells isolated from the foam glass was examined. In the case of the S.o./1AD strain the total number of yeast cells from the first, second and third columns was similar. In the first column we found 1.5×10^8 cells per 1cm^2 of the carrier (average for three levels) and in the third column an average of 1.2×10^8 cells. In the last column the number of cells was smaller – 8.8×10^7 (HSD 4.5×10^7). However, in the case of the strain S.o./1, the total number of cells was between 1.7×10^8-2.4×10^8 and differences both among columns and levels were within the margin of error (Table 2).

Table 2. Total cells number/1 cm^2 of carrier area, after the end of fermentor's work – direct counting
Tabela 2. Ogólna liczba komórek/1 cm^2 powierzchni nośnika po zakończeniu pracy fermentora – metoda liczenia bezpośredniego

Level Poziom	I column Kolumna I		II column Kolumna II		III column Kolumna III		IV column Kolumna IV	
	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1
Top Góra	1.6×10^8	1.9×10^8	1.6×10^8	2.9×10^8	1.4×10^8	1.7×10^8	9.9×10^7	1.4×10^8
Middle Środek	1.5×10^8	1.5×10^8	1.5×10^8	1.6×10^8	9.6×10^7	1.6×10^8	8.9×10^7	1.4×10^8
Low Dół	1.5×10^8	1.7×10^8	1.4×10^8	1.4×10^8	1.4×10^8	1.9×10^8	7.4×10^7	1.8×10^8
Average Średnio	1.5×10^8	1.7×10^8	1.4×10^8	2.0×10^8	1.2×10^8	1.7×10^8	8.8×10^7	2.4×10^8

S.o./1AD – NIR between columns – 4.5×10^7. PI number of cells between columns – 0.44.
S.o./1 – PI number of cells between columns – 1.0. Pl of number of cells between columns levels – 0.20.
S.o./1AD – NIR między kolumnami – $4{,}5\times10^7$. PI liczby komórek między poziomami – 0,44.
S.o./1 – PI liczby komórek między kolumnami – 1,0. PI liczby komórek między poziomami kolumn – 0,20.

The above results were obtained by the use of the counting method. Similar tendencies were observed by using the plate technique. In the case of the S.o./1AD strain the number of yeast cells isolated from the carrier from the third and fourth columns was significantly smaller in comparison with the number of cells from the foam glass from the first column. In the case of the S.o./1 strain these differences in the number of cells number were not observed. Moreover, in the first and in the second columns the number of viable cells was higher in the case of S.o./1AD than in the case of S.o./1. For instance there were 3.2×10^7 cfu of S.o./1AD yeast present per 1 cm^2 of the carrier from the first column, compared with 8.2×10^6 cfu of S.o./1 yeast. In the last column the number of viable cells on the foam glass was similar in the cease of both strains and amounted to 2.8×10^6 cfu/cm^2 and 2.7×10^6 cfu/cm^2 respectively (Table 3).

Table 3. Cells number of yeasts on carrier area, after the end of fermentor's work – plates methods, cfu/cm^3
Tabela 3. Liczba komórek drożdży na powierzchni nośnika po zakończeniu pracy fermentora – metoda płytkowa, jtk/cm^2

Level Poziom	I column Kolumna I		II column Kolumna II		III column Kolumna III		IV column Kolumna IV	
	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1
Top Góra	2.6×10^7	6.5×10^6	2.3×10^7	1.1×10^7	4.6×10^6	3.0×10^6	1.5×10^6	3.3×10^6
Middle Środek	4.4×10^7	7.2×10^6	1.2×10^7	3.6×10^6	4.4×10^6	4.2×10^6	1.8×10^6	1.5×10^6
Low Dół	2.5×10^7	4.2×10^6	1.4×10^7	1.0×10^7	4.6×10^6	3.3×10^6	5.1×10^6	3.2×10^6
Average Średnio	3.2×10^7	6.8×10^6	1.6×10^7	8.2×10^6	4.5×10^6	3.5×10^6	2.8×10^6	2.7×10^6

S.o./1AD – NIR between columns – 2.0×10^7. Pl of number of cells between levels – 0.86.
S.o./1 – Pl of number of cells between columns – 0.05. PI of number of cells between columns levels – 0.35.
S.o./1AD – NIR między kolumnami – $2{,}0\times10^7$. PI liczby komórek między poziomami – 0,86.
S.o./1 – PI liczby komórek między kolumnami – 0,05. PI liczby komórek między poziomami kolumn – 0,35.

The number of yeast cells on the porous carrier depends, among other things, on the number and size of the pores. Paterczyk et al. [1992] established that there were from 1.3 to 4.6×10^6 cells on 1 g of porous glass, depending on porosity. When the porosity of a carrier was 50% and the diameter of the pores was 250-500 μm, the number of cells on this carrier numbered 4.6×10^6/g. However when the pores's diameter was smaller, in the case 5-70 μm, the number of yeast cells decreased to 2.6×10^6/g. Shinonaga et al. [1992] determined that there were 1×10^9 yeast cells per 1 cm^3 of cross-linked chitosan beads and 2.2×10^9 cells per 1 cm^3 of the "SIRAN" glass carrier. Szajani et al. [1996] found 7.3×10^7 cells per 1 cm^3 of preformed cellulose beads.

In our present research, the part of viable cells of the S.o./1AD strain, an average from the three levels, of each of the four columns numbered: 43, 34, 20 and 11% respectively (HSD 6.2). In the case of the S.o./1 strain in the first and in the second columns the part of viable cells was similar – about 30%. In the third column the number was 20% and in the fourth column – the number was the lowest – 10% (HSD 6.3; Table 4).

Table 4. Part of viable cells on carrier from the following columns of fermentor after the end of fermentors work, %
Tabela 4. Udział komórek aktywnych życiowo na nośniku z kolejnych kolumn fermentora po zakończeniu fermentacji, %

Level Poziom	I column Kolumna I		II column Kolumna II		III column Kolumna III		IV column Kolumna IV	
	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1	S.o./1AD	S.o./1
Top Góra	46	26	38	32	21	19	11	10
Middle Środek	43	27	31	28	17	15	12	9
Low Dół	41	34	33	30	20	26	11	11
Average Średnio	43	29	34	30	20	20	11	10

HSD between columns: strain S.o/1AD – 6.2, strain S.o/1 – 6.3.
NIR między kolumnami: szczep S.o./1AD – 6,2, szczep S.o/1 – 6,3.

Lipiec [1969] and Lipiec and Krawczyk [1971] observed an increase of the part of unviable yeast cells in subsequent tanks after the end of continuous fermentation of apple wine with free cells. Godia et al. [1987] determined that after 20 days of continuous fermentation in a 1-column bioreactor, the viability of yeast entrapped in carageenan numbered 82% on the bottom level of the column and 70% on the top level. Pilkington et al. [1999] found that after 2 months of continuous fermentation, yeast viability was 76% in the case of the cells entrapped in kappa-carageenan gel and 93% in the case of the free-cell process. After 6 months of continuous fermentation, yeast viability decreased to under 50% in the first process and to 92% in the second. The above authors did not use high-sugar musts in their experiments. Roukas [1996] found that increasing the initial sugar concentration from 200 to 300 $g \cdot dm^{-3}$ resulted in a significant decrease of the number of yeast cells immobilized in Ca-alginate beads compared to the number of cells in the must containing 150 $g \cdot dm^{-3}$.

In this experiment we used must containing about 320 $g \cdot dm^{-3}$ sugars and *S. bayanus* S.o./1AD wine yeast strain. This strain was obtained from the S.o./1 strain by controlled selection during the processes of long-term adaptation to high sugar concentration and this S.o./1AD strain proved to be resistant to high sugar and ethanol content [Bugajewska and Wzorek 2000]. This is likely the reason for both: the higher number of the S.o./1AD yeast cells and the higher viability of these cells, compared to the S.o./1 strain as observed in the first and second columns of the bioreactor. Moreover, in the process of continuous fermentation in the high-sugar medium, it is more favourable to use the S.o./1AD yeast strain than the S.o./1 strain, because this results in higher ethanol concentration in fruit wine and more efficient process [Bonin and Wzorek 2004].

We should note, that when the yeast cells (isolated from the carrier) were observed under an electron-scanning microscope, at the end of fermentation, we found crushed pieces of foam glass with yeast attached (Fig. 1). The yeast cells covered the carrier in several layers. An especially high amount of yeast was observed in the hollows, from

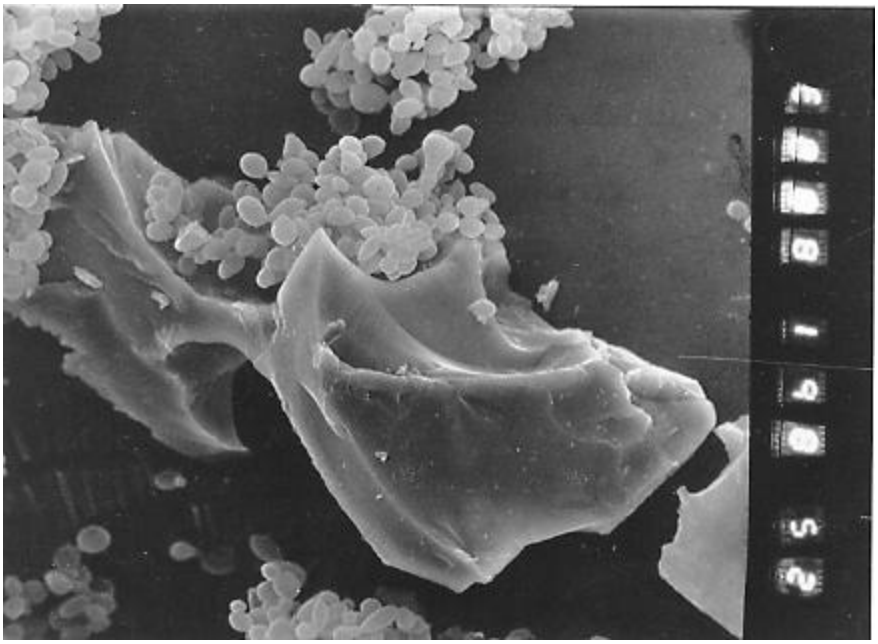

Fig. 1. Yeasts cells in the hollow of foam glass, magnification × 860
Rys. 1. Komórki drożdży w zagłębieniach szkła piankowego, powiększenie × 860

which it was more difficult to remove the cells in all the preparation procedures. This shows that the yeast cells were strongly attached to the foam glass. Therefore, both the number of yeast cells and yeast viability were commensurate with those cells that were isolated from the carrier after 2 hours of shaking. As we mentioned above, we determined a signiticant amount of yeast cells in the medium from each column, during the entire fermentation process. This shows a strong growth of yeast. The confirmation of this is the existence of budding cells as well as cells with bad scars, in cells that were isolated from the carriers from the first and the second columns.

Morphology of yeast cells

Each column, before fermentation, was filled with starter yeast culture, that did not show morphological changes. Cells were ovoid, single or budding and had many bad scars (Fig. 2).

Afer 3.5 months of continuous fermentation, we observed morphological changes of some yeast cells that were isolated from the foam glass. Moreover, we found the differences in morphology of yeast from each column's carrier as well as differences between both strains.

In the case of the S.o./1AD strain, in the first column there were many budding cells and cells with bud scars. Certain cells did not have normal shapes. We observed elongated and pear-like cells. However, these changed cells occurred only in some daugther cells, these cells were firmly bound to the mother cells (Fig. 3). In some instances these

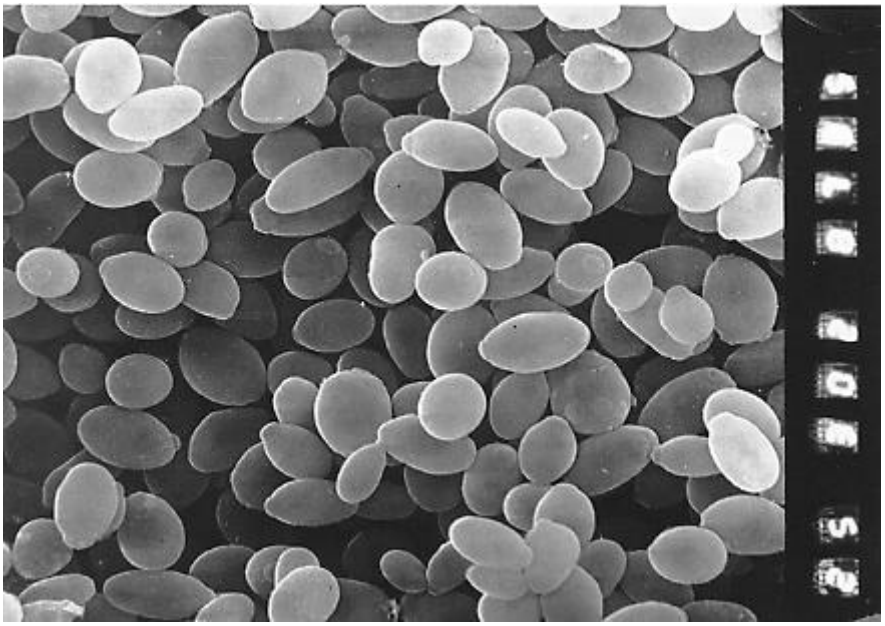

Fig. 2. Yeasts cells before immobilization, magnification × 3000
Rys. 2. Komórki drożdży przed immobilizacją, powiększenie × 3000

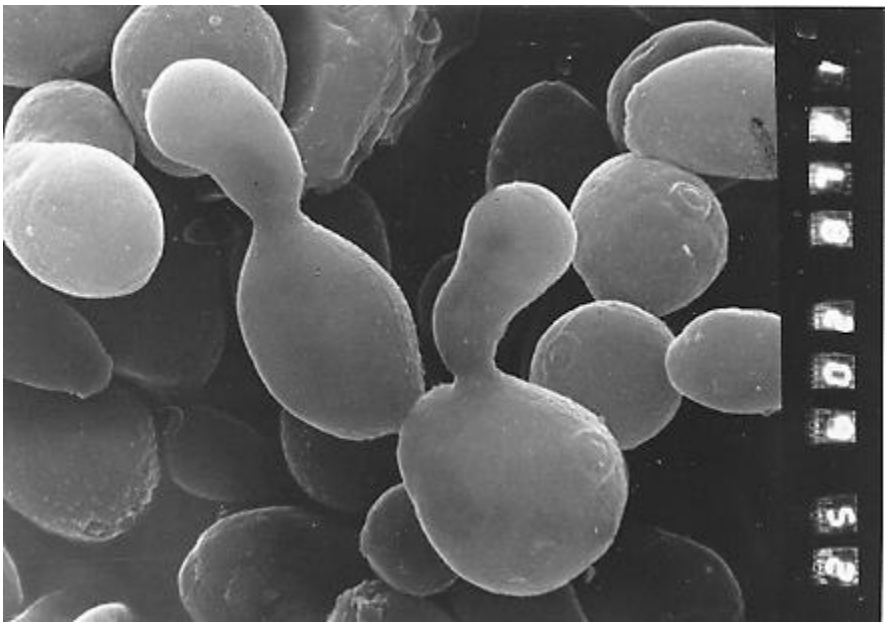

Fig. 3. Yeast S.o./1AD from the first column – bad scars and daughter cells in "pear-like" form, magnification × 6000
Rys. 3. Drożdże S.o./1AD z I kolumny – blizny po pączkowaniu i gruszkowate komórki potomne, powiększenie × 6000

firmly connected, changed daugther cells had buds on them and this resulted in forms of "pseudohypha". In the second column we observed cells that were still budding, cells with bud scars as well as elongated cells and "pseudohypha". However, pear-like cells were very rarely present. In the third and fourth columns we found a lower number of budding cells and cells with scars. Connected cells were often present, but their shapes were very deformed (Fig. 4). In the second, third and fourth columns we observed wrinkled or folded cells.

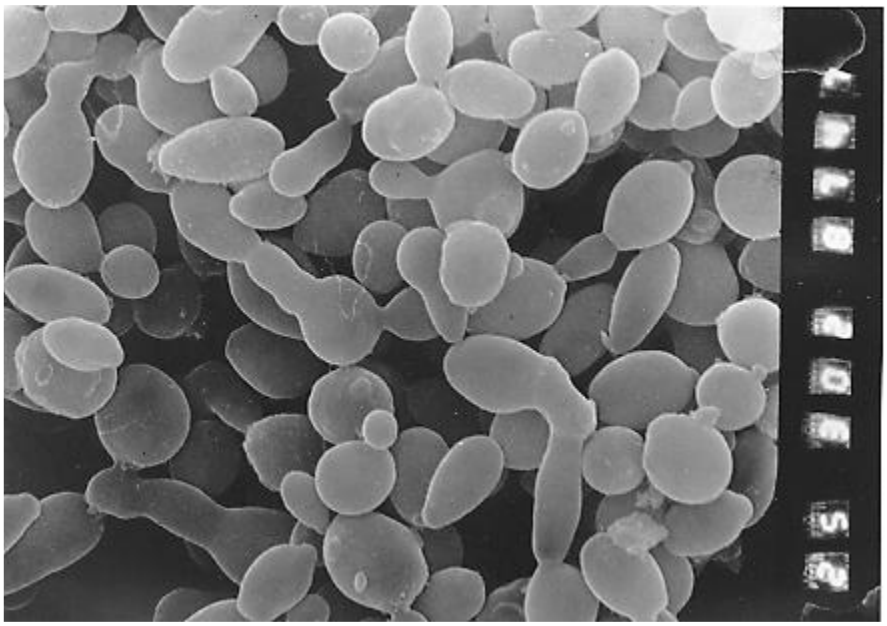

Fig. 4. Yeast S.o./1AD from the third column – diversity of cell forms, magnification × 3000
Rys. 4. Drożdże S.o./1AD z kolumny III – zróżnicowane kształty komórek, powiększenie × 3000

In the case of the S.o./1A strain, as in the case of the S.o./1AD strain, the changed shapes occurred only in some daugther cells. In all columns we observed wrinkled or folded cells, with an irregular, rough surface (Fig. 5). However, the amount of these cells varied depending on the column number. In the first column, we found the highest number of pear-like cells and the smallest number of wrinkled cells. In the second column, wrinkled cells were often observed, but in comparison with the first column, more cells had bad scars. Shapes of daugther cells were sometimes elongated or had a pear-like form, and mother cells produced two new buds at the same time (Fig. 6). In the two last columns, the highest number of yeast cells was wrinkled. Pear-like cells were observed rarely, however very elongated and deformed cells were more common (Fig. 7).

A comparison of the morphology of both strains found, that in the case of the S.o/1AD yeast, the highest number of budding cells and cells with bud scars were in the first column, however, in the case of the S.o./1 yeast, the highest number was in the second column.

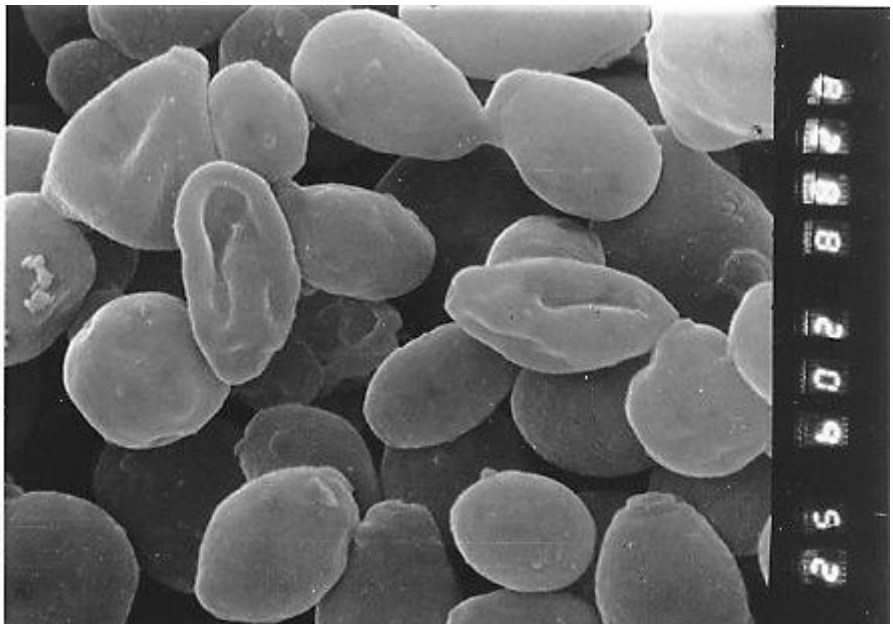

Fig. 5. Yeast S.o./1 from the fourth column – magnification × 6000
Rys. 5. Drożdże S.o./1 z kolumny IV – komórki zapadnięte, powiększenie × 6000

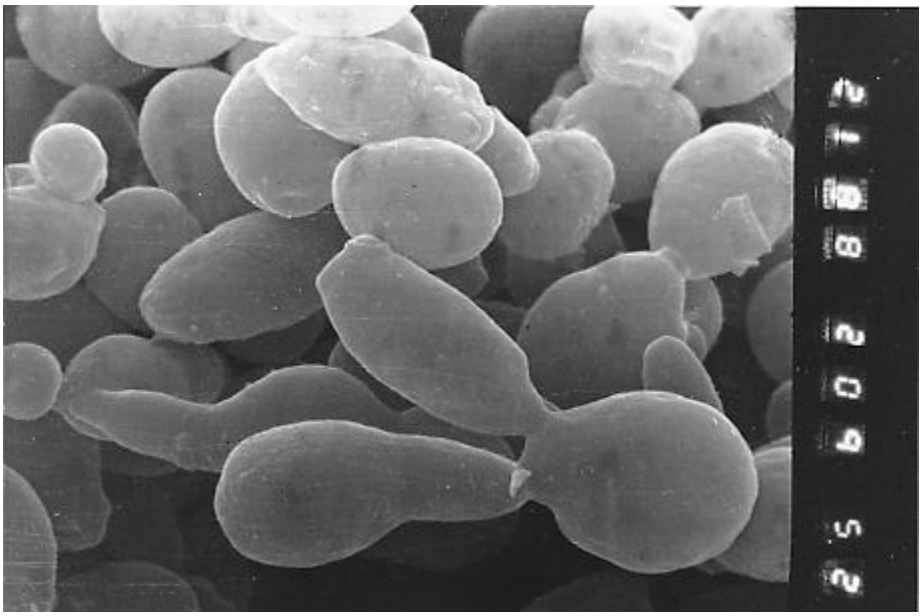

Fig. 6. Yeast S.o./1 from the second column – deformed cells and bud scars, magnification × 6000
Rys. 6. Drożdże S.o./1 z II kolumny – komórki zdeformowane i blizny po pączkowaniu, powiększenie × 6000

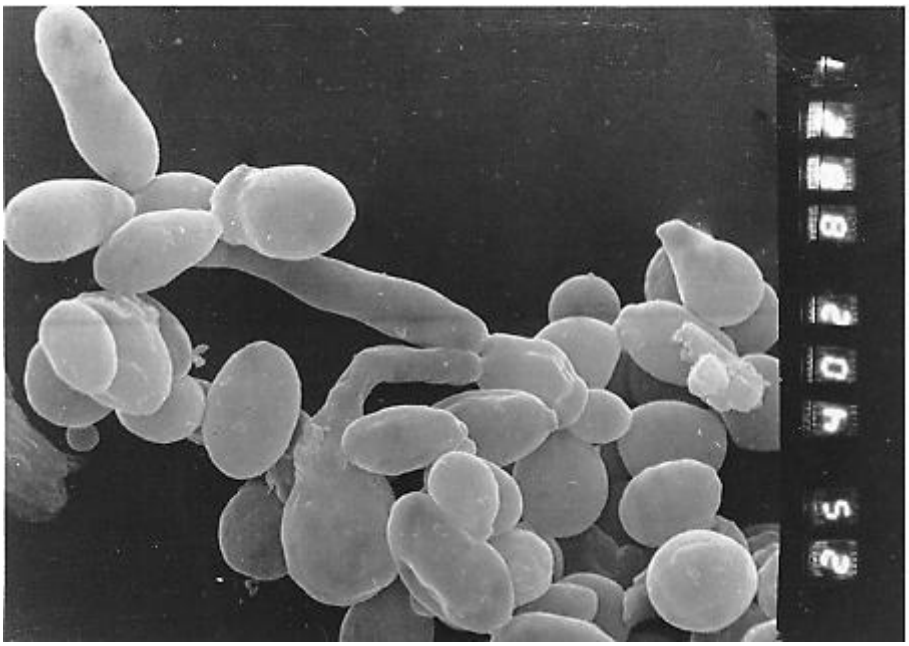

Fig. 7. Strongly deformed cells of yeast S.o./1 from the third column, magnification × 4000
Rys. 7. Silnie zdeformowane komórki drożdży S.o./1 z III kolumny, powiększenie × 4000

Moreover, as we mentoined above, in these columns we found the highest number of cells in the fermented medium during the time of fermentor's work, and the highest part of viable cells on the carrier after the end of the fermentor's work. In subsequent columns growth occurred in some unviable cells. Therefore the number of cells able to produce buds was smaller, and during microscopic observation we found few budding cells, "pseudohypha" as well as cells connected in the form of chains. The S.o./1AD strain cells were characterized by a higher number of budding cells and cells with bud scars and lower number of very deformed and wrinkled cells than those of the S.o./1 strain. However, we could not determine the cause of such different deformations of cells. Most likely, these morphological changes were the effect of simultaneous, long-term, continuous influence of ethanol, sugar, fermentation by-products, CO_2 and lack of oxygen. The observed changes of yeast morphology could be a result of distruptions of the cell function, or mutations.

In the case of the S.o./1AD strain, the use of computer image analysis enabled us to determine that the chain-like forms formed cells that did not separate after dividing. These yeast cells were firmly connected by their common cell wall. In some cases, the cell wall and cell membrane were not completely closed, and because of that, the cells also had a common cytoplasm [Bonin and Wzorek 2003]. Zalewski and Buchholz [1996] established that bud cells are characteristic during the exponential phase. In this time, aggregates of three or four cells are formed, and in both forms, an association of double cells or mother cells with two daugther cells, is observed. On the other hand, in the stationary phase, the majority of the cells are single cells. Singh et al. [1998] ob-

served that free yeast cells of *Saccharomyces cerevisiae* formed a pseudohypha of 5 or 6 cells when both mother and daughter cells produced buds and all of the cells remained firmly attached. It was caused by the activation of the DNA synthesis regulatory protein without the activation of the regulatory protein for bud site selection, as the cells respond to the nutrient deficiency signal. Alteriis et al. [2001] found slow growth of yeast entrapped in oxystarch-hardened gelatin discs. Moreover, cells would often divide asimetrically.

Furthermore, on the basis of our previous research we can assume that wrinkled or folded cells, are the same cells, which during computer image analysis were characterized by an irregular cell wall area. Martinez-Rodriguez et al. [2001] observed wrinkled or folded yeast cells after few months of aging in wine, using scanning electron microscopy. The above authors report that during autolysis, the cell membrane was destroyed. Cell membrane is the osmotic barrier, therefore turgor was lost, leading to a decrease in the cells diameter. The cell wall was not destroyed and the cell surface became rough – either wrinkled or folded.

CONCLUSIONS

1. Number of yeast cells in fermented medium decreased both with the column number and with every months of the fermentor's work. During all the time of the process, in fermented medium higher number of S.o./1AD yeast cells than S.o./1 was observed.

2. After the end of the fermentor's work, the total number of S.o./1 yeast cells was similar in all columns. In the case of the S.o./1AD strain we found less cells in the last column in comparison with the first and the second columns.

3. Yeast cells of S.o./1AD isolated from the carrier from the first column were characterized by the highest part of viable cells (43%). In the case of yeast isolated from the carrier from the third and the fourth columns, the part of viable cells was similar, for both strains: 20% and 10% respectively.

4. Long-lasting time of fermentor's work negatively influences yeast morphology. Yeast cells isolated from the carrier were morphologically changed, however cells of the S.o./1 yeast strain were more distorted.

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WPŁYW DŁUGOTRWAŁEJ CIĄGŁEJ FERMENTACJI WINIARSKIEJ NA DROŻDŻE IMMOBILIZOWANE NA SZKLE PIANKOWYM

Streszczenie. Celem pracy było porównanie wpływu długotrwałej, ciągłej fermentacji winiarskiej na immobilizowane na szkle piankowym drożdże *Saccharomyces bayanus* S.o./1 oraz *S. bayanus* S.o./1AD. Fermentacje prowadzono w 4-kolumnowym fermentorze przez ok. 3,5 miesiąca, a nastawy zawierały ok. 320 g·dm^{-3} cukrów ogółem. W czasie pracy fermentora stwierdzano zmniejszanie się liczby komórek w fermentującej cieczy w kolejnych kolumnach i miesiącach pracy fermentora, a liczba komórek S.o./1AD była większa w porównaniu z S.o./1. Po zakończeniu fermentacji liczba komórek aktywnych

życiowo na powierzchni nośnika była większa u szczepu S.o./1AD. Najwyższym udziałem komórek aktywnych życiowo (43%) charakteryzowały się drożdże S.o./1AD z kolumny I. W kolumnie III i IV udział komórek aktywnych życiowo był podobny u obu szczepów, wynosząc odpowiednio 20% i 10%. Ponadto obserwowano różnice morfologii niektórych komórek wyizolowanych z nośnika: m.in. drożdże wydłużone, gruszkowate, pomarszczone, kilkukomórkowe formy połączone. Bardziej zniekształcone były komórki drożdży S.o./1.

Słowa kluczowe: morfologia drożdży, drożdże immobilizowane, fermentacja ciągła, wino

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