# Selection of Yeast Strain *Kluyveromyces marxianus* for Alcohol and Biomass Production on Whey

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This paper emphasizes the suitability of five different strains of yeast *Kluyveromyces marxianus* for alcoholic fermentation on deproteinized whey. The selection of yeast strains was performed at different cultivation conditions: temperatures were 30 °C, 34 °C and 37 °C, respectively, and the concentration of lactose in the substrate varied from 5 % to 15 %. Cultivations were carried out in aerobic conditions on a rotary shaker, at pH 4,5-5,0. During cultivations the kinetics of alcoholic fermentations was analysed. Satisfactory results were achieved almost with all the yeast strains, but the best results were gained with *K. marxianus* VST 44 and ZIM 75, respectively. On the other hand, 34 °C seemed to be the optimal temperature for both strains. To optimize the process fed-batch cultivations were performed with the chosen strains of *K. marxianus* at 34 °C in aerobic/anaerobic conditions. The process ended after 12-14 hours. At the end of the process the biomass yield reached 10 g $L^{-1}$ and the ethanol fraction was $\phi$ = 7,31 %.

The results achieved in all experiments showed that the selected yeast strains, *K. marxianus* VST 44 and ZIM 75, could be succesfully used in the ethanol and yeast biomass production on whey.

*Key words:*
*Kluyveromyces marxianus*, whey, fermentation, ethanol, biomass, cultivation conditions

## Introduction

The world production of cheese has reached 11–12 × $10^6$ tons per year. The total amount of liquid whey produced in these processes is cca $10^8$ tons.[1] There are two kinds of whey, sweet and acid. This valuble by-product of cheese manufacture contains many of the nutrients from milk and can be almost completely utilized for different purposes (ethanol, yeast biomass, lactid acid).[2]

The problem of lactose utilization from milk or whey has been well described in literature.[3] From these data it is possible to see that there are many people, even whole populations, who are not able to hydrolise lactose, due to the lack of $\beta$-galactosidase in their digestive tract. The problem is mostly related to older people. Almost the same problem is the utilization of lactose from whey (or milk) by grown pigs, while young pigs can consume whey or milk. These problems have brought about many limitations in using whey as food or feed. An alternative of using whey is to hydrolyse its lactose by the enzyme lactase to a glucose and galactose.[4,5] The hydrolysis is very useful for the improvement of the processes for the production of ice cream or baby food. It can also be very interesting for the production of additives for human and animal food from whey.[3]

However, in spite of these advantages, most of the whey is discarded as waste in many countries around the world and is the cause of several pollution problems because of its high biological oxygen demand (BOD) which is 35–40·$10^{-9}$ kg $m^{-3}$.[1] BOD is due mainly to the lactose mass fraction which is usually 4,5–5 %. This lactose content is also present in the whey, it permeates after recovery of whey proteins by ultrafiltrations.[6]

The main problems of whey utilization are the economical solutions. Of course, the worst solution is the waste water treatment. It could be too expensive, particularly in the case of small cheese plants. The production of ethanol on original whey ($w$ = 4–5 % lactose) also demands high amount of energy because of the low ethanol yield.[7] The rentability of these processes is low, as well.

Since the price of molasses has almost doubled last two years, the substitution of this basic raw material in the production of ethanol and fodder yeast by another raw material which would justify the rentability of the process, is increasingly being considered. One of these raw materials, which has still not been used enough, is certainly whey. Besides the basic sugar lactose, whey also contains vitamins and minerals which improve the physiological

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activity of yeast cells. To achieve a good utilization of lactose from whey, it is especially important to choose a strain of yeast with suitable physiological characteristics.

For this reason, the goal of this work has been to test several strains of the yeast *K. marxianus* with the purpose to choose a strain that will, because of its physiological characteristics, be suitable for the production of alcohol and fodder yeast from deproteinized and concentrated whey.

## Materials and Methods

### Microorganisms

For the alcoholic fermentation of whey five strains of yeast *Kluyveromyces marxianus*, were used. *Kluyveromyces marxianus* strains VST44 and CC4 were taken from the culture collection of the Faculty of Food Technology and Biotechnology, University of Zagreb. *Kluyveromyces marxianus* strains ZIM75 and ZIM1867, were purchased from culture collection of Biotechnological Faculty, University of Ljubljana and *Kluyveromyces marxianus* DS12 was acquired from Faculty of Sciences, University of Zagreb.

The yeasts were maintained on a solid yeast medium (YM) containing (in g $L^{-1}$); lactose, 20; Bacto peptone, 10; yeast extract, 5; agar, 20.

### Inoculum preparation

For the preparation of inoculum, the yeast strains were reinoculated from agar slants into the test tubes containing each 10 mL of sterile liquid YM and incubated in a thermostat at 30 °C for 24 h. Then the sterile 200 mL of liquid YM in 500 mL Erlenmeyer flasks were inoculated with 5 % of the obtained inocula and the flasks were shaken on a rotary shaker by 150 rpm at 30 °C for 24 hours.

### Batch Process and Fed-batch process

For the production of ethanol a medium of diluted, dried whey was prepared. The composition of the media for alcoholic fermentation was (in g $L^{-1}$): Lactose, 50-150; yeast extract, 3.0; $(NH_4)_2HPO_4$, 1.0; $(NH_4)_2SO_4$, 2.0; $MgSO_4$, 0.5.

Batch fermentation was performed in Erlenmeyer flasks in an aerobic (shaker, OTR 9.6 mmol $O_2L^{-1}h^{-1}$) condition, at different temperatures (30, 34, 37 °C). Batch and fed-batch cultivations were carried out in 2 L bioreactor (working volume 1,5 L) with temperature and pH regulation. In the first 5 hours, i.e., until the sugar concentration dropped to one half of the starting value, the cultivation was conducted without feeding of substrate with the air flow rate of 5-10 $LL^{-1}h^{-1}$. Then the feeding started and the process was anaerobic. For feeding sterile clarified whey with sugar mass concentration of $\gamma$ = 250 g $L^{-1}$ was used. The feeding rate depended on the rate of sugar consumption during the fermentation process and it was between 150-200 mL $h^{-1}$ in all runs. The fed-batch process was run for 14 hours, and the feeding of substrate was stopped one hour earlier.

### Statistic procedure

The analysis of variance was used to establish the effect of different temperatures and three levels of lactose concentrations on the kinetics of alcoholic fermentation with 5 strains of *Kluyveromyces marxianus*.

## Results and Discussion

Biomass and ethanol yields, after cultivation with 5 %, 10 % and 15 % of lactose into the media by 5 yeast strains of *Kluyveromyces marxianus* in aerobic conditions at 30 °C, 34 °C and 37 °C, are illustrated in Figures 1 and 2, respectively.

In figures 1 and 2 it is possible to see that all yeast strains have fermented lactose at different mass fraction (5%-15%) in whey media. The strain *Kluyveromyces marxianus* DS12 in the same conditions have given the lowest yields. However, it seems that two yeast strains of *Kluyveromyces marxianus,* ZIM75 and VST44, have given the best results for alcoholic fermentation.

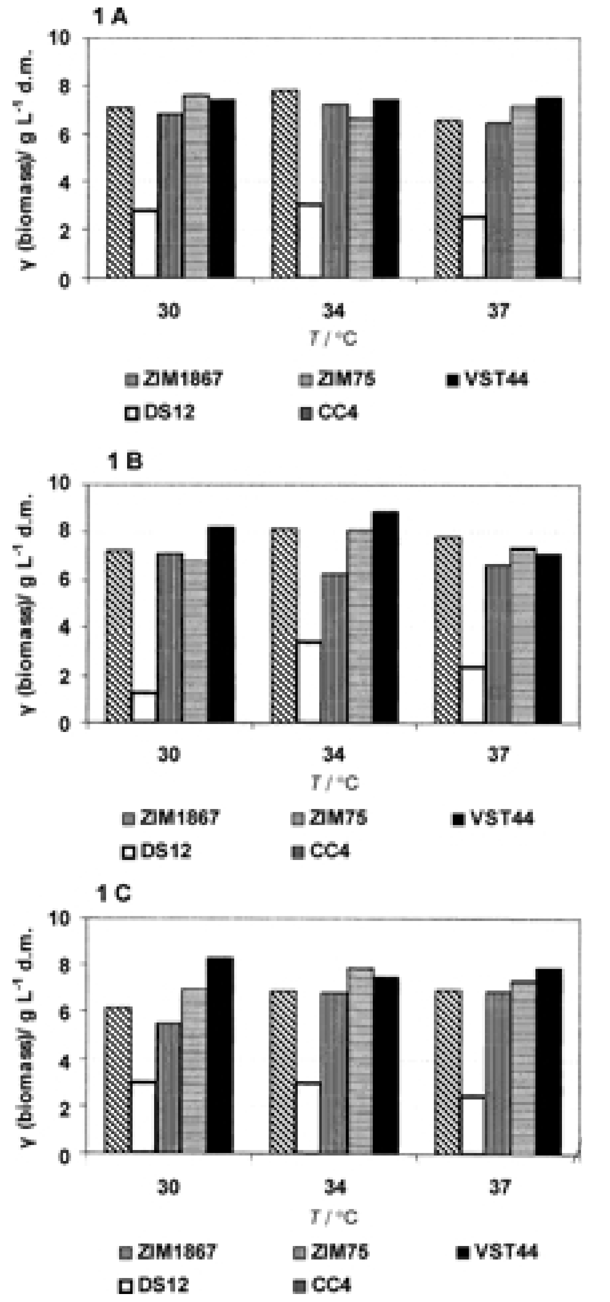
Statistical analysis of the data (Table 1) for the biomass yield after alcoholic fermentation in aerobic conditions at different temperatures has pointed out, that there is a significant difference ($p < 0.05$) between five strains of *Kluyveromyces marxianus* with regard to the biomass yield, but the temperature and lactose concentration have no significant influence on it.

The analysis of variance (Table 2) has also pointed out, that there is a significant difference

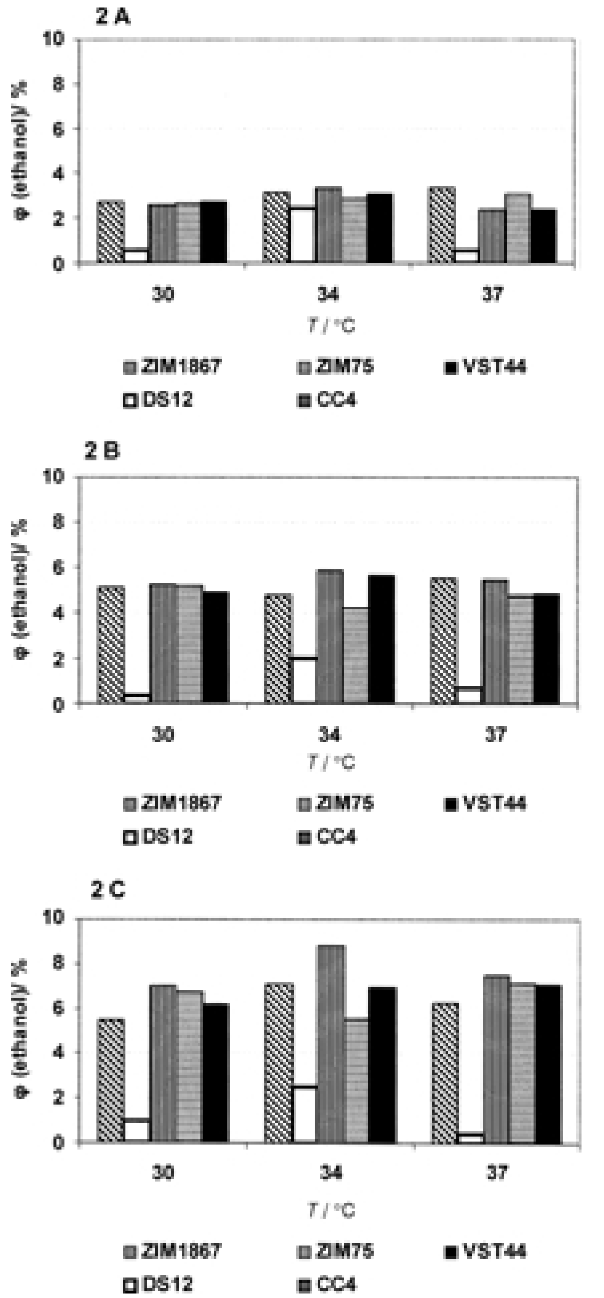
Table 1 – *Analysis of variance of data from fig. 1 (biomass yield in aerobic conditions)*

| Source of variation | *SS* | *df* | *MS* | *F* |
|---|---|---|---|---|
| Strains | 157.8700311 | 4 | 39.46750778 | 131.09284 |
| Temperature | 1.797817778 | 2 | 0.89890889 | 2.98576 |
| Lactose conc. | 0.422564445 | 2 | 0.211282223 | 0.701782 |
| Analytical error | 10.83835108 | 36 | 0.301065308 | |
| TOTAL | 170.9287644 | 44 | | |

$F_{0.05}$ (2/36) = 3.26 $F_{0.05}$ (4/36) = 2.63




F i g . 1 – *Biomass yield after alcoholic fermentation on whey (lactose conc. A–50 g L*$^{-1}$*, B-100 g L*$^{-1}$*, C–150 g L*$^{-1}$*) by 5 strains of Kluyveromyces marxianus in aerobic conditions at 30, 34 and 37 °C*




F i g . 2 – *Ethanol yield after alcoholic fermentation on whey (lactose conc. A–50 g L*$^{-1}$*, B-100 g L*$^{-1}$*, C–150 g L*$^{-1}$*) by 5 strains of Kluyveromyces marxianus in aerobic conditions at 30, 34 and 37 °C*

T a b l e 2 – *Analysis of variance of data from fig. 2 (ethanol yield in aerobic conditions)*

| Source of variation | *SS* | *df* | *MS* | *F* |
|---|---|---|---|---|
| Strains | 101.7727467 | 4 | 25.44318667 | 31.216156 |
| Temperature | 2.633884444 | 2 | 1.316942222 | 1.61575 |
| Lactose conc. | 76.37029778 | 2 | 38.18514889 | 46.84922 |
| Analytical error | 29.34232888 | 36 | 0.815064691 | |
| TOTAL | 210.1192578 | 44 | | |

$F_{0.05}$ (2/36) = 3.26 $F_{0.05}$ (4/36) = 2.63

($p < 0.05$) between the strains with regard to the ethanol yield. Lactose concentration also significantly influences ($p < 0.05$) the ethanol yield, but no significant influence of the temperature has been observed.

On the basis of these experiments two strains of *Kluyveromyces marxianus* were tested in controlled batch fermentations. The kinetics of the yeast growth and the ethanol production, as well as the utilization of $w$ = 10 % lactose from whey at 34 °C by two selected yeast strains of *Kluyveromyces marxianus*, is shown in Figure 3.

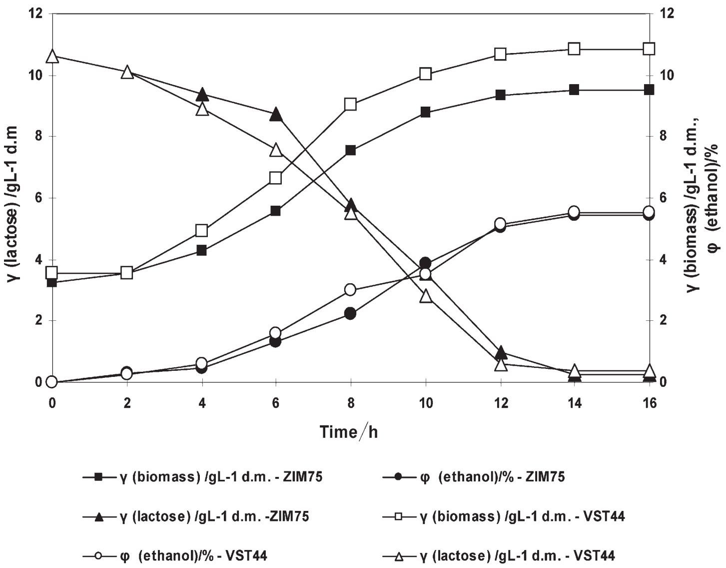



Fig. 3 – *Kinetic of biomass and ethanol production by selected yeast strains of Kluyveromyces marxianus VST44 and ZIM75, batch process at aerobic conditions*

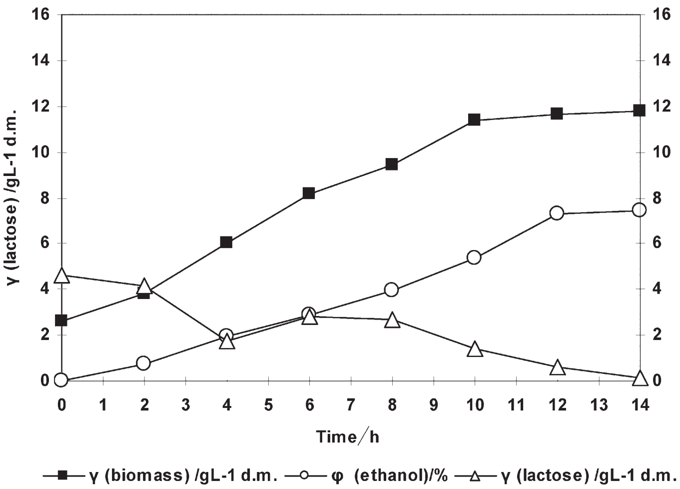



Fig. 4 – *Kinetic of biomass and ethanol production by yeast K.marxianus VST 44 in fed-batch process*

This result has assured us that yeast strains of *Kluyveromyces marxianus* VST44 and *Kluyveromyces marxianus* ZIM75, are suitable for industrial purposes because of the satisfactory kinetics and productivity of the process.

On the other hand, whey can be a biosubstrate for the production of bioyeast as a byproduct of ethanol production. For this reason, we have used a batch process with the substrate feeding which is illustrated in Figure 4.

Figure 4 shows that kinetic of the fed-batch process for alcoholic fermentation is very good and that molasses could be substituted by whey for the ethanol and yeast production. However, fed-batch process in aerobic/anaerobic conditions is favorable for this reason.

## Conclusion

Yeast strains *K.marxianus* VST44 and ZIM75 can be used for the production of ethanol and yeast biomass as byproducts on concentrated whey on the industrial scale. Also, whey could be used as GRAS substrate for the production of yeast biomass as byproduct of alcoholic fermentations

## List of symbols

$T$ – temperature, °C

$w$ – mass fraction, %

$\gamma$ – mass concentration, g $L^{-1}$

$\phi$ – volume fraction, %

$F$ – Fischer coefficient

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