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A STUDY ON THE FACTORS FOR IMPROVING THE ETHANOL PRODUCTION FROM JERUSALEM ARTICHOKE BY *KLUYVEROMYCES FRAGILIS*

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Abstract—This study is to investigate the factors influencing the alcohol production by *Kluyveromyces fragilis* using the juice of Jerusalem Artichoke tubers.

The cell growth rate and ethanol production rate were stimulated by aeration and by the addition of unsaturated fatty acids and the cell mass production and the ethanol production were substantially improved.

It was found that oxygen and unsaturated fatty acids added played a decisive role on the increase of alcohol tolerance of yeast.

INTRODUCTION

The oil price hike coupled with the relative price stability of renewable carbohydrate raw materials has stimulated worldwide interest in the utilization of ethanol produced by fermentation as an alternative liquid fuel. Ethanol alone or as a gasoline-ethanol mixture(gasohol) has been used successfully as an automobil fuel on a massive scale in Brazil[1].

In the fuel alcohol production, it is favorable to produce the high content alcohol with high productivity. However, the cell growth and alcohol production in the fermentation of alcohol by yeast are inhibited by the ethanol produced, resulting in the decrease of the yeast cell viability [2,3]. The inhibitions are partially induced by noncompetitive feedback inhibition intracellular ethanol on the glycerophosphate dehydrogenase and hexokinase enzymes in glycolysis[4]. Hence, this effect is increased as the intracellular ethanol concentration is elevated.

The ethanol concentration in cells is related to the content and the composition of unsaturated fatty acids in cell membrane[6]. The alcohol tolerance of yeast strains is increased by the high content of unsaturated fatty acids due to higher alcohol diffusion rate into the medium [6,7].

In this study, the various factors influencing alcohol tolerance of *Kluyveromyces fragilis* in alcohol fermentation using the juice of Jerusalem Artichoke tubers were examined.

MATERIALS AND METHODS

The organism used in the experiment was *Kluyveromyces fragilis* CBS 1555. The fermentation medium was prepared by pressing Jerusalem Artichoke tubers which were cooked at 121°C for 30 minutes. The total sugar concentration of medium was 180 g/*l*. The pH was adjusted to 5.5 using conc-H_3PO_4 and the media were sterilized by autoclaving at 121°C for 15 minutes. After sterilization, 1 m*l* of antifoaming agent(Sigma) was added.

All fermentations were performed in a 2 liter jar fermentor(New Brunswick) containing 1.5 liter of medium. The fermentation were carried out at 30°C with the agitation speed of 300 rpm.

The following five experimental conditions were established;

(i) Aerobic condition-fermentor was aerated at 0.01 VVM.

(ii) Partially anaerobic condition—air was not supplied during fermentation.

(iii) Strictly anaerobic condition—nitrogen gas was supplied at 0.01 VVM.

(IV) Addition of ergosterol—strictly anaerobic condition under which 17 mg of ergosterol per liter was added to the medium.

(V) Addition of unsaturated fatty acids—strictly anaerobic condition with 500 mg of linoleic acid and 500 mg of oleic acid were added to 1.5 liter of medium.

Ethanol was measured by gas chromatography and

the cell concentration was determined by measuring the optical densities with a spectrophotometer and the corresponding dry weights were obtained from an established standard curve of absorbance versus dry weight. The total sugar concentration was determined using the modified Anthrone method[8] and reducing sugar by the dinitrosalicylic acid method[9].

RESULTS AND DISCUSSION

The cell concentration during the fermentation is shown in Figure 1. The maximum spacific growth rate (μ_{max}), under aerobic, partially anaerobic and strictly anaerobic conditions were 0.356, 0.192 and 0.108 hr^{-1}, respectively (see Table 1). The aerobic condition also resulted in the highest maximum cell concentration (X_m) in the shortest time as compared to the other conditions. It was found out that the oxygen plays an important role in the growth of *K. fragilis* as well as *S. cerevisiae* whose growth was enhanced, due to not only the increased metabolism and energy production by respiration, but the synthesis of cell membrane in the presence of oxygen[10].

Figure 2 shows the relationship between ethanol production and fermentation time. Under the aerobic condition, maximum ethanol concentration (P_m) reached at 72.9 g/*l* after 22 hours fermentation, whereas under the partially anaerobic and strictly an-

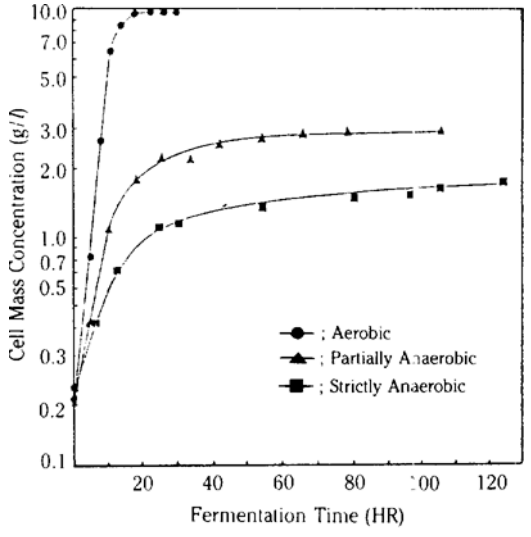

Fig. 1. Production of cell mass during the fermentation.

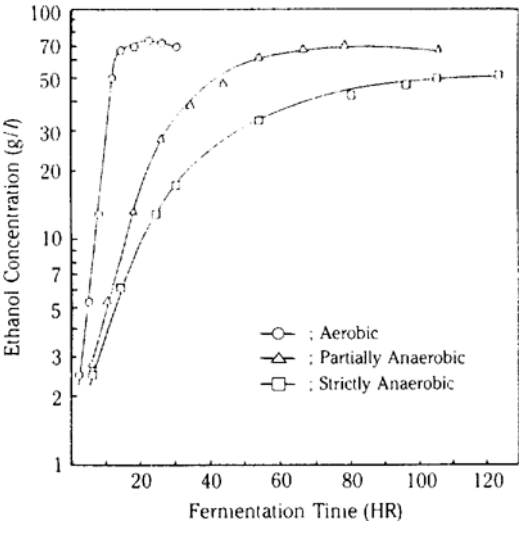

Fig. 2. Production of ethanol during the fermentation.

Table 1. The various kinetic parameters in each fermentation conditions.

Fermentation Condition	μ_{max} (hr^{-1})	X_m (g/*l*)	P_m (g/*l*)	P (g/*l*-h)	q_p (g/g-h)	Y (g/g)	F (%)
Aerobic	0.356	9.70	72.9	3.31	2.31	0.47	79.8
Partially Anaerobic	0.192	2.99	70.1	0.90	0.54	0.48	76.8
Strictly Anaerobic	0.108	1.77	51.9	0.42	1.19	0.49	58.2
Strictly Anaerobic with Ergosterol	0.068	3.94	65.5	0.62	0.96	0.48	71.7
Strictly Anaerobic with Linoleic&Oleic Acid	0.118	4.06	71.8	0.79	1.27	0.48	77.7

μ_{max} : Maximum specific growth rate
q_p : Specific ethanol production rate
X_m : Maximum cell concentration
P_m : Maximum ethanol concentration
P : Overall ethanol productivity
Y : Ethanol Yield
F : Fermentability

aerobic conditions, P_m of 70.1 g/*l* and 51.9 g/*l* were obtained after 78 hours and 124 hours fermentation, respectively. The overall ethanol productivities (P) were calculated based on the Figure 2 and the values were shown in Table 1. The highest productivity of 3.31 g/*l*-hr, was obtained under the aerobic condition. On the other hand, ethanol yield was 0.49 under the strictly anaerobic condition, which was 96% of theoretical value. The aerobic condition resulted in slightly lower yield than strictly anaerobic condition because, under aerobic condition, not only sugar was consumed for cell growth but also *K. fragilis* utilized the ethanol for its growth at the later stage of fermentation as reported by Guiraud et al. [11] and Magaritis et al. [12]. However, the highest fermentability, of about 80%, was obtained under the aerobic condition.

As a result, the cell growth rate and ethanol production rate were increased by aeration and it is suggested that the oxygen participates in the synthesis of sterol, unsaturated fatty acids and its precursors[7,10, 13]. Therefore aeration increases the alcohol tolerance of *K. fragilis* as in *S. cerevisiae* [14].

The effects of ergosterol and unsaturated fatty acids on the cell concentration (Fig. 3) and ethanol concentration (Fig. 4) were plotted as a function of fermentation time. With the addition of unsaturated fatty acids, P_m was 71.8 g/*l* after 90 hours fermentation and, although μ_{max} was slightly higher, X_m and P were increased by about 2-fold as compared to a strictly anaerobic condition (Table 1). With the addition of ergosterol, cell growth was somewhat inhibited in the early stage of fermentation (Fig. 3), while X_m, P_m and P increased as compared to a strictly anaerobic condition. Therefore ergosterol or unsaturated fatty acids seem to be directly used in the synthesis of cellular components.

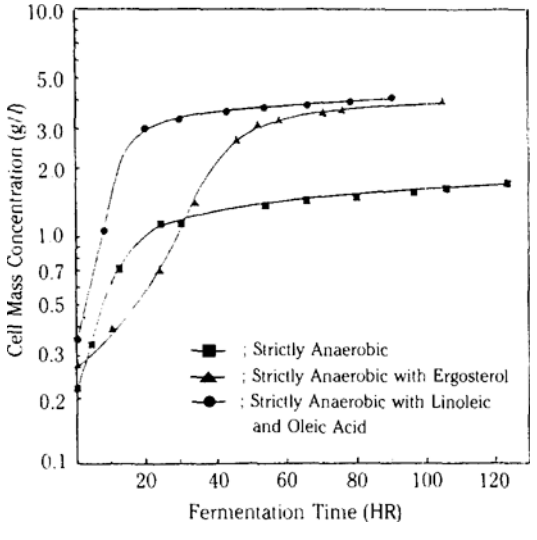

Fig. 3. Production of cell mass during the fermentation.

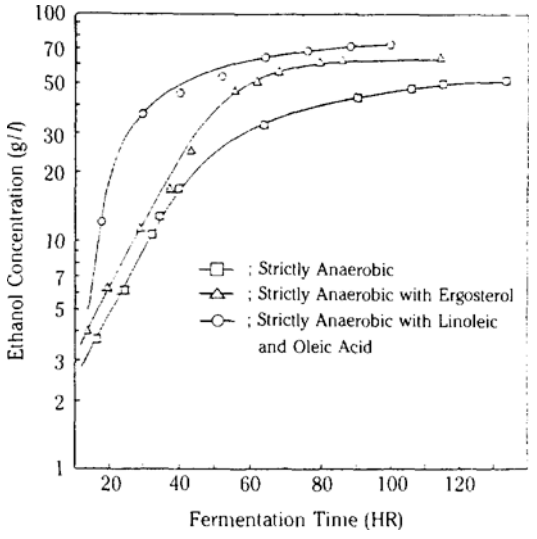

Fig. 4. Production of ethanol during the fermentation.

As a result, even under a strictly anaerobic condition, alcohol tolerance of *K. fragilis* increases with the addition of ergosterol and unsaturated fatty acids, especially, linoleic and oleic acids, which acts as pre-

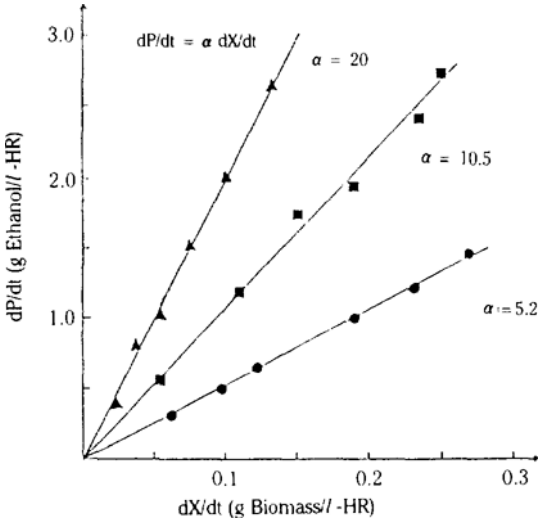

Fig. 5. Plot of ethanol production rate against biomass production rate during the exponential growth phase of (●) aerobic condition, (■) unsaturated fatty acid and (▲) ergosterol added.

cursors of the liquid synthesis of cell membrane [15-17].

Figure 5 shows that the plot of ethanol production rate against biomass production rate during the exponential growth phase. Under the aerobic condition and strictly anaerobic condition with ergosterol or unsaturated fatty acids, ethanol production is a growth -associated form as reported by Magaritis et al.[18].and stoichiometric constants were 5.2, 10.5, and 20.0, respectively.

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