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Effect of Fermentation Temperature on the Production of high content Alcohol

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고농도 알코올 생성을 위한 온도의 영향

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ABSTRACT

The effect of fermentation temperature on the production of high content alcohol has been investigated with high substrate concentration. The maximun specific growth rate, μmax was 0.461hr^{-1} at 35°C which was the highest, whereas the maximun biomass concentration was 8.7 g/*l* at 25°C, at the growth rate lower than that at 35°C.

Approximately 140 g/*l* of ethanol was produced in the temperature range of 20 to 25°C with nearly complete consumption of the substrate. Extended fermentation time has been required at lower temperatures, however, for the maximum values of biomass concentration and alcohol content, hence higher ethanol productivity, as the temperature was elevated to 40°C.

The viability of yeasts was greatly improved by lowering the fermentation temperature down to 25°C and also extended survival of the cells has been observed at lower fermentation temperatures, although the ethanol concentration of broth was higher.

INTRODUCTION

The alcohol fermentation process depends on environmental factors and cellular characteristics of the organism used. The important factors affecting alcohol fermentation could be numerous; the substrates, their concentrations, the yeast concentration, the fermentation temperature, pH, the aeration, the nutrient supplementation, and others. Among these factors, the fermentation temperature is one of the most crucial environmental factors affecting the growth and the viability of yeasts for the production of alcohol.

When one of the yeast genus, *Saccharomyces cerevisiae*, is used for the fermentation, it has been the general practice to maintain the temperatures at around 30°C and 8~15°C in the alcohol production and the brewing industuies respectively.

In alcohol fermentation, the fermentation temperature affects the growth rate of yeasts and the production rate of ethanol; both gro-

wth rates and the ethanol production rates were increased as the temperature were elevated to 35~42°C (Cysewski, 1976; Williams, *el al.*,1981). The viability of yeasts, however, has fallen at higher temperatures (Nagodawithana, *et al.*, 1974; Ryu, 1980). The fermentation temperature is also directly related to the concentration of intracellular ethanol (Navarro and Durand, 1978), which inhibits the catabolism of glucose (Nagodawithana, *et al.*, 1977) and synthesis of cellular components. In particular,in the synthesis of unsaturated fatty acids, the acid content has been increased at lower fermentation temperature(Pfister, *et al.*, 1977; Woodbine, 1959), and the unsaturated fatty acids content has been related to the ethanol tolerance and viability of yeasts(Thomas, *et al.*, 1978).

For the production of high content alcohol, high substrate concentration is needed, which in turn inhibits the cell growth (Ryu, 1980) and fermentation rate (Chen, 1981), resulting in the extended fermentation period (Chen, 1981). Hence, it would be extremely difficult to study the quantitative effects all factors in alcohol fermentation.

As pointed out, the effect of temperature appeared a crucial factor and the work of Hayashida, *et al.*, (1974) reported the production of highly concentrated alcohol by fermentation using *Saccharomyces sake* at 20°C.

Hence, in this study, the effect of fermentation temperature on the production of high content alcohol has been investigated using 30% (W/V) glucose substrate in batch alcohol fermentation.

MATERIALS AND METHODS

1. Organism

Saccharomyces cerevisiae STV 89, reported to produce high content ethanol (Ryu, 1980), was obtained from INSA of Toulouse in France, and maintained by transferring to fresh Sabiraud agar slant every month and storring at 4°C.

2. Fermentation medium

The fermentation medium consisted of 300g of glucose (Anhydrous), 1.15g of KCl, 1.5g of KH_2PO_4, 1.5g of NH_4Cl, 0.065g of $MgSO_4 \cdot 7H_2O$, 0.015g of $MnSO_4$, 0.1g of $Zn(NO_3)_2 \cdot H_2O$, 7.5g of yeast extract (BBL) and 7.5g of peptone (Mikuni) in 1.0 liter of tap water. The pH was adjusted to 5.0 with H_3PO_4 after sterilization.

3. Inoculum

The concentration of glucose in the above medium was reduced to 100g/l for the inoculum medium. Cells were incubated aerobically in shaking Erlenmeyer flask at 30°C for 16 hrs.

4. Analytical methods

The biomass concentration, in g/l was determined by measuring the absorption with the photoelectric colorimeter (Jouan model AE -11) at 650nm with washing cells by the centrifugation,and the corresponding dry weight was obtained from an estabilished standard curve of absorbance versus dry weight.

The ethanol concentration, in g/l was measured by gas chromatography using a Girdel 3000 Chromatograph with a flame ionization detector. A 2.0×2.4mm column was packed with Chromosorb W/AW (60~80 mesh) impregnated with 20% Carbowax 20M. The temperature of injector and detector was maintained at 250°C and the column oven was operated isothermally at 90°C. The internal standard was used with 2% V/V n-propanol.

5. Experimental procedures

The alcoholic fermentations were carried out in a batch system by 4 liter fermentor loaded with 3 liter fermentation medium. The fermentor and its contents were sterilized in an autoclave at 110°C for 30 min. to avoid caramelization of the glucose. The fermentor was

inoculated with a 2% V/V inoculum and agitated at 300 rpm without aeration. The fermentor was equipped with a refrigerated circulator (Model Facis) in order to control the temperature in 20~40°C range by circulating cooling water.

RESULTS AND DISCUSSION

1. Effect of fermentation temperature on the growth of cells.

In each fermentation temperature, the maximum specific growth rates, μmax have been estimated from the logarithmic phase of the growth curves and the maximum concentation of biomass, Xm, has been obtained at the early stationary phase in which no further cell growth has been observed.

As shown in Figure. 1, the optimum temperature for the fastest cell growth was 35°C for the strain used, but the value of Xm was the highest at the temperature of 25°C, 10 degrees lower than the optimum temperature for growth rate, even though extended fermentation has been required for Xm values at lower fermentation temperatures.

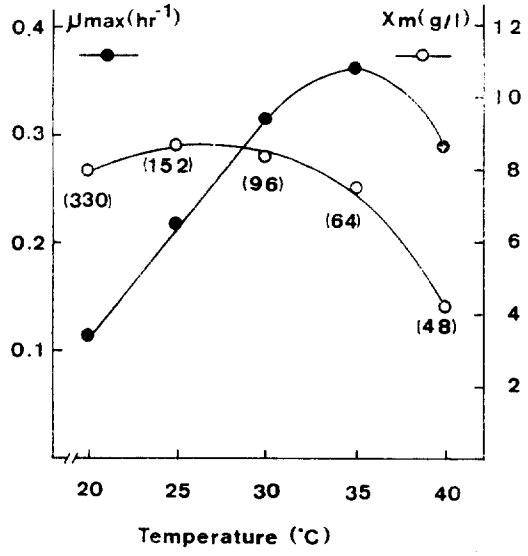

Fig. 1. Effect of fermentation temperature on the μmax and Xm. The numbers in parenthesis indicated the time (hr) required for Xm values.

The results have shown that the Xm values were independent on the μmax which was affected by the fermentation temperature. When the nutrient was supplemented, however, the Xm values were dependent on the μmax (Ryu, 1980) as the supplemented nutrient could directly be used as the precursors for the synthesis of cellular components.

A simple Arrhenius-type formula, $\mu max = A\exp(-\Delta E/RT)$, has been employed to investigate the tendency of the activation energy, ΔE. As shown in Figure 2, the activation energy has fallen with the increase of fermentation temperature up to 35°C, possibly due to the internal diffusion limitation (Engasser and Horvath, 1976) and the product inhibition (Eseber, *et al.*, 1981).

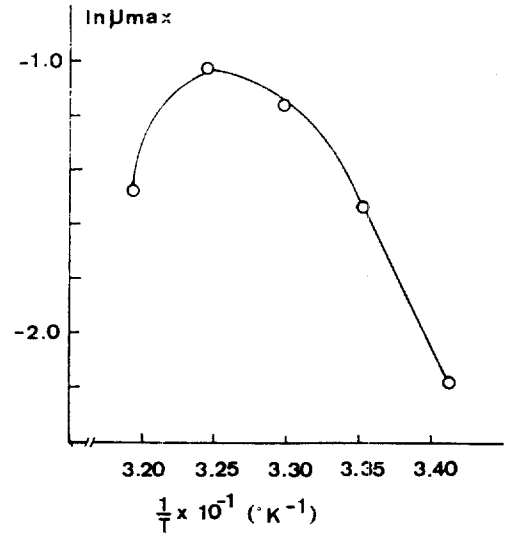

Fig. 2. Natural logarithmic of μmax versus the reciprocal of absolute temperature

2. Effect of fermentation temperature on the ethanol production

The ethanol content has reached its maximum value, Pm, at the completion of the production at all temperatures (Fig. 3).

As shown, the value of Pm remained constant at approximately 140 g/l in the temperature range of 20 to 25°C with nearly complete consumption of the substrate. At the temperatures higher than 25°C, it started to decline linearly as the fermentation temperature was elevated.

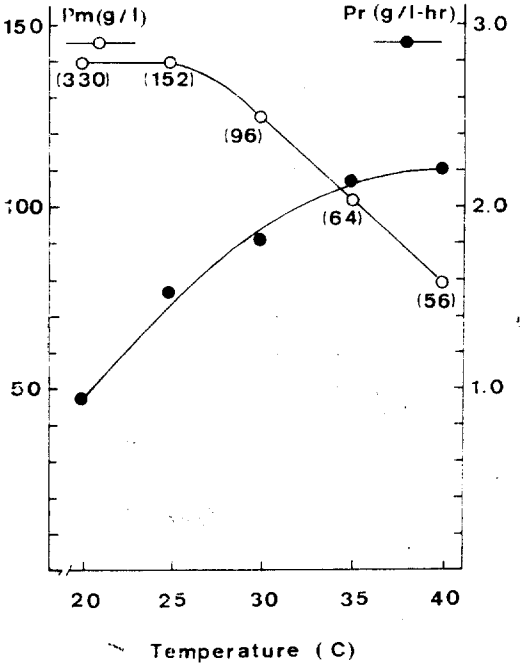

Fig. 3. Effect of fermentation temperature on the Pm. The numbers in parenthesis indicates the time (hr.) required for Pm values

In general, elevated fermentation temperatures enhance the cellular activity in the early stage of fermentation, resulting in higher growth rate of the yeasts, and the accumulation of ethanol in cells (Navarro and Durand, 1978) as well, which in turn accelerate the inhibition of glucose catabolism (Nagodaxithane, *et al.*, 1977) and the synthesis of cellular components.

As a consequence, the optimal temperature, 25°C, for the high content ethanol, differs substantially from that for cell growth rate, 35°C.

The yeast growth has been inhibited when substrate concentration was high. The ethanol production has continued, however, without further cell growth, and as shown in Table 1. the amount of ethanol produced during this period has been approximately ten fold by lowering the fermentation temperature from 40 to 20°C.

Table 1. The quantity of ethanol produced without further cell growth in all fermentation temperatures

Temperature, °C	20	25	30	35	40
Ethanol produced, g/l	50.7	45.8	22.5	8.7	5.0
Ethanol produced/Pm. %	36.4	32.7	18.1	8.5	5.8

The ethanol productivity, Pr, in g Ethanol/1-hr, estimated from the ethanol production curves at given fermentation temperatures, however, has increased as the temperature was elevated up to 40°C (Fig. 3).

The following conclusion could be drawn;

Regarding the effect of fermentation temperature, the production of ethanol in high content has not been related with the cell growth rate, although the optimal ethanol production has always been associated with high growth

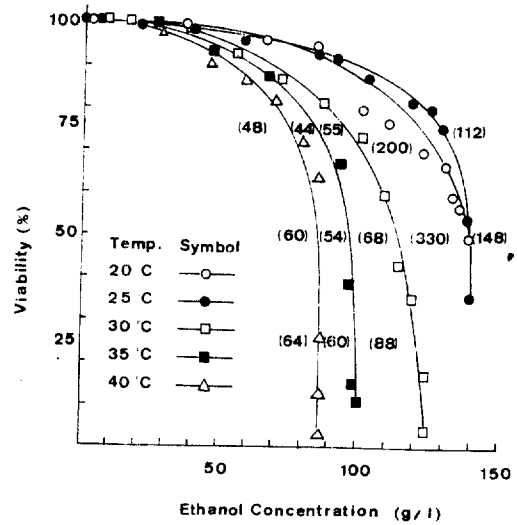

Fig. 4. The viability of yeasts as function of ethanol concentration in broth at fermentation temperatures. The numbers in parenthesis indicates the fermentation time(hr) at 75% 50%, 25% viability

rate under the condition that there was no limitation of yeast growth (Cysexski, 1976). However, cell production to high concentration has been required (Fig. 1, 3) when nutrients were supplemented (Ryu, 1980).

3. Effect of fermentation temperature on the viability of yeasts

The viability, the fraction of viable cells over the total cells (over 500 cells counted) has improved as the unsaturated fatty acid content in cells (Day, *et al.*, 1976) was augmented by lowering the fermentation temperature (Pfister, 1977). And the presence of the unsaturated fatty acids in plasma-membrane has increased the ethanol tolerance for *Saccharomyces cerevisiae* (Thomas, *et al.*, 1977).

As shown in Fig. 4, the viability of yeasts has been substantially improved by lowering the fermentation temperature down to 25°C, which has also been reported by Nagodawithana, *et al.*, (1974) in the fast fermentation. At lower temperatures, the survival of the cells has also been extended although the ethanol concentration in broth was higher.

摘 要

본 연구에서는 고농도 알콜을 생성하기 위한 온도의 영향을 검토하였다. 효모의 최대 성장속도 μmax는 0.461 hr^{-1} 로 35°C에서 최대값을 나타냈다. 반면에 균체의 농도는 8.7 g/*l*로써 25°C에서 최대값을 보였고, 알콜의 생성농도는 거의 모든 기질을 소모하면서 20°C와 25°C에서 약 140 g/*l*이었다. 이중 효모의 성장이 중지된 후 생성된 알콜의 농도는 20°C와 25°C에서 각각 50.7 g/*l*와 45.8 g/*l*이었다.

일정한 온도에서 최대 알콜 생성 농도를 위한 발효 시간은 온도의 감소에 따라 길어졌으나, 반면 효모의 생존도는 발효온도가 낮을수록 향상되었다. 따라서 효모가 높은 알콜 농도에서 더 오래동안 생존할 수 있으므로, 고농도 알콜 생성을 위한 최적 발효 온도는 효모의 성장속도가 가장 높은 35°C보다 낮은 25°C였고, 이 온도는 최대 균체농도를 생성하는 온도와 동일하였다.

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