The Effect of $K_L a$ on the Ethanol Yield for Xylose Fermentation
(氧氣質傳係數與 P. STIPITIS 菌體在五碳糖醱酵產率之研究)

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Abstract

D-xylose was bioconverted into ethanol by yeast of *Pichia stipitis* CBS 5773. The similar tendency of oxygen transfer coefficient, $K_L a$, on ethanol yield was observed among various configurations of bioreactors, namely, shake flask (250 ml, 500 ml), spinner flask (500 ml), and stirred tank (5 L). The ethanol yield could reach about 0.37 g ethanol/g xylose if $K_L a$ lower than 0.004 sec^{-1}. But the yield fell down rapidly as $K_L a$ increased.

Introduction

D-xylose, a kind of pentose, is abundant in hemicellulose and can be fermented to ethanol. Since one fourth of ample agricultural wastes is hemicellulose [1], it is an attractive idea to include hemicellulose for ethanol production in addition to cellulosic material. The economic impact of converting D-xylose to ethanol was examined and found that the price of ethanol could be significantly affected by yield and final ethanol concentration [2]. However, only a few of bacteria [3, 4], yeast [5], and fungi can ferment D-xylose. Yeast was found to be potentially suitable for industrially application.

In present study, the relationship between ethanol yield and $K_L a$ was explored among various configurations of bioreactors.

Materials and Methods

Organism and Medium

The yeast *Pichia stipitis* CBS 5773（or *Pichia stipitis* NRRL- Y7124）was obtained from Food Industry Research & Development Institute. The modified YM medium contained 3 gL^{-1} yeast extract, 5 gL^{-1} peptone, 10 gL^{-1} xylose and was sterilized at 121℃ for 15 min. A loopful of culture from a modified YM plate was inoculated into the medium and was cultivated in a rotary shaker at 125 rpm and 30℃ for 24 hr. This shaken broth was then transferred to the bioreactors by the ratio of 1:10 in the xylose fermentation experiments.

Experimental conditions for xylose fermentation

Three types of bioreactors were evaluated, i.e. shake flasks of 250 ml and 500 ml, spinner flask of 500 ml（Bellco, 1965-00500）, and fermenter of 5 L（Electrolab Ltd. 300 series）. All fermentations were maintained at 30℃ and pH 4.5. A citric acid buffer mainly controlled the pH in shake flasks and

spinner flasks. On the other hand, pH was adjusted automatically with 0.2 M H_2SO_4 and 1 M NaOH in the 5 L fermenter. Fermentation studies were focused on the ethanol yield at different levels of oxygen mass transfer coefficient (K_La). In shake flasks K_La was tuned by changing broth volumes at constant agitation rate of 125 rpm. For spinner flasks and fermenters, adjusting the aeration rate controlled the degree of KLa. Gassing-out method developed by Wise (1951) was adopted to measure K_La in all bioreactors [6]. The agitation rates in 500 ml spinner flask and 5 L fermenter were fixed at 160 rpm and 200 rpm, respectively. However, all K_La's in various bioreactors were measured only containing the cultural medium for simplicity.

Experimental conditions for newspapers fermentation Preparation of acid hydrolyzate of newspapers

Ten pieces of newspapers (76 cm*54 cm) were sliced and mixed with 1500 ml distilled water and 20 ml concentrated H_2SO_4 (96 wt %). Hydrolysis was then carried out in an autoclave at 121℃ for 30 min. The mixture was then blended into slurry and 500 ml distilled water was added. Further hydrolyzation was then done at 121℃ for another hour. The hydrolyzate was filtered and the filtrate was adjusted to pH 4.5. Final volume of hydrolyzate about 1.2 L contained ca. 7.5 g xylose /L and 2.5 g glucose /L. That is, a single piece of newspaper had about 0.9 g xylose and 0.3 g glucose.

Adaptation of *P. stipitis* to hydrolyzates

Adaptation was carried out to select the yeast accommodated to the newspaper hydrolyzates. The strains were transferred to fresh medium after 24 hr cultivation with stepwise increasing ratio of hydrolyzates. After four cycles, the broth was preserved on the agar plate containing 3 g yeast extract, 5 g peptone, 20 g agar in 1 L hydrolyzate.

Analytical procedures

Fermentation samples were determined the optical densities at 620 nm and the data were converted to cell mass by a pre-calibrated curve. Xylose and ethanol concentrations were measured with an HPLC equipped with a refractive index detector（Perking Elmer series 200） and a interaction CHO-620 carbohydrate column. The column was operated at 70℃ with a mobile phase of water. The dissolved oxygen concentration in the medium was monitored with a DO probe (Ingold, type 170).

Results and Discussions

Xylose fermentation study

Fig.1 shows the typical K_La vs. culture medium volume in 250 ml and 500 ml shake flasks. Since agitation rate of 125 rpm was fixed, the lager broth volume means a smaller K_La because of less gas-liquid interphase for oxygen transfer. Fig. 2 demonstrates typical batch fermentation for synthetic medium, while Fig. 3 elucidate the fermentation of newspaper hydrolyzate. Fig.4 shows

summarized results of the ethanol yield vs. K_La for three types of bioreactors. It was found that although these bioreactors have different geometric shape, they have similar tendency in the relationship between ethanol yield and K_La. That is, when K_La is less than ca. 0.004 sec^{-1}, ethanol yield seems to maintain at high yield of yield falls down rapidly. Fermentation in spinner flask with and without n f average 0.37 g-ethanol/g xylose. Once K_La exceeds 0.004 sec^{-1}, ethanol

Conclusions

Experimental results showed that the relation between K_La and ethanol yield is independent of the geometric type of bioreactors. To obtain high ethanol yield, K_La must be controlled between 0 and 0.004 sec^{-1}. Since high aeration could promote the growth rate of yeast, K_La is preferably set at the margin value, 0.004 sec^{-1}, to attain the highest yield in a short time. On the other hand, growth rate of *P. stipitis* is inhibited by the high concentration of substrate. Therefore fed-batch is applicable to maintain suitable substrate concentration. Furthermore, it is also suggested that high aeration be adopted in the initial stage of fermentation for fast growth of yeast, and then lower the aeration to consume substrate effectively to produce ethanol. For newspaper hydrolyzates fermentation, strain adaptation and selection were shown capable to overcome the possible inhibitory effects. The relation between the ethanol yield and K_La in hydrolyzate medium was found similar to that in synthetic medium.

References

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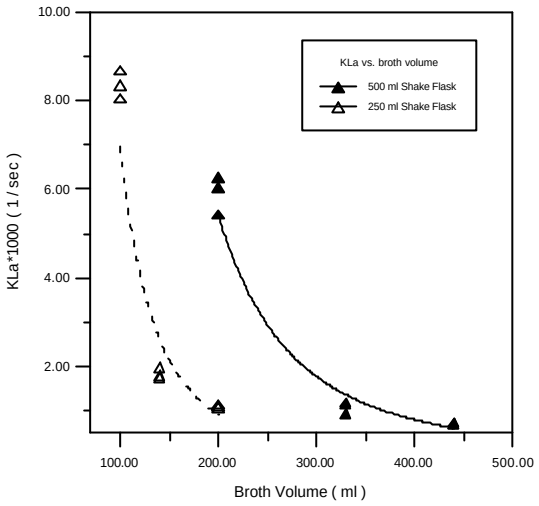

Fig.1 Relation between K_La and broth volume in shake flasks.(agitation rate : 125 rpm)

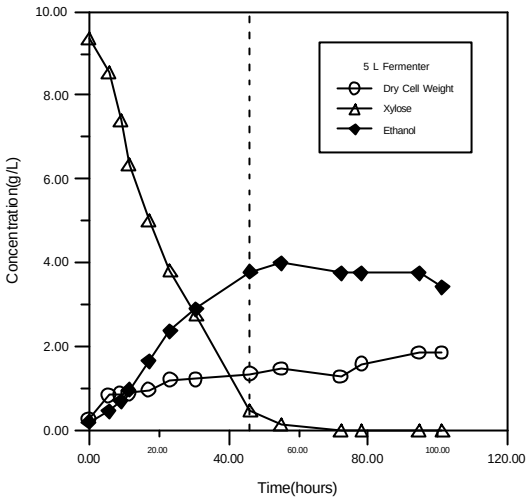

Fig.2 Fermentation in synthetic medium

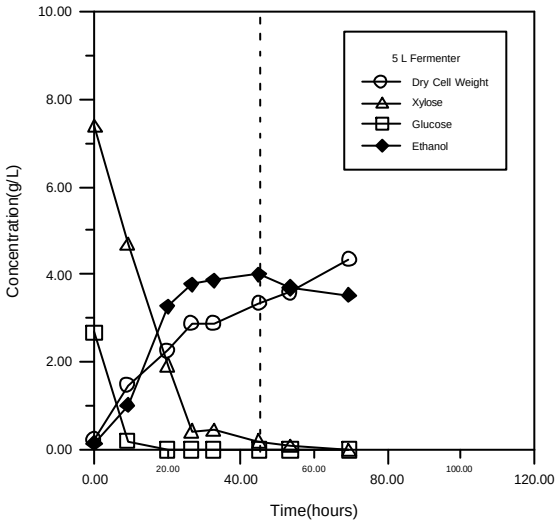

Fig.3 Fermentation in newspaper hydrolyzates

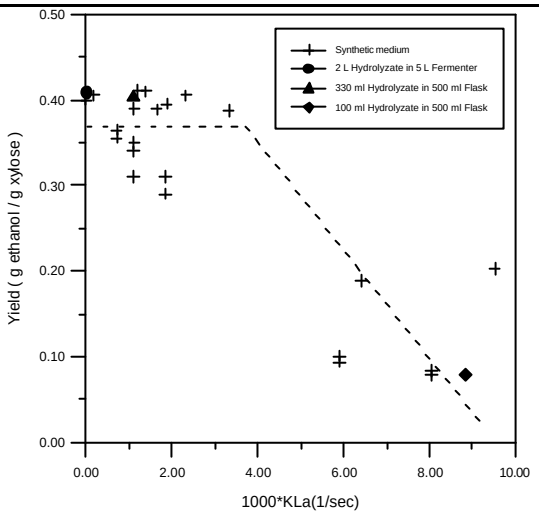

Fig.4 Comparison of the yield tendency for both synthetic medium and newspaper hydrolyzates