INFLUENCE OF AERATION TIME ON FERMENTATION PERFORMANCE OF THE IMMOBILIZED YEAST IN HIGH GRAVITY BREWING

ẢNH HƯỞNG CỦA THỜI GIAN SỤC KHÍ ĐẾN KHẢ NĂNG LÊN MEN CỦA NẤM MEN CỐ ĐỊNH TRONG QUÁ TRÌNH LÊN MEN BIA NỒNG ĐỘ CAO

Tran Quoc Hien, Le Van Viet Man
Ho Chi Minh City University of Technology

Hoang Kim Anh
Institute of Tropical Biology

ABSTRACT

An adequate oxygen supply is essential for brewer yeast growth and the brewing fermentation can complete rapidly. In this paper, batch fermentation system was used to study the influence of the aeration time on high gravity brewing using Saccharomyces cerevisiae immobilized in calcium alginate gel. During the first period of fermentation, when the aeration time increased from 0 to 20 h, the fermentation time decreased 41.67%. However, the ethanol concentration in green beer decreased 9.46%. The suitable aeration time after pitching was 12h in high gravity brewing.

Key words: calcium alginate gel, high gravity brewing, immobilization, *Saccharomyces cerevisiae,* wort oxygenation.

TÓM TẮT

Cung cấp oxy đầy đủ vào dịch nha là điều kiện cần thiết cho sự phát triển của nấm men bia và quá trình lên men bia có thể kết thúc nhanh chóng. Trong nghiên cứu này, hệ thống lên men gián đoạn được dùng để khảo sát ảnh hưởng của thời gian cung cấp oxy đến quá trình lên men bia nồng độ cao sử dụng nấm men Saccharomyces cerevisiae cố định trong gel alginate. Trong giai đoạn đầu của quá trình lên men, khi thời gian sục khí oxy vào trong dịch nha gia tăng từ 0 đến 20 giờ thì thời gian lên men giảm 41,67 %. Tuy nhiên, nồng độ etanol trong bia non giảm 9,46%. Trong quá trình lên men bia nồng độ cao, thời gian sục khí thích hợp cho dịch nha sau khi cấy giống là 12 giờ.

I. INTRODUCTION

High gravity brewing (HGB) involves preparation and fermentation of wort with initial density higher than 12^{o}P. By increasing the wort density, higher level of ethanol per given plant capacity can be achieved and substantial savings can be attained by the brewer; the plant efficiency and capacity are increased; labour, energy and capital costs are reduced (13). On the other hand, production of beer using immobilized cells has also been considered as a very promising technology. The use of immobilized cells in the fermentation process and their potential advantages over the free cell systems have been widely studied and reviewed (8).

In addition, the optimal oxygen delivery strategy in the brewing fermentation enhanced HGB fermentation performance as attenuation commences more rapidly, the yeast utilize less free amino nitrogen (FAN) as well as higher cell viabilities throughout the fermentation (7). The major role of the dissolved oxygen in brewery fermentation is to promote the biosynthesis of unsaturated fatty acids and ergosterol (4,9,10,12). These compounds, known as anaerobic growth factors, are essential for importance in promoting cell growth (3,4,7).

In traditional brewing, wort is aerated before pitching until the oxygen concentration in wort reaches 6-8ppm. This oxygen content is enough for yeast growth and the fermentation can complete rapidly (2). However, some researchers aerated the wort before pitching and during the first hours of brewing fermentation; their results showed that the metabolic activities of the free yeast were improved (4,10,12).

In addition, the oxygen requirement of brewer yeast varies in a wide range and depends on the fermentation method (10). In this study, the influence of the aeration time on high gravity brewing using yeast immobilized in alginate gel was examined.

II. MATERIAL AND METHODS

Materials

- *Saccharomyces cerevisiae* (lager strain) was supplied by Tien Giang Foster Company (Vietnam).

- Na-alginate was supplied by Biotechnology Center, Nha Trang University (Vietnam). The ratio of mannuronic acid to guluronic acid (M/G) was 1.2. The viscosity (2% alginate solution, 25^{o}C) was 423.6cp.

- Malt was supplied by Duong Malt Company (Vietnam), the extraction yield was 80%.

- Other chemicals used in this study were supplied by Merck (EU).

Inoculum preparation

Precultures were prepared by two successive inoculations: 1) in 100ml Erlenmeyer Shake-flask containing 15ml of 8%(w/w) malt wort; and 2) in 500ml Erlenmeyer Shake-flask containing 150ml of 8%(w/w) malt wort. For both periods, the preculture was grown at 30^{o}C and 100rpm for 24h.

Immobilization

A volume of yeast suspension (100.10^6 cell mL^{-1}) was added to an equal volume of sodium alginate solution (50g/l) and homogenized. This mixture was then dropped into a 3% (w/v) $CaCl_2$ solution for formation of calcium alginate gel beads. The cell concentration in the gel bead was $50x10^6$cell/cm^3. The residence time of the gel beads in calcium chloride solution was 4 hours at 4^{o}C for increasing the gel strength. Then the gel beads were washed in sterile water.

Fermentation conditions

Batch fermentation was carried out in 4l tank containing 1.8l of 24% (w/w) malt wort. The fermentation temperature was 17^{o}C. The inoculation rate was $10x10^6$ cells/ml of wort. 6 samples were examined. Before pitching, the initial dissolved oxygen concentration of wort in all samples was 7.5ppm. After pitching, aeration was carried out during the first hours of the fermentation. The aeration time of the 6 samples was 0, 4, 8, 12, 16 and 20h, respectively. During the aeration, the dissolved oxygen concentration of wort in all samples was maintained at 7.5 ppm.

The fermentation was considered as it was completed when the degree of attenuation reached 75% (11). The degree of attenuation was calculated by the reducing sugar content in the initial wort and in the green beer.

Analytical methods

- Dissolved oxygen was measured with a Sension 8-HacH. The unit was calibrated prior to each use and the reading (in ppm) was obtained after the value stabilized (7).

- Yeast concentration and viability: The Ca-alginate gel beads containing yeast cells were dissolved in a 3% (w/v) EDTA solution. The yeast concentration was then determined by haemocytometry using Thoma counter chamber. Yeast viability was determined by methylene blue test (11).

- Reducing sugar and free amino nitrogen (FAN) were measured by spectrophotometric method, using 3.5 dinitrosalicylic acid and ninhydrin, respectively (7).

- Diacetyl was determined by spectrophotometric method using o-phenylenediamine (1).

Ethanol and volatile compounds were determined by gas chromatography (Agilent technologies 6890N) using a flame ionization detector (FID) and a HP-FFAP column (19091F-413), with 30m length, 0.25µm film thickness and 0.32mm internal diameter. The working conditions were as follows: injection temperature is 200^{o}C; oven temperature is at 45^{o}C for 2 min, increase at 7^{o}C/min to 150^{o}C, hold for 2 min; detector temperature is 200^{o}C. The carrier gas was hydrogen (6).

- Statistical analysis: The results were averaged over three independent experiments. The averaged results were subjected to analysis of variance (ANOVA), $p<0.05$ using Statgraphics plus, version 3.2.

III. RESULTS AND DISCUSSION

Yeast growth

Fig.1 presents the evolution of viable cell concentration in the culture. In general, the

maximum of viable cell number changed when the total content of oxygen supplied to wort was altered. When the aeration time increased from 0 to 12h, the yeast concentration reached maximum after the first 48h of fermentation. Meanwhile, the total cell number attained maximum after the first 72 h of fermentation when the aeration time increased from 16 to 20h.

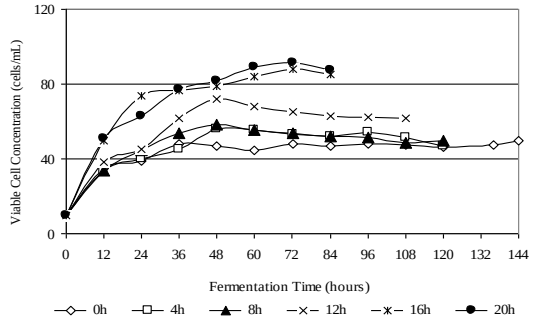

Fig.1 The effect of aeration time on viable cell concentration during high gravity brewing using immobilized yeast

Under aerobic conditions, oxygen and sugars are transformed into CO_2, water and energy. A part of this energy was used for the production of yeast biomass. When the content of oxygen supplied to wort increased, the yeast growth was better and the exponential phase prolonged. In consequence, the maximum yeast cell concentration increased. When the aeration time augmented from 0 to 20h, the maximal yeast concentration increased from $48.1x10^6$cell/ml to $91.5x10^6$cell/ml (Table 1).

Substrate utilization

The reducing sugar and FAN consumption rates of the immobilized yeast are showed in Table 1. It can be offered that the presence of oxygen in wort stimulated substrate utilization. When the aeration time increased from 0 to 20h, the reducing sugar and FAN consumption rates increased from 0.95 g/l.h to 1.66 g/l.h and from 1.36 mg/l.h to 3.03 mg/l.h, respectively. The immobilized yeast utilized carbon & nitrogen substrates (sugar & free amino nitrogen) for cell multiplication. It can be confirmed that the longer of aeration time after pitching, the higher of yeast concentration in the culture.

According to O'Connor-Cox (9), wort aeration during the first hours of fermentation was more effective to supply oxygen for yeast growth than wort aeration before pitching. Our results showed that increase in aeration time after pitching shortened the primary fermentation. So, selection of suitable aeration time after pitching could decrease the primary fermentation time in high gravity brewing with the immobilized yeast.

Ethanol formation

Fig.2 presents the kinetics of ethanol formation of the immobilized yeast of the 6 samples.

The results show that the ethanol concentrations in green beer reduced slightly from 8.56 to 7.75 %v/v when the aeration time increased from 0 to 20h. In general, the longer the aeration time after pitching, the lower the ethanol concentration in the green beer because high oxygen content in wort inhibited ethanol formation (2).

Table 1. Effect of aeration time on primary fermentation parameters of high gravity brewing using immobilized yeast

	Aeration time (h)					
	0	4	8	12	16	20
Fermentation time (h)	144^d	120^c	120^c	108^b	84^a	84^a
Maximum of yeast concentration (10^6cell/ml)	48.13^f	56.04^e	58.13^d	72.08^c	87.92^b	91.46^a
Sugar consumption rate (g/l.h)	0.95^d	1.15^c	1.14^c	1.27^b	1.66^a	1.66^a
FAN consumption rate (mg/l.h)	1.36^e	1.95^d	1.97^d	2.22^c	2.86^b	3.03^a
Ethanol production rate (g/l.h)	0.47^d	0.54^c	0.54^c	0.60^b	0.74^a	0.73^a

Various small letters in row represent statistically significant difference at the level of p=0.05

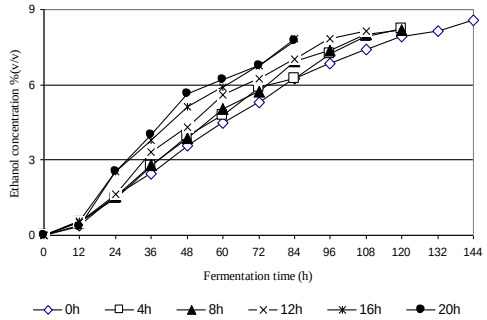

Fig.2 Effect of aeration time on ethanol concentration during high gravity brewing using immobilized yeast

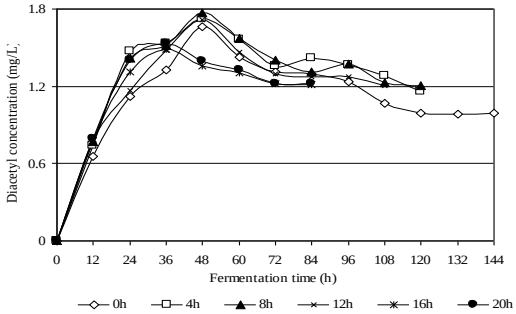

Fig.3 Effect of aeration time on diacetyl concentration during high gravity brewing using immobilized yeast

However, Table 1 show that aeration after pitching resulted in significantly faster ethanol production rate. The improvement in ethanol production rate can be primarily attributed to the reduction in time required to complete the fermentation.

It can be concluded that the suitable aeration time is 12h in high gravity brewing with the immobilized yeast. In this case, the fermentation time and ethanol concentration in the green beer reduced 25% and 4.67% in comparison with those of the sample only aerated before pitching.

Diacetyl formation

Fig.3 indicates the kinetics of diacetyl formation in the culture during the fermentation. Diacetyl was produced by yeast at the beginning of the fermentation; it was then reduced. The results illustrate that the maximal concentration of diacetyl in wort increased when the aeration time increased from 0 to 12h and the peak of diacetyl concentration occurred after the first 48h of fermentation. In contrast, the maximal concentration of diacetyl in wort decreased when the aeration time varied from 16 to 20h and the peak of diacetyl concentration occurred after the first 36h of fermentation. However, when the aeration time prolonged after pitching, the diacetyl concentration in green beer increased (Table 2). Therefore, prolongation of aeration time after pitching slows down diacetyl reduction of the immobilized yeast.

Formation of other volatile products

Table 2 illustrates the concentration of some volatile compounds in the green beer diluted to 5% (v/v) ethanol. Acetaldehyde flavour is considered as beer defect when this compound presents in excessive concentration. Its content in the green beer increased when aeration time prolonged. However, the acetaldehyde concentration in the 6 samples was below the flavour threshold (25mg/l) (7). Concerning higher alcohol, their concentration varied in a similar range like that reported by Jone H. L.(7).

The major ester in beer is ethyl acetate. Its concentration increased 61.01% when the aeration time increased from 0 to 8h. When the aeration time increased from 8 to 20h, the ethyl acetate concentration reduced 17.29%. According to Fujii T.(5), Willaert R.(14) and Verstrepen K. J.(15), too low or too high aeration time decreased alcohol acetyltransferase (AATase, EC 2.3.1.84) of *S. cerevisiae*. This enzyme synthesizes acetate ester from ethanol and acetyl coenzyme A.

Table 2. Concentration of volatile compounds (mg/l) in the green beers obtained by using immobilized yeast with different aeration times (the green beer was diluted by water to 5% ethanol content)

Volatile product (mg/l) in the green beer	Aeration time (h)					
	0	4	8	12	16	20
Diacetyl	0.58[a]	0.70[b]	0.73[c]	0.74[c]	0.77[d]	0.79[d]
Acetaldehyde	14.70	13.88	14.39	16.06	16.21	19.22
Ethyl acetate	15.44	15.84	24.86	21.07	20.74	20.56
1-Propanol	12.62	12.07	15.50	16.73	16.76	16.36
Isoamyl alcohol	66.08	58.09	70.09	54.93	65.73	82.54

Various small letters in row represent statistically significant difference at the level of p=0.05

IV. CONCLUSIONS

It was demonstrated that batch beer fermentation conducted under HGB with immobilized yeast and oxygen supplementation show good results. When the aeration time after pitching increased from 0 to 20 h, the fermentation time decreased 41.67%. However, the ethanol concentration in the green beer reduced 9.46%. The suitable aeration time is 12h in high gravity brewing with the immobilized yeast. In this case, the fermentation time reduced 25% compared to the control sample which was only aerated before pitching.

REFERENCES

1. *Analytica-EBC***;** Verlag Hans Carl Geranke-Fachverlag, Nurnberg, Germany, method 9.24.1 (1998)
2. *Boulton C. and Quain D.*; Brewing yeast and Frermentation, Blackwell Science Ltd, USA, 644p (2001)
3. *Depraetere S. A., Winderickx J. and Delvaux f. R.*; MBAA TQ 40(4), pp. 283–289 (2003)
4. *Fornairon-Bonnefond C., Demaretz V., Rosenfeld E. and Jean-Michel S.*; J. Biosci. Bioeng. 93(2), pp.176-182 (2002)
5. *Fujii T., Kobayashi O., Yoshimoto H., Furukawa S., and Tamai Y.*; Appl Environ Microbiol. 63(3) pp. 910–915 (1997)
6. *Gil M., Cabellos J. M., Arroyo T.; Prodanov M.,* Anal. Chim. Acta 563, pp. 145-153 (2006)
7. *Jones H. L., Margari tis A., Stewart R.*; J. Inst. Brew. 113(2), pp. 168-184 (2007)
8. *Kourkoutas Y., Bekatorou A., Banat I. M. ; Marchant R., Koutinas A. A.,* Food Microbiol. 21, pp. 377-397 (2004)
9. *O'connor-Cox E. S. C. and Ingledew W. M.*; J. Am. Soc. Brew. Chem. 48(1), pp. 26-32 (1990)
10. *O'connor-Cox E. S. C., Lodolo E. J. and Axcell B. C.*; J. Am. Soc. Brew. Chem. 51(3), pp. 97-107 (1993)
11. *Patkova J., Smogrovicova D., Domeny Z. and Bafrncova P.*; Biotechnol Lett 22, pp. 1173-1177 (2000).
12. *Rosenfeld E., Beauvoit B., Blondin B. and Jean-Michel S.*; Appl Environ Microbiol. 69 (1), pp. 113–121(2003)
13. *Silva D. P., Brányik T., Dragone G., Vicente A. A., Teixeira J. A. and Silva A. E.*, Chemical Papers 62 (1), pp. 34–41(2008)
14. *Willaert R., Nedovic V.*, J Chem Technol Biotechnol (81), pp. 1353-1367 (2006)
15. *Verstrepen K. J., Derdelinckx G., Dufour J. P., Winderickx J., Thevelein J. M., Pretorius I. S., Delvaux F. R.*; J. Biosci. Bioeng. 96(2), pp. 110-118 (2003)

Contact: Le Van Viet Man - Tel: (+848) 3864.6251, Email: lvvman@dch.hcmut.edu.vn
Dep. of Food Technology, Ho Chi Minh City University of Technology
No. 268, Ly Thuong Kiet Str., D.10, Ho Chi Minh City, Vietnam