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Application of fed-batch fermentation in high-gravity brewing

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Abstract. Economic demands to intensify the brewing process and increase the fermenter productivity have stimulated interest in high-gravity brewing. However, increasing wort sugar concentration can have a detrimental effect on fermentation performance, adversely affecting yeast physiology and altering the physical and flavor properties of the beer product. Many methods such as: higher pitching rates, higher fermentation temperatures, more efficient aeration than in conventional brewing, and immobilised yeast were used to improve this process.

This study focused on the application of fed-batch fermentation in high-gravity brewing. Two fed-batch cultures with different specific gravity of the supplemented worts were examined. The specific gravity after the supplementation was the same. Despite the higher primary fermentation time, both fed-batch cultures achieved higher concentration of ethanol and lower diacetyl content than those in batch fermentation. These resulted in the possibility of increasing the final beer volume and of shortening the maturation time.

Keywords: Fed-batch fermentation, high gravity brewing, *Saccharomyces cerevisiae*.

1. Introduction

The traditional brewing is generally applied to ferment worts with 11–12°Bx specific gravity to produce beers of 4–5% (v/v) ethanol. With the need to increase productivity and consequently competitiveness on the market, many breweries are changing their methods and processes for producing beer. A major innovation in brewing is high gravity brewing technology–the fermentation of wort containing 16 g or more of dissolved solids per 100 g of wort. This technology has become popular due to such advantages as increasing plant efficiency and capacity, reducing energy, labour and capital costs, as well as increasing in ethanol yield per unit of fermentable extract. Moreover, it can help improve beer stability, produce smoother taste and greater flexibility to the final product [1-4]. On the other hand, the drawbacks of this process include decreased material efficiency, as well as reduced foam stability and flavour matching [5]. In addition, during fermentation of high-gravity wort, the yeast are exposed to extreme conditions: increased osmotic pressure and increased toxicity of produced ethanol [2,6,7], nutrient limitation especially concerning oxygen and assimilable nitrogen [1].

The arising problems have been solved by using higher pitching rates, higher fermentation

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temperatures [1], more efficient aeration than in conventional brewing [8] and immobilised yeast [2]. One of the possibilities to reduce the detrimental factors acting in high-gravity brewing could be fed-batch fermentation. This technique is commonly used in microbial fermentation because it combines the advantages of both batch and continuous fermentations. Fed-batch fermentation is used to prevent or decrease substrate-associated growth inhibition by controlling nutrient supply. Process is started as a batch process with a small amount of biomass and substrate in the fermentor. Then the substrate feed is applied when most of the initially added substrate has been consumed. This procedure helps to increase the total substrate content in the fermentor, while always maintaining a low substrate concentration during fermentation for reducing the negative effect of osmotic pressure on yeast. Thus, fed-batch fermentation allows decreasing the initial yeast concentration and the cost of inoculum propagation.

This study focused on the primary fermentation in brewing. Fed-batch cultures were examined in order to increase ethanol content in the green beer.

2. Materials and methods

2.1. Wort production

16 °Bx wort was produced from 80% barley malt (imported from Australia) and 20% rice (Viet Nam), by decoction mashing. The 28 °Bx and 24 °Bx worts were prepared by vacuum concentration at 60°C from the 16 °Bx wort.

2.2. Microorganism and fermentation conditions.

Yeast used in this study was a lager strain of *Saccharomyces cerevisiae*, supplied by Foster Tien Giang Ltd. Company. The pre-inoculum was obtained from yeast cultures maintained on malt-agar slants at 4°C. The inoculum propagation was carried out in the 10°Bx wort. The pitching rate was about 10×10^6 viable cells mL^{-1}. All fermentations were performed in a bioreator BIOSTAT B (B.Braun Biotech International) at 17°C with 1,5 L of 16°Bx wort. In fed-batch fermentation, 2 samples were examined: 1,5 L of fermenting wort was supplemented by 1,5 L of 28°Bx wort or 2,5 L of 24°Bx wort. In the 2 fed-batch samples, the specific gravity in the medium after supplementation was the same (16°Bx).

2.3. Analytical methods

During the fermentation, the evolution of pH, total number of yeast cell and viability, wort specific gravity, reducing sugar, free amino nitrogen, ethanol and diacetyl contents were examined. The number of yeast cell was determined by using an Improved Neubauer Haemocytometer at x400 magnification with a light microscope. Yeast viability was determined by using the methylene blue staining [9]. After removing yeast biomass by centrifugation (6000 rpm, 15 mins, 4°C), the specific gravity was measured by a refractometer. Concentration of reducing sugars was quantified by spectrophotometric method using the dinitrosalicylic acid reagent [10]. Free amino nitrogen (FAN) content was measured by spectrophotometric method, using ninhydrin reagent [11]. Ethanol concentration was determined by a method based on distillation and density quantification [12]. Concentration of diacetyl was determined by spectrophotometric method using O – phenylendiamin reagent [11]. The sugar uptake rate (g/L.h) was calculated as the ratio of the reducing sugar content (g/L) consumed by yeast to the fermentation time (h).

3. Results and discussion

3.1. Yeast growth

Yeast growth during the fermentation is presented in Fig. 1.

In the batch fermentation, during the first 60 hours, yeast cell number in the culture increased quickly because the wort was rich in oxygen and nutrients. The maximum concentration of cells (approximately 120 × 10^6 cells/mL) was obtained after 108 hours. After that, the biomass decreased progressively. Thus the 108th fermenting hour was chosen as the moment for wort supplementation in the two fed-batch cultures.

In fed-batch fermentation, after fresh wort feeding, the yeast concentration in sample 2 was lower than that in sample 1. This was due to the higher volume of wort supplementation. Our experimental results showed that the maximum yeast concentration reached in the two samples was similar; however, the growing time of yeast in sample 2 was 24 hours longer than that in sample 1 (Fig. 1).

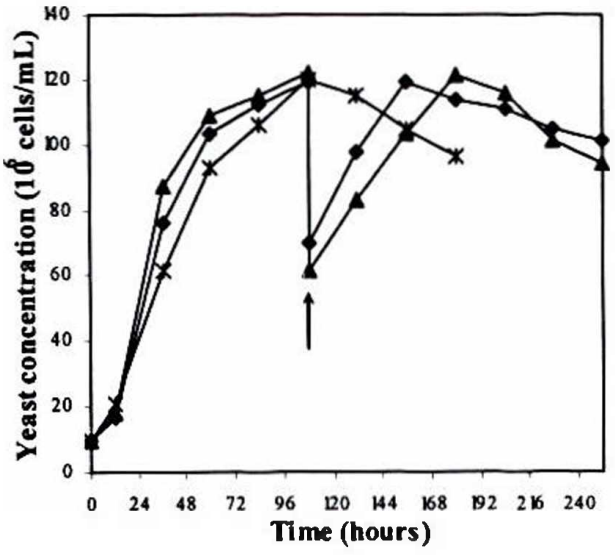

Fig. 1. Kinetics of yeast growth during the fermentation of 1,5 L of 16 °Bx worts: (✱) batch culture, (◆) fed-batch culture 1: supplemented with 1 L of 28 °Bx wort after 108th fermenting hours, (▲) fed-batch culture 2: supplemented with 1,5 L of 24 °Bx wort.

3.2. Substrate assimilation

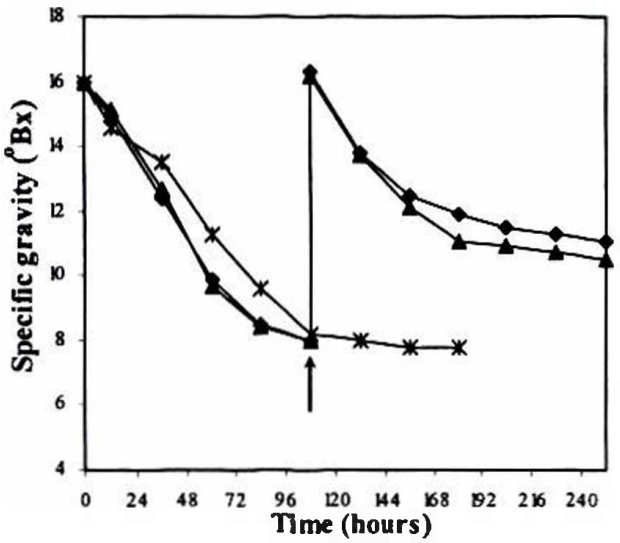

Fig. 2. Kinetics of specific gravity during the fermentation of 1,5 L of 16 °Bx worts: (✱) batch culture, (◆) fed-batch culture 1: supplemented with 1 L of 28 °Bx wort after 108th fermenting hours, (▲) fed-batch culture 2: supplemented with 1,5 L of 24 °Bx wort.

Fig. 2 shows the evolution of the specific gravity during the batch and fed-batch fermentations.

In the batch culture, after 108 fermenting hours, the specific gravity did not change significantly. It was due to the low concentration of substrates and high concentration of some toxic metabolites (ethanol and other higher alcohols) produced by yeast in the medium. It can be confirmed that the chosen moment – the 108th fermenting hour for fresh wort supplementation was reasonable.

In the fed-batch cultures, fresh wort supplementation increased the nutrient contents and decreased ethanol concentration in the fermenting medium. Therefore, the yeast propagation continued (Fig. 1) and the specific gravity decreased significantly, particularly during the next 48 hours after supplementing. From the 156th hour, the specific gravity decreased with a slower rate: the substrate assimilation rate in fed-batch culture 1 was lower than that in fed-batch culture 2. At the end of the primary fermentation, the specific

gravity of green beer in both fed-batch cultures were still rather higher than that in the batch culture. Perhaps the increased concentration of ethanol in the medium caused a harmful effect on yeast cells, which led the fermentation to be sluggish or even stuck.

In this study, the primary fermentation was considered to have completed when the ethanol concentration increased less than 0,3 % (v/v) in the successive 24 fermenting hours. The fermentation time in fed-batch cultures 1 and 2 was 204 hours and 228 hours respectively. The final specific gravity in fed-batch culture 2 was lower than that in fed-batch culture 1 (10,7 °Bx vs 11,5 °Bx). Thus, it can be concluded that in fed-batch culture 2, yeast consumed more nutrients than in fed-batch culture 1.

Yeast growth involves the uptake of Free Amino Nitrogen (FAN) for the synthesis of cellular protein (Fig. 3).

Fig. 3 indicated that during the first 84 hours, FAN concentration decreased sharply in the 3 cultures. After that, it began to increase slightly in batch fermentation, that may be due to the autolysis of dead cells. This phenomenon led to a release of intracellular nitrogen based compounds.

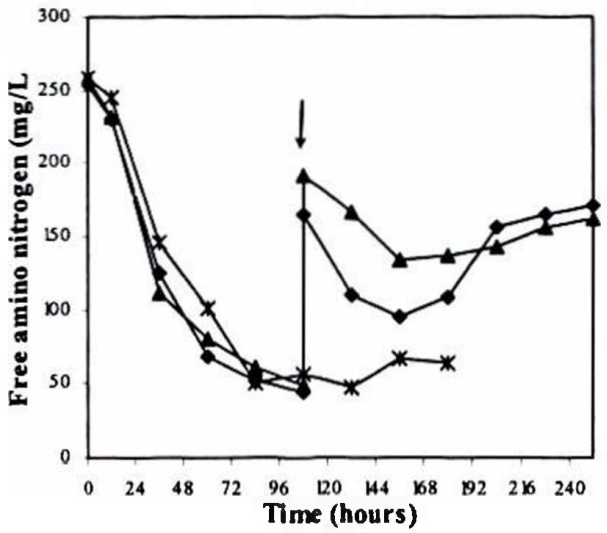

Fig. 3. Kinetics of free amino nitrogen assimilation during the fermentation of 1,5 L of 16 °Bx worts: (✱) batch culture, (◆) fed-batch culture 1: supplemented with 1 L of 28 °Bx wort after 108th fermenting hours, (▲) fed-batch culture 2: supplemented with 1,5 L of 24 °Bx wort.

After wort supplementation, the FAN concentration in fed-batch culture 2 was higher than that in fed-batch culture 1. During the next 48 hours, FAN concentration decreased due to the cell growth. Then it augmented when the death phase of yeast began (after the 108th fermenting hour – Fig. 3) and the autolysis took place. High ethanol concentration was another harmful factor to the yeast's viability, increasing the number of dead cells.

During the last hours of the primary fermentation, the FAN level in fed-batch culture 1 increased more strongly than that in fed-batch culture 2. It could be reasoned that at the 156th hour, the death phase of yeast in fed-batch culture 1 began while in fed-batch culture 2, the yeast growth still continued. This phenomenon affirmed the results obtained in figure 2: after 156 fermenting hours, the yeast activity in fed-batch culture 2 was higher than that in fed-batch culture 1 and the specific gravity in fed-batch culture 2 was lower than that in fed-batch culture 1.

3.3. Metabolite production

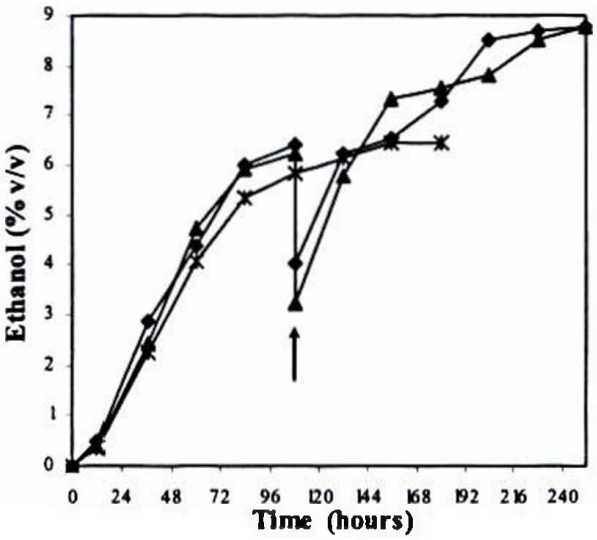

Fig. 4. Kinetics of ethanol production during the fermentation of 1,5 L of 16 °Bx worts: (✱) batch culture, (◆) fed-batch culture 1: supplemented with 1 L of 28 °Bx wort after 108th fermenting hours, (▲) fed-batch culture 2: supplemented with 1,5 L of 24 °Bx wort.

Fig. 4 showed that in batch fermentation, the final ethanol concentration could reach only 6,46 % (v/v) due to the low nutrient content and high ethanol level. In fed-batch fermentation, those harmful effects could be overcome, resulting in higher final ethanol content.

The final ethanol concentrations were 8,52% (v/v) in both fed-batch cultures, although the residual sugar content was still rather high. It can be supposed that the activity of the *Saccharomyces cerevisiae* strain used in this study decreased remarkably at that ethanol concentration.

The obtained results in figure 4 could explain for the data in figure 3. In fed-batch culture 1, from the 180th hour to the 204th hour, FAN concentration increased sharply (Fig. 3); during this period, the increase in ethanol content from 7,27 % (v/v) to 8,52 % (v/v) was observed (Fig. 4). Thus, it was evident that the increase in ethanol content in the culture had a bad effect on yeast viability. In fed-batch culture 2, the same phenomenon was also observed. From the 204th hour to the 228th hour, the ethanol concentration increased from 7,79 % (v/v) to 8,52 % (v/v), the FAN concentration also augmented in the medium due to the autolysis of dead cells.

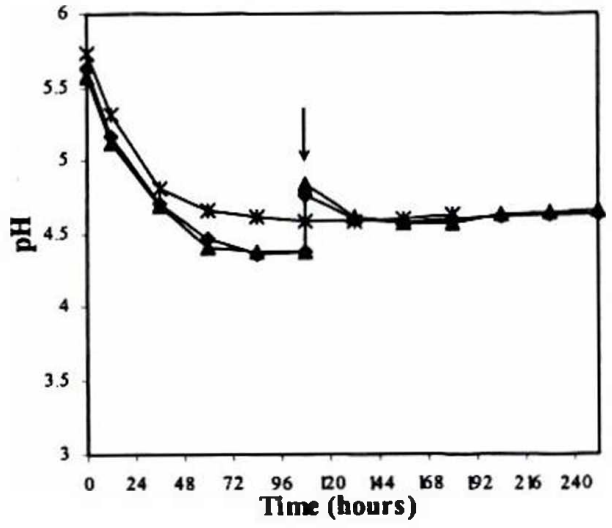

Fig. 5. Change in pH during the fermentation of 1,5 L of 16 °Bx worts: (∗) batch culture, (◆) fed-batch culture 1: supplemented with 1 L of 28 °Bx wort after 108th fermenting hours, (▲) fed-batch culture 2: supplemented with 1,5 L of 24 °Bx wort.

During the alcoholic fermentation, the decrease of pH is related to the formation of CO_2 and organic acids of yeast cells. Besides, the increase of pH marks the beginning of yeast autolysis.

Fig. 5 presented the evolution of pH in batch and fed-batch fermentations. There was no significant difference in pH values of the green beers in the 3 cultures.

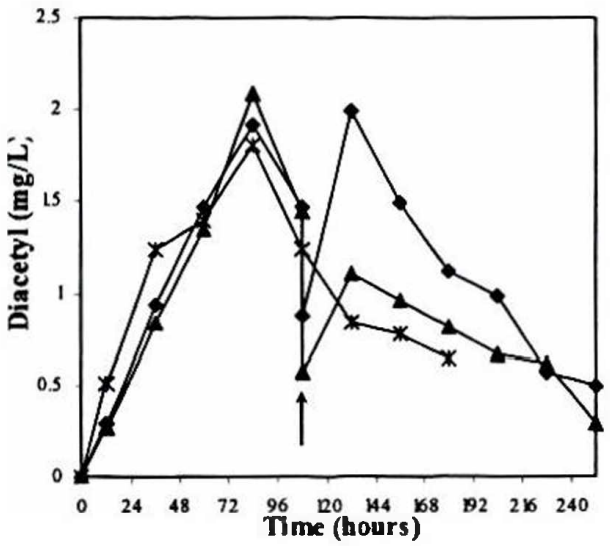

Fig. 6. Evolution of diacetyl concentration during the fermentation of 1,5 L of 16 °Bx worts: (∗) batch culture, (◆) fed-batch culture 1: supplemented with 1 L of 28 °Bx wort after 108th fermenting hours, (▲) fed-batch culture 2: supplemented with 1,5 L of 24 °Bx wort.

Diacetyl is one of the most important by – products in alcoholic fermentation. At low levels, it gives beer a slick mouthfeel; at higher levels, the flavor becomes buttery and that decreases the sensory properties of the final product.

Fig. 6 showed that the diacetyl level increased during the first 84 hours and reached the maximum concentration (approximately 2 mg/L). Many authors affirmed that diacetyl formation is closely related to the yeast growth, particularly to the valine biosynthesis [13]. In

comparison with the obtained results in Fig. 1, the intensive growth of yeast was observed during this period. Then the diacetyl content in the fermenting media decreased by reduction reaction catalyzed by the enzyme system in yeast. This result was similar to those in many previous studies [13,14].

After fresh wort supplementation, diacetyl concentrations in the 2 fed-batch cultures increased again due to yeast growth and then decreased. It can be noted that the second peak of diacetyl formation in fed-batch culture 2 was much lower than that in fed-batch culture 1. The explanation can be that the FAN content in fed-batch culture 2 was much higher than in fed-batch culture 1. Low diacetyl level in green beer is an advantage in brewing because the maturation time will be shorter.

Table 1 presented the parameters of the primary fermentation in beer production using batch and fed-batch cultures.

In the fed-batch cultures, in spite of the longer primary fermentation time, the sugar contents assimilated by yeast were higher than that in the batch culture. Besides, the sugar uptake rate in fed-batch culture 1 and 2 were 30,2 % and 20,6 % higher than that in the batch culture.

The final ethanol concentration in the fed-batch fermentations was 32 % higher than that in the batch fermentation. This is a very important advantage of high gravity brewing because the higher concentrated beer we achieve, the more volume of final beer we produce. The results from table 1 showed that, the volume of final beer in the fed-batch fermentations was much higher than that in the batch fermentation.

Table 1. Parameters of the primary fermentation in beer production using batch and fed-batch cultures

	Batch fermentation[a]	Fed-batch fermentation 1[b]	Fed-batch fermentation 2[c]
Fermentation time (h)	156	204	228
Sugar content assimilated by yeast (g/L)	97,69	167,05	173,32
Sugar uptake rate (g/L.h)	0,63	0,82	0,76
Ethanol concentration in green beer (g/L)	50,9	67,2	67,2
Diacetyl concentration in green beer (mg/L)	0,8	1,0	0,6
Volume of final beer after being diluted to ethanol concentration of 5 % (v/v)[d].	1,50 L of 16 °Bx wort → 1.94 L of beer. 2,50 L of 16 °Bx wort → 3,23 L of beer. 3 L of 16 °Bx wort → 3,88 L of beer.	1,50 L of 16 °Bx wort and 1,00 L of 28 °Bx wort → 4,26 L of beer	1,50L of 16 °Bx wort and 1,50 L of 24 °Bx wort → 5,11 L of beer.

[a] The initial volume of the wort was 1,50 L, the initial specific gravity was 16 °Bx.

[b] The initial volume of the wort was 1,50 L, the initial specific gravity was 16 °Bx. The culture was supplemented with 1 L of 28 °Bx wort. The specific gravity after supplementing was 16 °Bx.

[c] The initial volume of the wort was 1,50 L, the initial specific gravity was 16 °Bx. The culture was supplemented with 1,50 L of 24 °Bx wort. The specific gravity after supplementing was 16 °Bx.

[d] It was supposed that the ethanol content in the green beer did not augmented during the secondary fermentation.

Although the ethanol content in the two fed-batch cultures was similar, fed-batch culture 2 was considered to be more efficient than fed-batch culture 1 for two reasons. Firstly, lower diacetyl of green beer in fed-batch culture 2 made the maturation time become shorter. Secondly, the pitching rate in fed-batch culture 2 was lower than in fed-batch culture 1 if the volumes of final beer in the two cultures were similar.

4. Conclusion

Fed-batch cultures have shown to be more advantageous than batch fermentation in high gravity brewing. In fed-batch fermentation, the green beer contained higher ethanol concentration and lower diacetyl content. In this study, 2 fed-batch cultures were realized. It was concluded that, the ratio 1 : 1 of the volume of wort for supplementing to the initial volume of wort in the fermentor was more efficient than the ratio 2 : 3.

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