



Development of the Fed-batch Culture of Brewing Yeast

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Agenda

- 1. Background of This Study**
- 2. Outline of the Fed-batch Culture**
- 3. Application of Fed-batch Culture to Brewing Yeast**
- 4. Fermentation Performance of Fed-batch Cultured Brewing Yeast**
- 5. Conclusions**

Background of This Study

Scale-up Scheme of the Brewing Yeast Culture

The typical culture scheme needs many scale-up steps.

Typical

scale-up

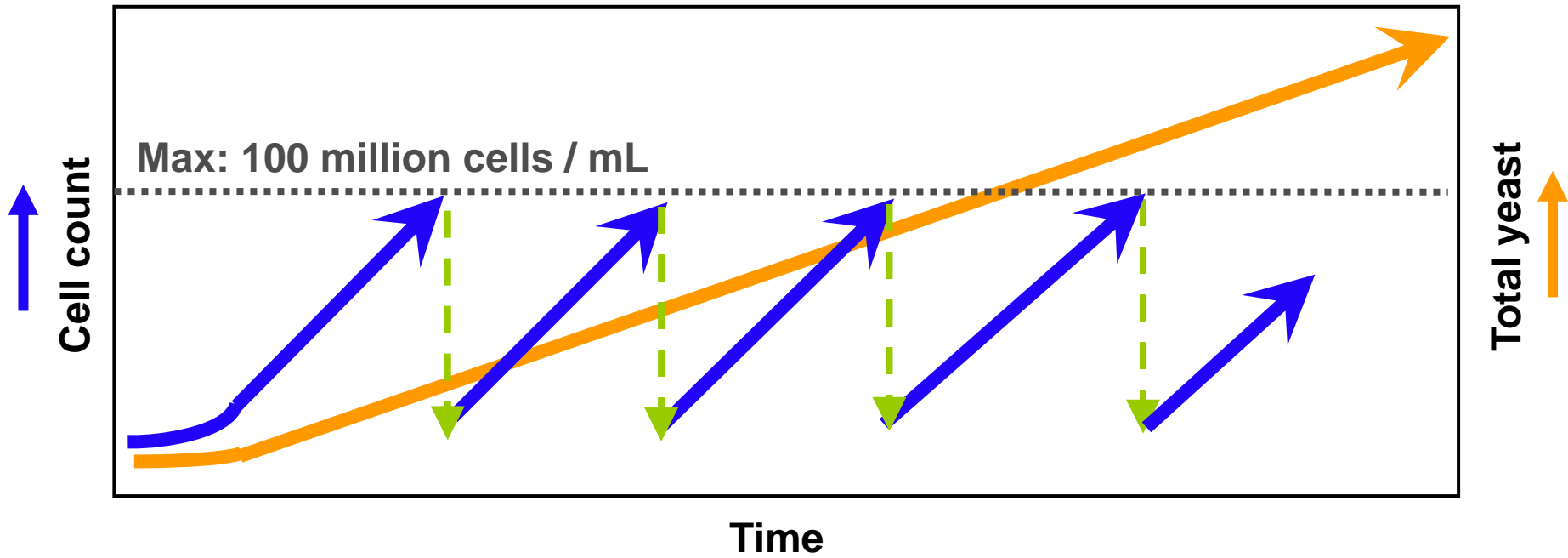
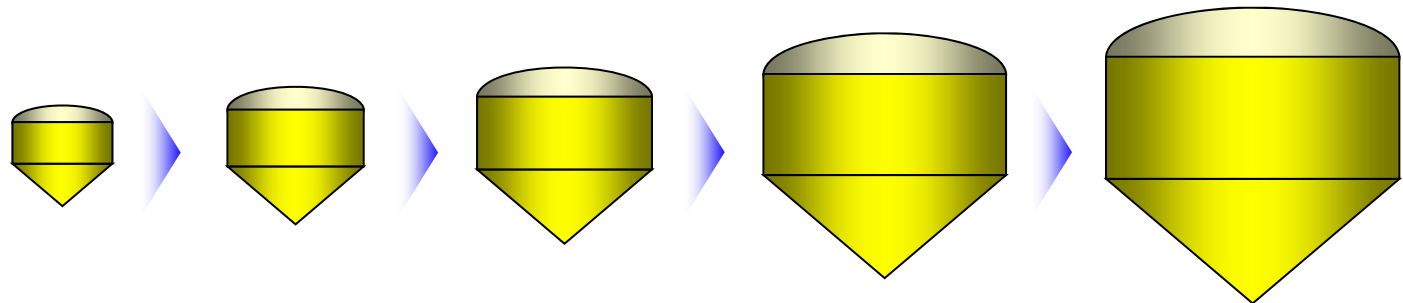


Figure 1. The typical scale-up scheme of the brewing yeast culture

Scale-up Scheme of the Brewing Yeast Culture

If the maximum cell count is elevated, the number of scale-up steps can be reduced.

Ideal

scale-up

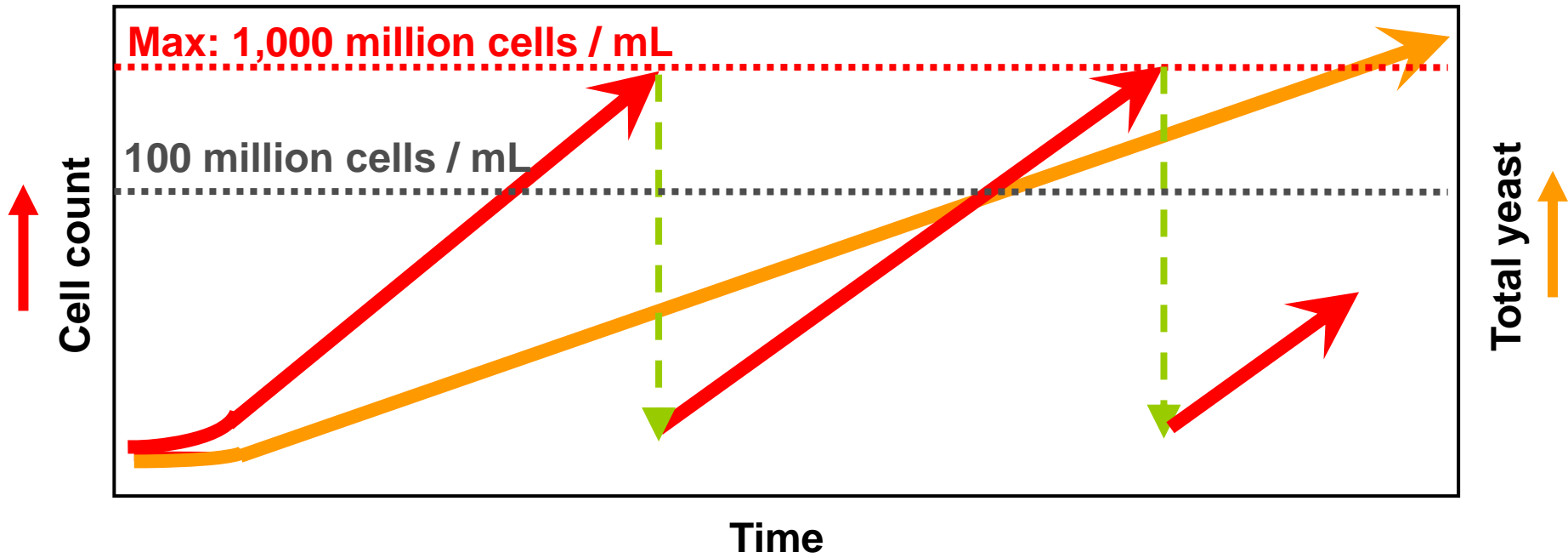
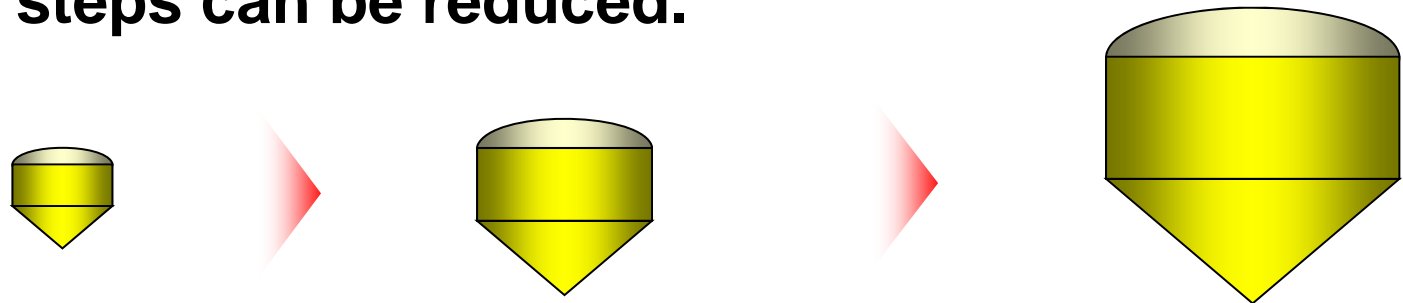


Figure 2. The ideal scale-up scheme of the brewing yeast culture

Need of Wort in Yeast Culture

We need fresh wort at every scale-up step of yeast culture. Yeast culture and wort production are processes in time constraint with respect to each other.

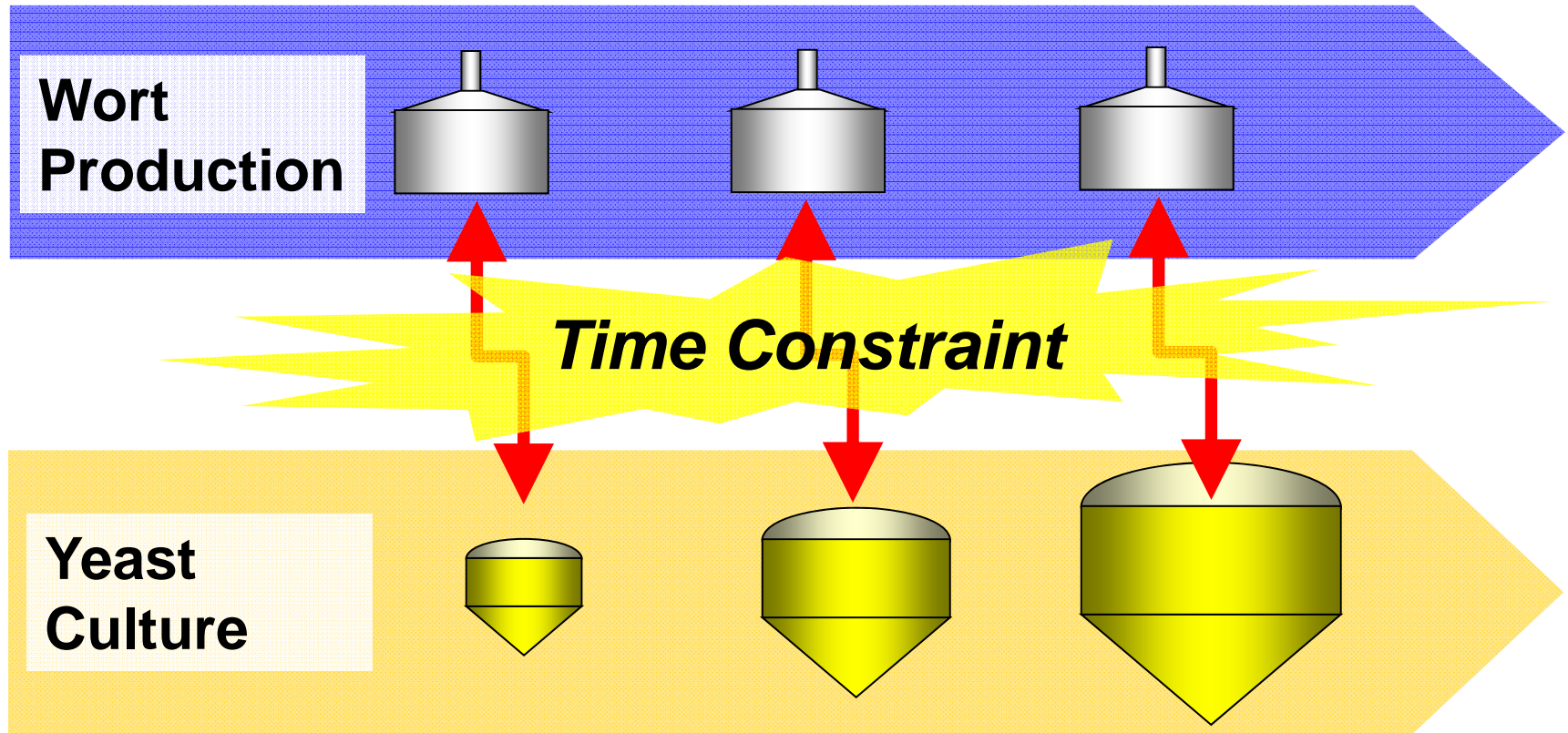


Figure 3. Relationship between the wort production and the yeast culture

Objectives of This Study

Conventional yeast culture process

- Operation : Need a lot of scale-up steps
- Flexibility : Restriction by wort production timing

Objectives of this study :

1. Elevating maximum cell count to reduce scale-up steps
2. Developing a culture medium that is independent of wort production

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Table 1. Comparison between batch and fed-batch culture.

	Conventional batch culture	Fed-batch culture
Medium	wort	synthetic medium
Yield	low	high
Maximum cell count	low	high

Focus on fed-batch culture method without wort

Outline of the Fed-batch Culture

The Principle of Fed-batch Culture in Yeast Culture

In a fed-batch culture of yeast, sugar concentration is kept very low ($\ll 0.1$ Plato) to avoid the ethanol fermentation which inhibits cell growth.

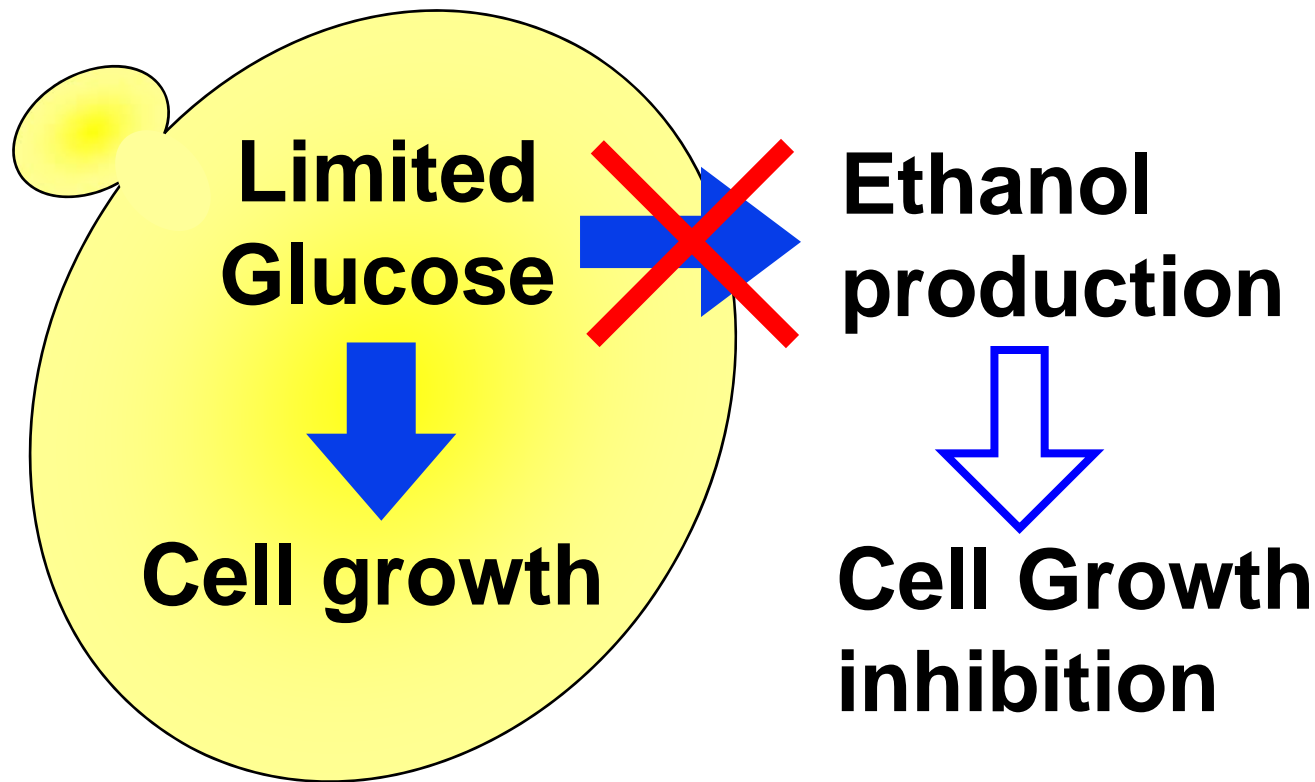


Figure 4. Maximization of the sugar utilization to cell growth in the fed-batch culture

The System of Baker's Yeast Culture

Baker's yeast is cultured by the fed-batch culture system. Feed medium, which contains sugar, is properly added into culture medium in order to keep sugar concentration very low.

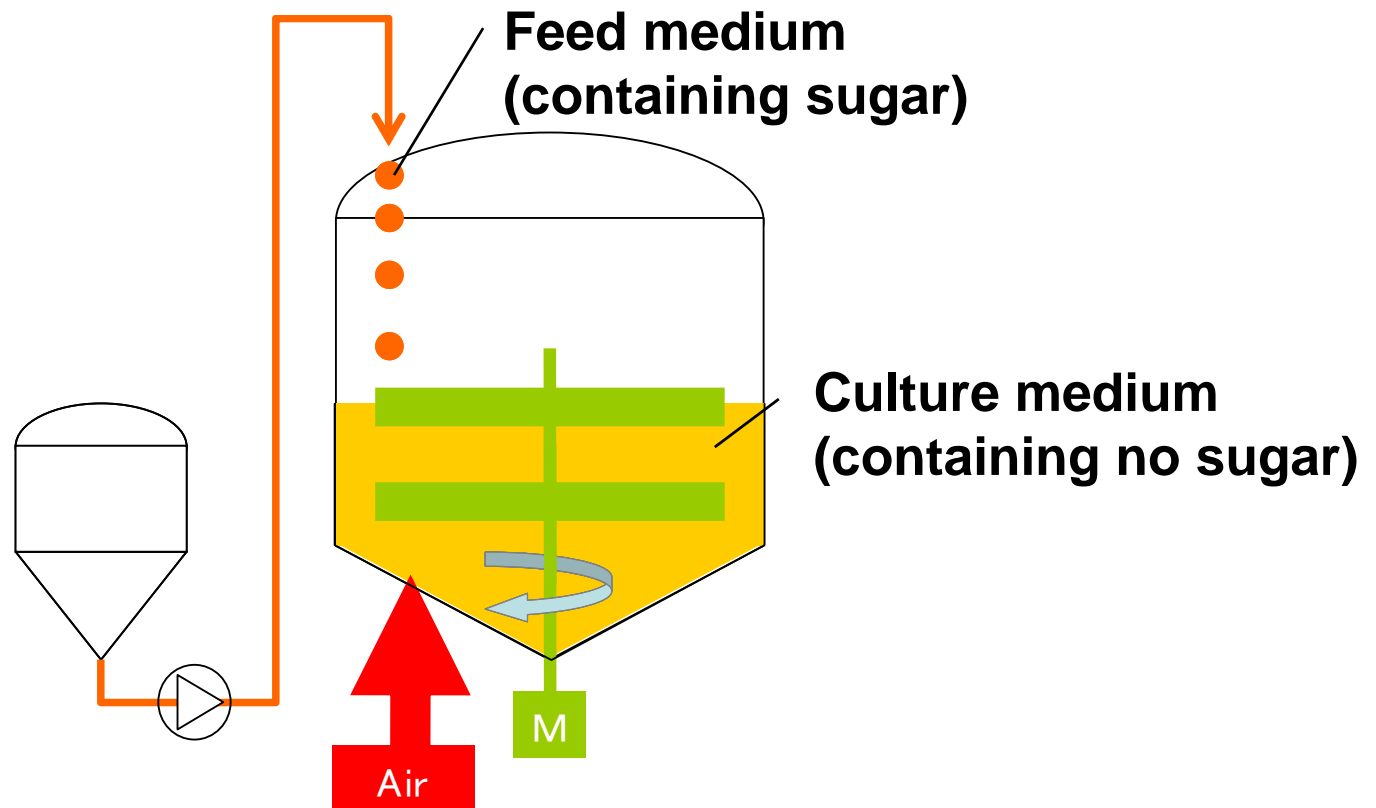


Figure 5. Outline of the fed-batch culture system.

Application of Fed-batch Culture to Brewing Yeast

Fed-batch Culture Trial of Brewing Yeast

We tried to apply fed-batch culture to brewing yeast culture. In reference to baker's yeast culture, we use molasses, which is by-product of the refining of sugarcane or sugar beets into sugar, as feed medium.

Table 2. Composition of the fed-batch medium.

Feed	Molasses
Culture medium	Corn steep liquor
	Ammonium sulfate
	Ammonium phosphate
	Other salts, minerals, and vitamins

Growth Profile of Brewing Yeast

In fed-batch culture, the maximum cell count of the brewing yeast reaches about 1,000 million cells/ml which is 10 times higher than with the conventional batch culture method.

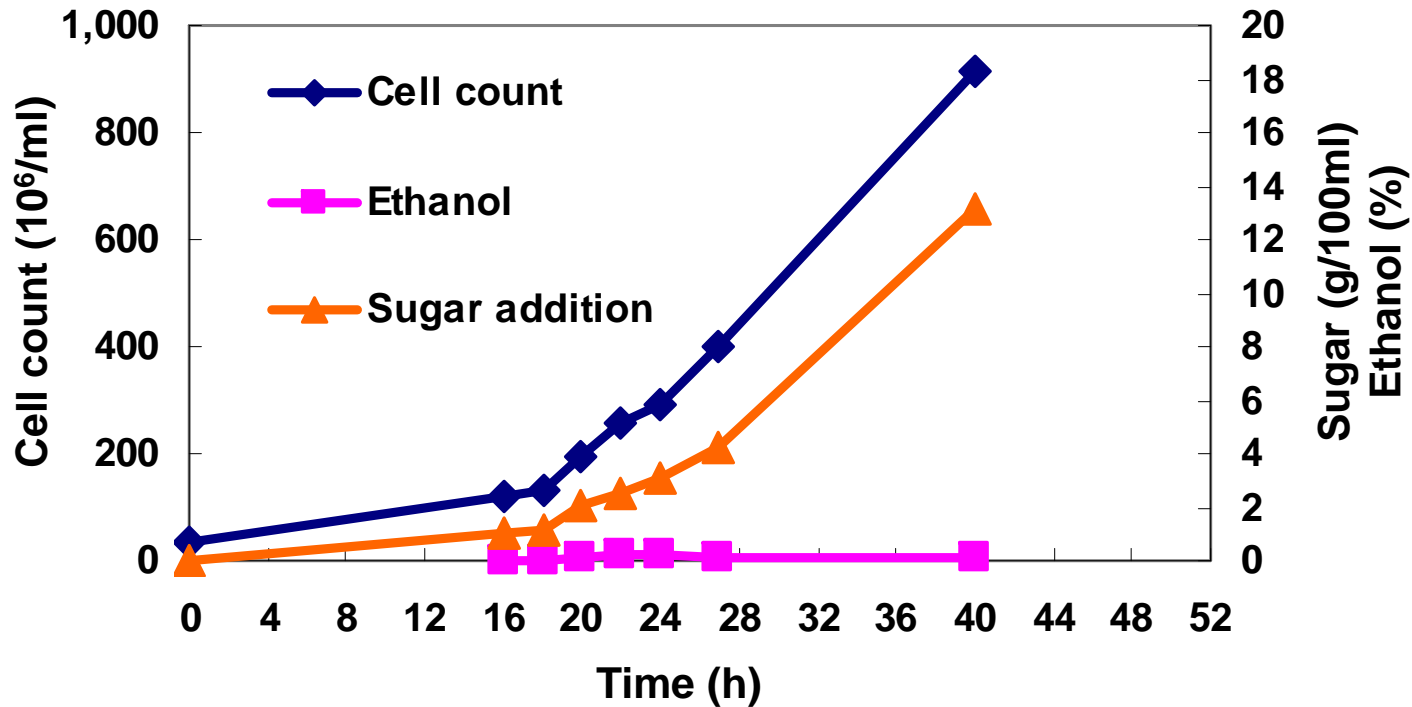


Figure 6. Growth profiles of bottom-fermenting yeast in fed-batch culture.
Conditions: 20L scale propagator, 30°C, pH 4.2, 20L/min air, 300rpm stir, bottom- fermenting yeast strain

Improvement of Medium Composition

Molasses varies considerably in quality and its viscosity is too high for it to be handled.

To solve these problems, we developed a fed-batch medium without molasses.

Table 3. Composition of the Improved fed-batch medium without molasses.

Feed	Sugar
Culture medium	Yeast extract
	Ammonium sulfate
	Ammonium phosphate
	Other salts, minerals, and vitamins

Growth Profiles in the Improved Medium

In the improved medium instead of molasses, the maximum cell count reached about 1,000 million cells/ml as well.

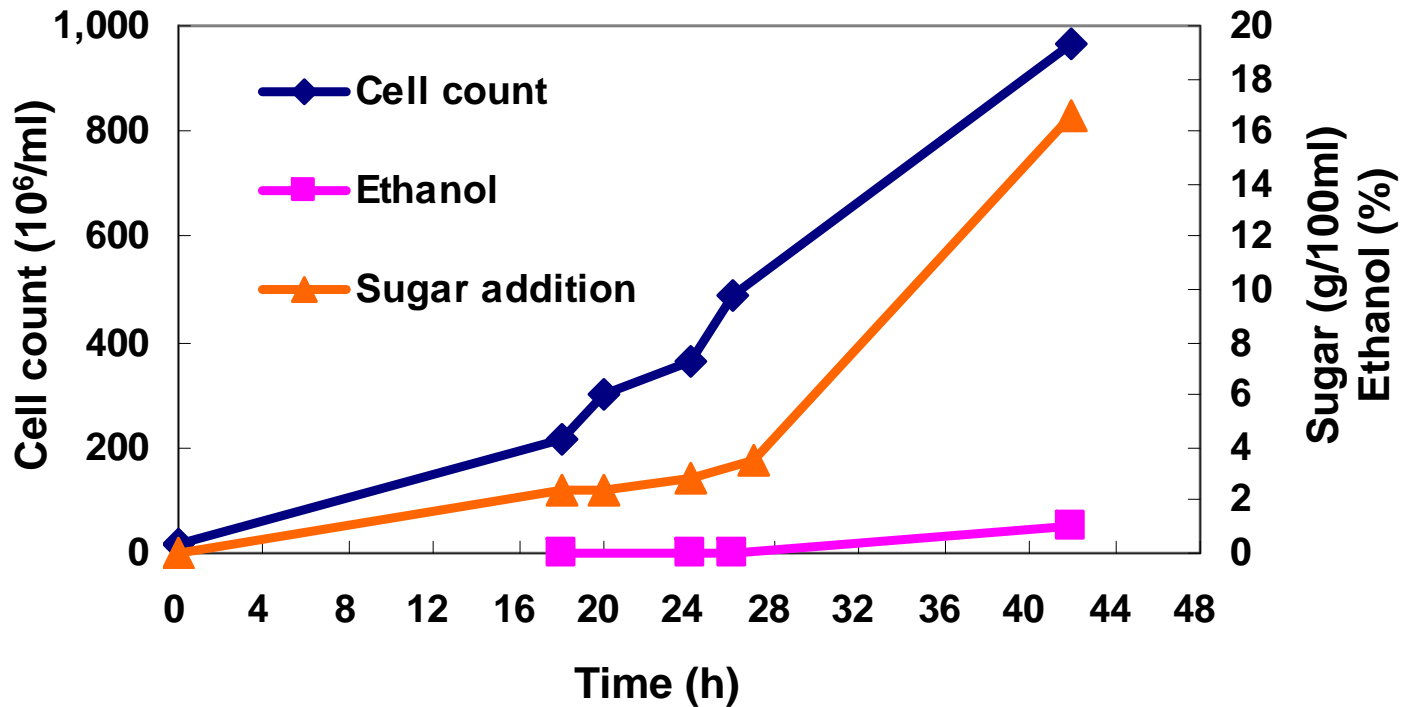


Figure 7. Growth profiles of bottom fermenting yeast in fed-batch culture using the improved medium.

Condition: 20L scale propagator, 20°C, pH 4.2, 20L/min air, 300rpm stir bottom- fermenting yeast strain

Comparison with Conventional Culture Method

Fed-batch culture improved yield and maximum cell count compared to conventional batch culture.

Table 4. Major technical data in the brewing yeast culture.

Condition: 10L scale propagator, 20°C, 5L/min air, 800rpm stir

	Conventional batch culture	Fed-batch culture
Medium	wort	synthetic medium
Yield (million cells/ml per Plato)	34.8	105.2
Maximum cell count (million cells/ml)	120	989
Viability (%)	99.4	98.8

Summary of Fed-batch Yeast Culture

Advantages of the fed-batch culture are that

- (1) Maximum cell count is very high and the number of scale-up steps can be drastically reduced.
- (2) Yeast culture schedule is independent of wort production.

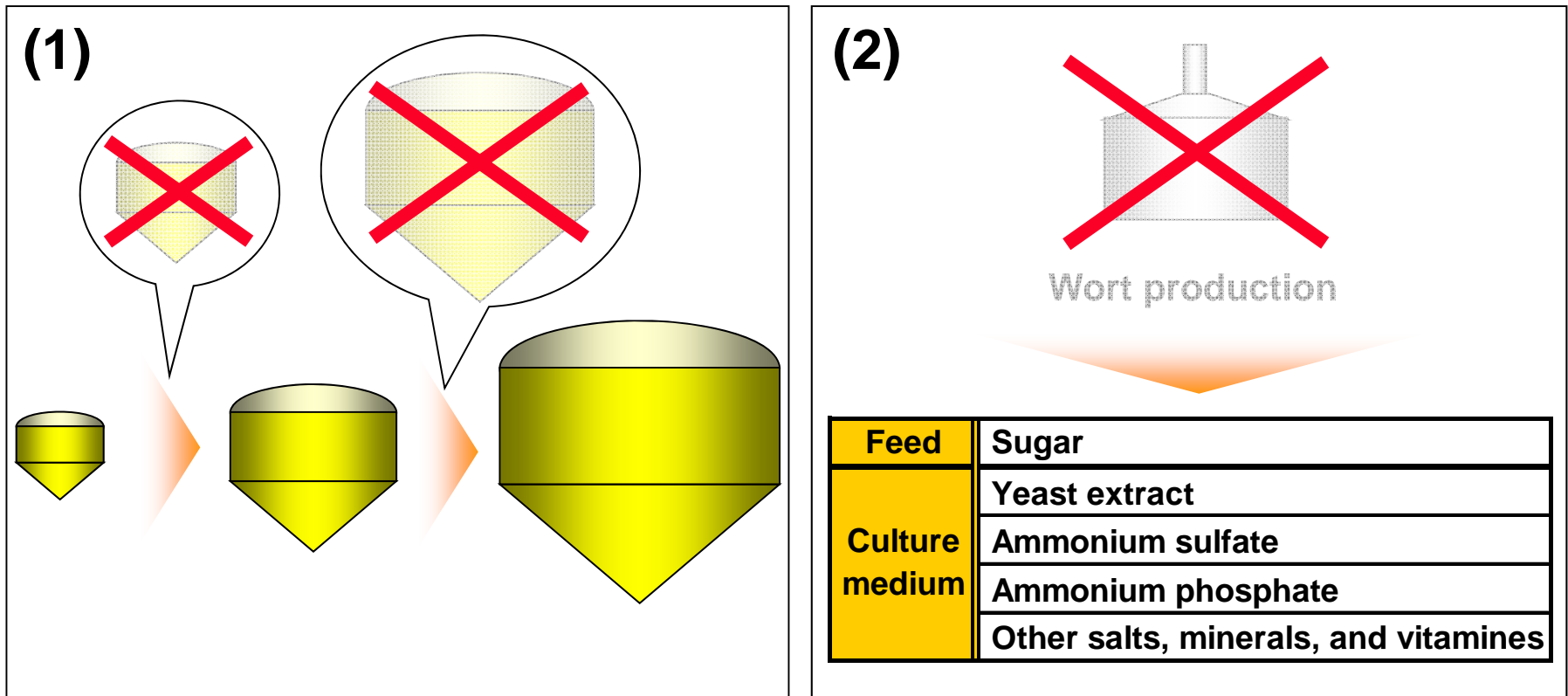


Figure 8. Advantages of the fed-batch culture method in the brewing yeast culture.

Fermentation Performance of Fed-batch Cultured Brewing Yeast

Fermentation Performance

Fed-batch cultured yeast can ferment beer as well as cropped yeast though it shows slightly slower attenuation speed.

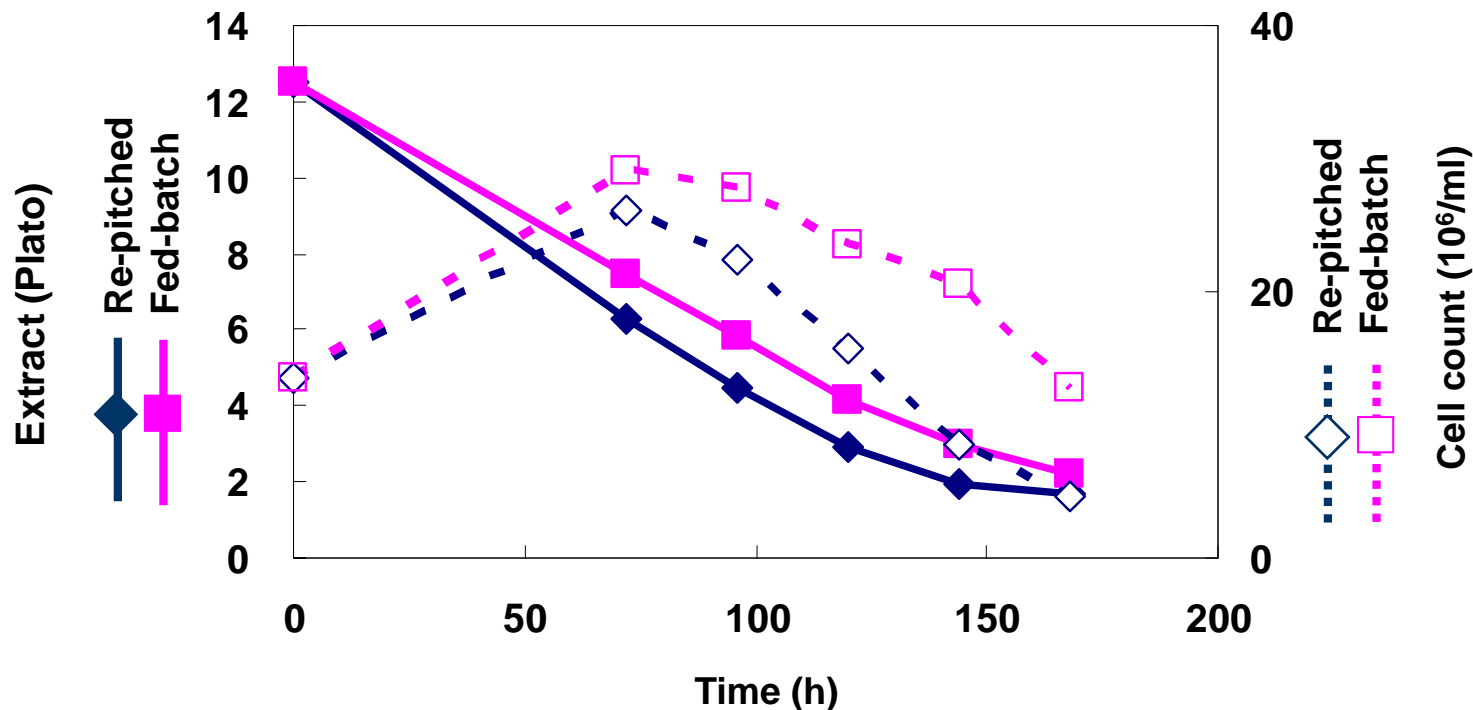


Figure 9. Fermentation performance of fed-batch cultured yeast.

Yeast was re-pitched from all malt beer or fed-batch cultured in the improved medium. Fermentation conditions: All malt wort, Pitched at 12×10^6 cells, 12° C, 500mL scale, Yeast: re-pitched from all malt beer fermentation (blue, control) and fed-batch cultured yeast (pink, test). Both are bottom-fermenting yeast strains.

Characterization of flavor profile

Typical fermentation flavor compounds and sensory evaluation are almost equal between fed-batch and re-pitched yeast.

Table 5. Young beer analysis and sensory evaluation results.

*Yeast were re-pitched from all malt beer or fed-batch cultured in improved medium.
Fermentation condition: All malt wort, Pitched at 12×10^6 cells, 12° C, 200L scale.*

		Fed-batch	Re-pitched
Acetaldehyde	mg/l	12.7	12.8
Ethyl acetate	mg/l	21.8	19.4
Isoamyl acetate	mg/l	1.8	1.7
Total amino acid	mg/l	852	812
Sensory evaluation		Estery Sharp Fatty	Sweaty Fatty

Future work

For further improvement of fermentation performance of fed-batch cultured brewing yeast, we will investigate culture and habituation condition.

Culture condition

- **Temperature**

Habituation conditions (after cultivation)

- **Temperature**
- **Aeration**
- **Conditioned medium**

Conclusions

Conclusions

- 1. By the fed-batch culture method, the maximum cell count can be increased by 10 times in comparison to conventional batch culture method.**
- 2. It is expected that the number of yeast scale-up steps can be drastically reduced by applying the fed-batch culture method to the brewing yeast.**
- 3. We developed the synthetic fed-batch medium without molasses that enabled easy-handling for us in the brewhouse operation.**
- 4. It was suggested that the fed-batch cultured yeast can produce beer as well as cropped yeast.**

Thank you very much for your attention !

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