0273-2289/95/5303—0277$05.40

Rapid Fermentation of Beer Using an Immobilized Yeast Multistage Bioreactor

Control of Sulfite Formation

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Received June 13, 1994; Accepted September 15, 1994

ABSTRACT

The characteristics of yeast sulfite metabolism in a multistage bioreactor system for beer fermentation were investigated. No sulfite was produced in the continuous stirred-tank reactor (CSTR). However, large amounts were produced in the packed-bed reactor (PBR). Production of sulfite in the PBR seems to be inevitable when it is operated continuously. In order to control the sulfite level in the young beer, the yeast needs to be reactivated into the growth phase. One possible strategy to achieve this is to aerate and periodically remove yeast clogged in the reactor once every 6–7 months before the sulfite level exceeds a given concentration (e.g., 20 mg/L).

It was confirmed that sulfite production is closely related to the growth condition of the yeast and is therefore important to consider in the control strategy for sulfite when using the immobilized yeast reactor for beer production.

Index Entries: Immobilization; bioreactor; yeast; sulfite, sulfur dioxide; beer brewing.

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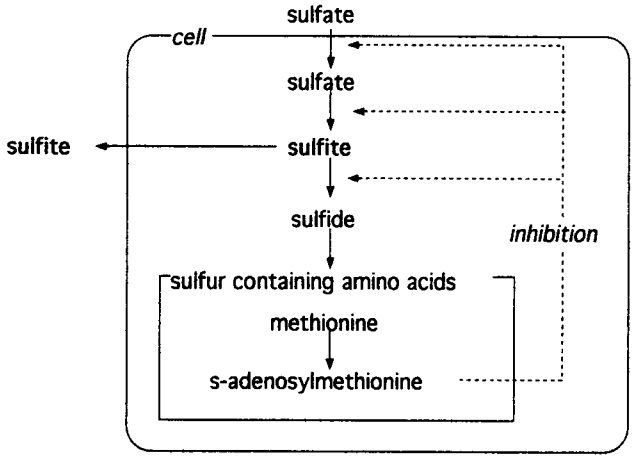

Fig. 1. Schematic pathway of sulfite-related metabolism.

INTRODUCTION

Sulfite (or sulfur dioxide or bisulfite depending on pH value) is a minor component in beer, and its concentration in a conventionally brewed beer is normally very low (below 10 mg/L). Control of sulfite concentration in beer is important, however, because the sulfite ion acts as a flavor stabilizer (it works as an antioxidant) (*1*). It is sometimes added to beer for this purpose, and in Britain, sulfur dioxide is a permitted preservative in beer (up to 70 mg/L) (*2*).

Sulfite in beer is mostly produced during fermentation and is present in a bound form, such as acetaldehyde (carbonyl) bisulfite (*3*). It is an intermediate in the biosynthesis of the sulfur-containing amino acids (cystein and methionine) (*4*). The sulfate in wort is taken in by the yeast, reduced to sulfite, and then further reduced to sulfide for biosynthesis of sulfur-containing amino acids (Fig. 1). This biosynthetic pathway is regulated by feedback inhibition of enzyme activity and also is genetically controlled (*4*).

Factors affecting the sulfite level in beer in a conventional process have been studied (*5–8*), and several models have been proposed to interpret the phenomena of sulfite accumulation in beer during fermentative yeast growth (*9–11*). According to Dufour et al., any condition that stimulates yeast growth (lipids, oxygen, and so forth) should decrease the sulfite content of a young beer. The sulfite level in young beer is dependent on yeast growth as soon as methionine in the wort has been consumed.

In a novel multistage bioreactor system, the yeast is principally subdivided into growth and nongrowth phases (*12,13*) using a continuous stirred-tank reactor (CSTR) and a packed-bed immobilized yeast reactor.

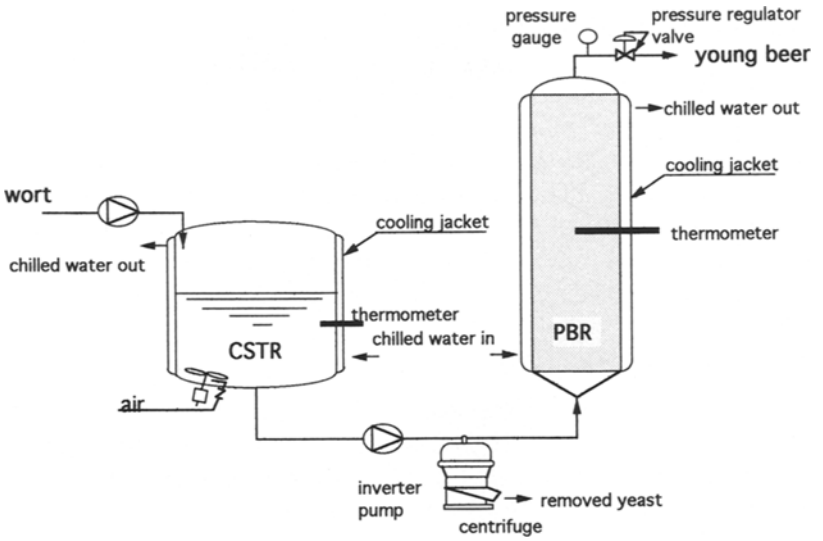

Fig. 2. Schematic diagram of the multistage fermentation process.

No report has as yet been made available describing sulfite production in an immobilized yeast reactor. Sulfite formation in the CSTR and PBR was investigated, and also a control strategy for sulfite production in the system is discussed.

MATERIALS AND METHODS

Microorganisms and Media

Saccharomyces cerevisiae SMA (culture collection of Kirin Brewery Co., Ltd. Japan) was used for all experiments. This strain is a typical bottom-fermenting brewer's yeast for pilsner-type beer. Typical brewer's wort (11% extract) was used for the brewery fermentation.

Multistage Continuous Fermentation

A schematic diagram of the main fermentation process, which consists of two reactors, is shown in Fig. 2.

The first stage is a CSTR equipped with marine impeller at the bottom. An air sparger was installed at the base of the agitation impeller. Wort was supplied along the inner wall of the reactor to prevent foaming. Wort was fed continuously after passing through the continuous sterilizer (at 70–80°C, 20–30 min). The reactor temperature was controlled at 13°C, and the air sparging rate was 0.017 vvm. The reactor was controlled such that extract concentration was maintained at 8.0%. Yeast cells in the wort were continuously removed after the CSTR by centrifugation to below 1.0 $\times$ 10^6 cells/mL in order to eliminate any effect of these suspended cells on fermentation in the second stage.

The second stage is an immobilized yeast packed bed reactor (PBR) consisting of a cylindroconical-type vessel (1.9-L vol, diameter-to-length ratio 1:4) fitted with cooling jackets. Porous spherical glass beads (3-mm diameter Bioceramics®, Kirin, Japan) were used as a carrier material. The central pore size was 10–20 μm, with a bulk density of 0.35 g/cm^3 and surface area of 3.13 m^2/g. The void volume of the reactor was 40% (vol/vol). The PBR was operated at 8°C, and the pressure was controlled at 0.01 kg/cm^2 to avoid external microbial contamination. The flow rate was controlled so as to maintain the desired residual extract concentration (1.8–2.5%).

Analytical Methods

Amino acids were measured using an HPLC amino acid analyzer (Hitachi, L-8500, Japan). Sulfite was also determined by an HPLC method. EDTA solution (0.25 mL, 0.1*M*) was added to 5 mL of sample; 1.9 mL of buffer solution (0.5*M*, pH 8.8) and 0.3 mL of *N*-(9-acridinyl) maleimide solution were added to 0.3 mL of the previously prepared sample solution, and then left at room temperature for 15 h. Sulfite was measured by HPLC (Model LC-6A, Shimadzu, Japan, column NOVA PAK C18, 3.9 × 150 mm, Waters, USA) with fluorescence detector (Model RF-535, excitation wavelength 360 nm, detection wavelength 435 nm).

The alcohol content and extract concentration of a sample were determined by the EBC method (Analysis Committee of the EBC, 1975). A beer sample (100 g) was distilled, the alcoholic content was determined from the specific gravity of the distillate diluted to 100 g, and the extract was determined from the specific gravity of the residue diluted to 100 g. The major component of the extract was maltose.

RESULTS AND DISCUSSION

Sulfite Formation in the CSTR and the PBR

The results of continuous operation of the multistage reactor system are shown in Fig. 3. Almost no sulfite was produced in the CSTR. However, the production rate of sulfite in the PBR gradually increased with time and stabilized at a rate between 20 and 30 mg/L. In a conventional process, sulfite is usually produced during the early stages of fermentation, and when yeast growth stops, it is also produced at a nearly constant rate (*11*).

According to the model proposed by Dufour (*11*), sulfite excretion did not occur in the CSTR because of sulfite-metabolizing enzyme inhibition and repression by wort methione. As shown in the previous article (*13*) easily assimilable amino acids, including methionine, are mainly consumed in the CSTR, and hence, this phenomena is in line with the model prediction. Effects contributing to sulfite excretion are cessation of yeast

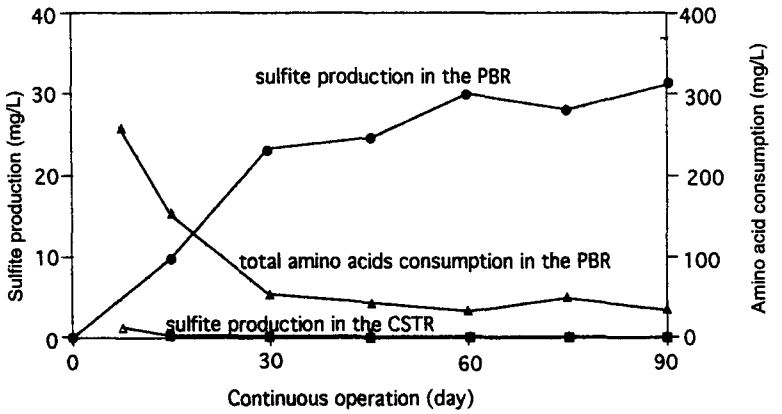

Fig. 3. Sulfite production in the CSTR and the PBR. ● Sulfite production in the PBR, ■ sulfite production in the CSTR. ▲ total amino acids consumption in the PBR, and △ methionine consumption in the PBR.

growth and a low level of the enzyme sulfite reductase. These factors slow down the metabolic flux and biosynthesis of sulfur-containing amino acids from sulfite. Since extract is still present in the wort, however, assimilation of sulfate from the wort continues to proceed, and sulfite formation occurs in the cell, with the result that sulfite is excreted owing to metabolic overflow. Sulfite production in the PBR may be explained by this model, because the immobilized cells are in the stationary phase and at the same time wort extract is available. The gradual increase in sulfite productivity in continuous operation reflects the transition from growth phase to stationary phase. The decrease in total amino acid consumption could also be observed during this transition. However, consumption of methionine did not occur because it had already been consumed in the CSTR. It is still unclear whether the lack of methionine in the wort is the cause of sulfite excretion in the PBR.

Effect of Declogging by Air Pressure for Reduction of Sulfite Formation

In order to decrease the sulfite formation in the PBR, the effects of aeration and declogging were investigated (Fig. 4). All of the fermentation broth in the PBR was withdrawn and declogged by first applying 1.0 kg/cm^2 of air pressure to the PBR and then releasing the pressure. When the system was restarted, sulfite production decreased from 10 to 4 mg/L, and also the total amino acid consumption in the PBR increased. However, the consumption of methionine remained the same. This is possibly because the yeast activity was recovered by this procedure and effected a switch from stationary phase to growth phase. The wort methionine seems not to be a decisive factor in repressing sulfite production as long as the yeast is in a growth phase.

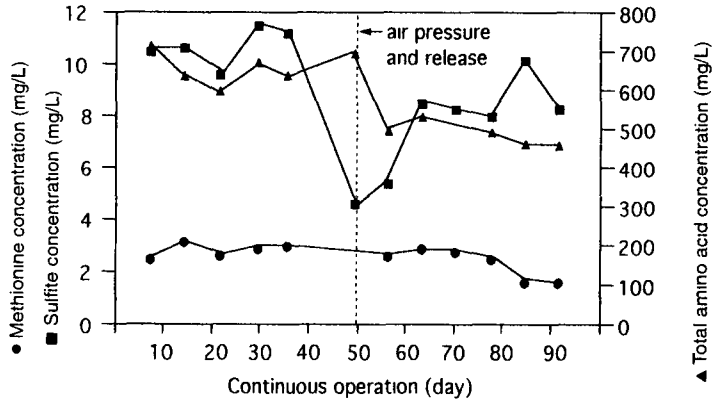

Fig. 4. Effect of air washing for reduction of sulfite.

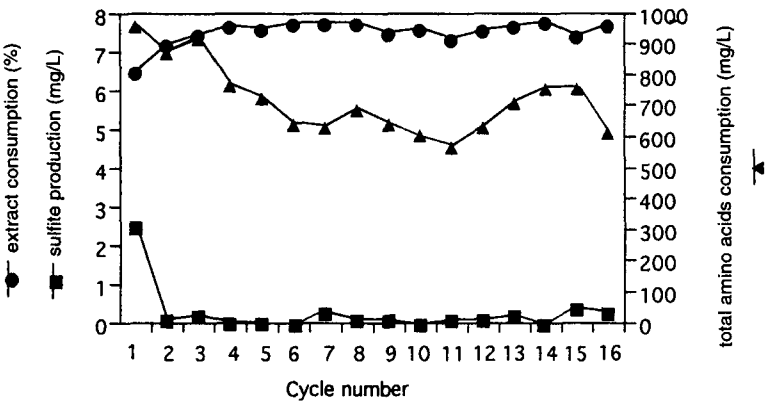

Fig. 5. Sulfite production by repeated batch fermentation using the immobilized yeast reactor.

Repeated Batch Operation of the Immobilized Yeast Reactor

Another possible method for repressing sulfite production by the immobilized yeast is repeated batch operation. Wort was added to the immobilized yeast reactor and fermented at 8°C for 48 h. In this way, young beer and surplus yeast were recovered after every cycle. The results are shown in Fig. 5. Extract consumption and total amino acid consumption were maintained for 16 cycles, and virtually no sulfite production was observed. This may be because the immobilized yeast can be maintained in a practical growth phase by adding aerated fresh wort and the effect of surplus yeast removal every time (declogging effect).

SUMMARY

Sulfite formation by yeast is derived from exogenous sulfate. It does not occur, therefore, when passing the maltose solution through the PBR, which does not contain sulfate. The phenomenon of producing a larger amount of sulfite by the immobilized yeast through fermentation of the wort is reported in the article, and the possibility of controlling the sulfite formation is discussed.

ACKNOWLEDGMENT

The authors would like to thank H. Yokoyama and M. Mawatari, Kirin Brewery Co., Ltd., for permission to publish, Professor I. Karube of The University of Tokyo and K. Morimoto, Kirin Brewery Co., Ltd., for encouragement, and J. M. Dicks, a Japan Society for the Promotion of Science postdoctoral research fellow at RCAST, for useful discussions and help in preparing the manuscript.

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