Modeling growth of *Saccharomyces rosei* in cucumber fermentation†

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Objectives of this study were to assess the effects of key variables involved in cucumber fermentation on growth of the yeast, Saccharomyces rosei, *and to develop a mathematical description of those effects. The growth medium for the studies was cucumber juice. Effects of concentrations of lactic, acetic, and hydrochloric acids and sodium chloride on growth at 30°C were determined in batch culture. Effect of substrate concentration on the specific growth rate was also defined. The specific growth rate decreased from $0.355 \; h^{-1}$ at pH 6.0 to $0.189 \; h^{-1}$ at pH 3.2. The undissociated form of lactic acid was more inhibitory than that of acetic acid. A predictive equation for specific growth rate was developed for predicting growth of* S. rosei *in batch culture. The molar yield of ethanol was 1.75 (±0.07) mM ethanol per mM hexose. Malate was not utilized, and glycerol was produced. The apparent biomass yield under anaerobic condition was 12.2 (±1.3) g cells/mol hexose. Aerobically, the biomass yield was 30.7 g cells/mol hexose. Similar specific growth rates were observed anaerobically ($0.358 \; h^{-1}$) and aerobically ($0.352 \; h^{-1}$). The predictive model for growth of* S. rosei *in cucumber juice should prove useful in modeling the mixed culture (yeast and lactic acid bacteria) fermentation of brined, whole cucumbers.*

Introduction

In cucumber fermentation, preservation results from the conversion of the fruit sugars into lactic acid and other compounds and by a lowering of the pH. Cucumber nutrients available for the fermentation must diffuse through the tissue and skin of the fruit into the surrounding brine. Also, sodium chloride and acetic acid added in the begin-

ning of the process diffuse into the fruit. The concentration of these components will exert selective effects on growth of the natural microflora and/or on the starter culture during fermentation. In addition, the mass transfer rate of solutes affects the growth rate of micro-organisms.

Fermentations by homolactic acid bacteria alone result in concentrations of lactic acid which are too high for direct consumption† of the fermented product. A minimum of 0.6% (66.7 mM) lactic acid has been recommended to insure preservation (Etchells and Hontz 1972). Excessive acidity levels, however, can adversely affect the texture (Thompson et al. 1979) and flavor (Daeschel et al. 1988) of the fermented cucumbers. In addition, sugar may remain after primary fermentation by lactic

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acid bacteria, resulting in subsequent fermentation and bloater damage by acid-tolerant, fermentative yeast.

The use of buffer components or neutralization of the acid produced to allow complete conversion of sugar to end products by lactic acid bacteria has been recommended (Etchells et al., 1973). The addition of a fermentative yeast, *Saccharomyces rosei*, has been suggested as a means of partially utilizing fermentable sugars to avoid excessive acid production by lactic acid bacteria (Daeschel et al. 1988). Although yeast and other gas-forming bacteria can cause bloater damage to the cucumber, brines can be purged of the CO_2 produced using air or N_2 to avoid this problem (Fleming et al. 1975).

The objective of this research was to develop a mathematical model to predict the growth of *S. rosei*. Effects of lactic acid, acetic acid, NaCl, and hydrogen ions on the specific growth rate were considered. Growth equations developed for *S. rosei*, when combined with similar equations defined for *Lactobacillus plantarum* and equations for diffusion of soluble components in/out of the fruit, will provide a kinetic model for mixed culture fermentations of whole cucumbers.

Materials and Methods

Culture and growth medium

The yeast used in the study was *S. rosei*, isolated from fermenting cucumber (Daeschel et al. 1988) and stored in YM broth containing 16% glycerol at −70°C. Isolated colonies were picked from YM agar streak plates of the frozen culture and grown twice in cucumber juice for 12–15 h at 30°C. The inoculum growth medium was supplemented with acetic acid (40 mM), lactic acid (40 mM), or NaCl (4%) as needed to be consistent with the growth medium. The inoculum culture was diluted to an optical density ($OD_{630 nm}$) of 0·4–0·5, and 1·0% (by volume) in the growth medium to give initial cell levels approximating 5×10^5/ml.

Cucumber juice for growth studies was prepared as previously described (Passos et al. 1993). The chemicals used in the study

were hydrochloric acid, DL-lactic acid, acetic acid (Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A.) and sodium chloride (Fisher Scientific, Pittsburgh, PA, U.S.A.).

Cucumber juice at different dilutions was used to test the effect of substrate concentration on the specific growth rate of *S. rosei*. Media containing 0·1 to 50·0% undiluted cucumber juice were prepared.

Fermentors

Water-jacketed jars from Wheaton (Millville, NJ, U.S.A.), with 200 ml working volume, were used as uncontrolled pH batch growth systems. The growth medium was agitated by a magnetic stirrer and maintained at 30°C. Compressed N_2 was humidified, sterilized (0·22 μm Millex-FG$_{50}$ filter, Millipore Corp., Bedford, MA, U.S.A.), and released into the head space of the fermentor at a rate of 2·5 l/h to assure anaerobic conditions in all the experiments. During batch growth, 3-ml samples were removed aseptically by syringe from the 200 ml initial broth volume at intervals of 1–2 h (depending on the fermentation rate) until growth ceased, used for optical density and pH measurement, and then frozen for future HPLC analysis.

Analytical methods

Cell growth was followed by measurement of the OD of the medium in a 1·5 ml glass cuvette using a Novaspec II spectrophotometer (Pharmacia LKB, Piscataway, NJ, USA). The linear range extended to OD readings of 0·30. During growth, if the OD was higher than 0·25, the sample was diluted to within a range of 0·10 to 0·25 using distilled water. Standard curves were used to relate OD, dry weight (g/l) and cell number (CFU/ml). For dry weight determination a 500 ml cell suspension (around 0·6 OD) was washed two times with an equal volume of sterile water, concentrated 25× by centrifugation, and 4 samples of 3 ml each were then dried to constant weight in a vacuum oven at 80°C. Viable cells were enumerated in YM agar (Difco Labs, Detroit, MI, U.S.A.), using the same cell suspension used for dry weight. One unit of OD was equivalent to 0·39 g