Modeling growth of *Saccharomyces rosei* in cucumber fermentation†

Frederico V. Passos¹, Henry P. Fleming²*, Richard M. Felder³ and David F. Ollis³

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 beginning 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

Received: 17 October 1996

¹Department of Food Science, Universidade Federal de Viçosa, Viçosa, MG, Brazil 36570.
²Food Fermentation Laboratory, U.S. Department of Agriculture, Agricultural Research Service, and North Carolina Agricultural Research Service, Department of Food Science, North Carolina State University, Raleigh, North Carolina U.S.A. 27695-7624
³Department of Chemical Engineering, N. C. State University, Raleigh, NC, U.S.A. 27695-7905.
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 CO2 produced using air or N2 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 (OD₆₃₀ nm) of 0.4-0.5, and 1.0% (by volume) in the growth medium to give initial cell levels approximating 5×10⁵/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 N2 was humidified, sterilized (0.22 µm Millex-GF 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
cells/l and 1.5×10^7 CFU/ml, for dry weight and cell number, respectively. All the chemicals used were described in Passos et al. (1993). Initial specific growth rates were calculated from linear regression analysis of the exponential portion of the initial growth curves.

Model development

To develop a mathematical representation of the specific growth rate of *S. rosei* as a function of the dynamic chemical variables during cucumber fermentation (pH, lactic acid, acetic acid, and NaCl concentrations), the effect of each variable was studied individually. The kinetics of cell growth can be described by modeling the cell batch specific growth rate μ, defined as:

\[
\mu = \frac{1}{X} \frac{dX}{dt}
\]  

(1)

where X=cell concentration. Assuming that the influences of the four inhibitor variables are independent, we may write

\[
\mu = \mu_0 f_1 ([S]) f_2 ([H^+]) f_3 ([HLa]) f_4 ([HAc]) f_5 ([NaCl])
\]  

(2)

where \(f_1, f_2, f_3, f_4, \) and \(f_5\) refer to the presumably independent influences of substrate limitation ([S]), protons ([H^+] ), undissociated forms of lactic and acetic acids ([HLa] and [HAc], respectively), and [NaCl]. This independence is tested following the establishment of suitable functions for \(f_i\) through \(f_5\).

To define the mathematical relationship between specific growth rate and component concentrations, models were fitted to the data using non-linear or linear regression and goodness-of-fit criteria. Resultant data indicated the best model for establishing relevant coefficients. Analysis was performed with SAS software using the iterative, modified Gauss–Newton method for non-linear analyses (proc NLIN) and the principle of least squares for linear analyses (proc REG) (SAS 1988). Each model was selected using the value of the sum of squares as criteria.

Results

Substrate limitation (S)

The Monod model (equations 3 and 4) was applied to describe the hexose concentration effect on the specific growth rate of *S. rosei*. The values of \(K_m\) and \(\mu_{max}\) were determined as 0.86 mM and 0.359 h\(^{-1}\), respectively, using a Lineweaver–Burk plot (Bailey and Ollis 1986). Figure 1 shows the experimental data (symbols) and fitted model (line) for the growth rate (μ), where

\[
\mu = \mu_{max} f_1 ([S])
\]  

(3)

and

\[
f_1 ([S]) = \frac{[S]}{K_m + [S]}
\]  

(4)

Proton concentration effect ([H^+])

Hydrochloric acid (HCl) was used to vary the initial medium pH for measurements of initial cell growth rate vs pH in the absence
of other inhibitors. The data obtained (Fig. 2) are fit best by an inhibition function proposed by Levenspiel (1980). The solid line in Fig. 2 was calculated using the function

$$\mu = \mu_{\text{max}} f_2 ([H^+])$$

(5)

where $\mu_{\text{max}} = 0.359 \, \text{h}^{-1}$ and

$$f_2 ([H^+]) = \left(1 - \frac{[H^+]}{[H^+]_{\text{max}}} \right)^{\alpha}$$

(6)

where $[H^+]$ is expressed in mM, $[H^+]_{\text{max}} = 2.50$ mM, and $\alpha = 2.13$.

Undissociated lactic acid concentration effect ([HLa])

Kuhn (1991) recently established that effects of acid-product-inhibited *Escherichia coli* could be decomposed into the separate influences of pH and the undissociated forms of the products (acetate and formate). A similar approach was used to evaluate the effect of acetic and lactic acids on the growth of *L. plantarum* (Passos et al. 1993). Yabannavar and Wang (1991) showed that for *L. delbrueckii* the growth-inhibiting effect of the ionized form of lactic acid is extremely small when compared with that of non-ionized lactic acid and proposed a model relating specific growth rate to concentrations of hydrogen ion and the non-ionized form of lactic acid. It is well known that only the uncharged forms of organic acids can penetrate the cell membrane (Ingram et al. 1956); thus, [HLa] and [HAc] were of primary interest in this study. To model the influence of [HLa], we used the acid ionization equilibrium,

$$[\text{HLa}] = [H^+] + [\text{La}^-]$$

(7)

to calculate [HLa] from measurements of total lactic acid ([HLa] + [La^-]). The corresponding effect of [HLa] on the measured overall specific growth rate was estimated after subtracting out the $[H^+]$ effect. A simple linear inhibition model provided the best fit in the range studied, giving

$$\mu = \mu_{\text{max}} f_2 ([H^+]) f_3 ([\text{HLa}])$$

(8)

where [HLa] (mM) is the concentration of non-ionized lactic acid,

$$f_3 ([\text{HLa}]) = \left(1 - \frac{[\text{HLa}]}{[\text{HLa}]_{\text{max}}} \right)$$

(9)

and $[\text{HLa}]_{\text{max}} = 156$ mM. The experimental data (symbols) and predicted values from Eqn 8 (solid line), are shown in Fig. 3.
where [C] is the stimulatory-inhibitory component concentration. As seen in Fig. 4, Eqn 12 provided a good fit of the data, giving

\[ \mu = \mu_{\text{max}} f_5 ([\text{NaCl}]) \] (13)

where [NaCl] is the weight percent of NaCl,

\[ f_5 ([\text{NaCl}]) = \left(1 + \frac{\beta [\text{NaCl}]}{K_{\text{NaCl}} + [\text{NaCl}]}\right) \]

\[ \left(1 - \frac{[\text{NaCl}]}{[\text{NaCl}]_{\text{max}}}\right) \] (14)

and \( \beta = 0.8, K_{\text{NaCl}} = 4.1\% \), and \([\text{NaCl}]_{\text{max}} = 16.9\% \).

Test of independence of [H⁺], [HL], [HAc], and [NaCl] effects

The functions \( f_2-f_5 \) of Eqn 2 were determined from experimental cell growth measurements in which only a single inhibiting component was present in the growth medium. The presumed independence of the inhibiting effects was tested by measuring growth rates in the presence of combinations of the four components and correlating the data with Eqn 2, without adjusting the four factors from their individually determined values. The results are shown in Table 1. The goodness-of-fit of the observed values against the predicted values was subjected to a \( \chi^2 \) test; the fit was significant at the 0.005 level. The excellent agreement between the predicted and measured growth rates over the range of conditions tested justifies the assumption of independence.

Model-predicted variations in growth rate over ranges of pH, NaCl, and lactic and acetic acid concentrations are shown in Fig. 5. In Fig. 5a, undissociated acetic acid, NaCl, and hexose concentrations were kept constant, and the effect of pH was demonstrated for different undissociated lactic acid concentrations. In Fig. 5b, undissociated lactic acid, NaCl, and hexose were kept constant, and the effect of pH was demonstrated for different undissociated acetic acid concentrations. In Fig. 5c, undissociated lactic acid, undissociated acetic acid, and hexose concentrations were kept constant, and the effect of

**Non-ionized acetic acid concentration effect ([HAc])**

The same equation used for [HL] (Eqn 9) was also used to describe the inhibitory effect of [HAc]:

\[ \mu = \mu_{\text{max}} f_2 ([H^+]) f_4 ([\text{HAc}]) \] (10)

where

\[ f_4 ([\text{HAc}^+]) = \left(1 - \frac{[\text{HAc}]}{[\text{HAc}]_{\text{max}}}\right) \] (11)

The fitted parameter was \([\text{HAc}]_{\text{max}} = 218 \) mM. The experimental data (symbols) and predicted values from Eqn 10 (broken line) are shown in Fig. 3.

**NaCl concentration effect**

NaCl addition first increased, then decreased the specific growth rate of \( S. \) rosei. Thus, salt functions as stimulant or inhibitor of growth, depending upon its concentration. A similar behavior was observed for \( L. \) plantarum (Passos et al. 1993) and the same function was used to account for such dual functional behaviour:

\[ f([C]) = \left(1 + \frac{\beta [C]}{K_M^0 + [C]}\right) \left(1 - \frac{[C]}{[C]_{\text{max}}}\right) \]

(12)
Table 1. Combinations of acetic and lactic acids and NaCl used to test growth prediction Eqn 2.

<table>
<thead>
<tr>
<th>pH</th>
<th>[La], mM</th>
<th>[Ac], mM</th>
<th>NaCl, %</th>
<th>$\mu_{obs}$</th>
<th>$\mu_{pred}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.38</td>
<td>0</td>
<td>18</td>
<td>3</td>
<td>0.362</td>
<td>0.362</td>
</tr>
<tr>
<td>4.15</td>
<td>0</td>
<td>35</td>
<td>3</td>
<td>0.259</td>
<td>0.330</td>
</tr>
<tr>
<td>3.74</td>
<td>15</td>
<td>18</td>
<td>6</td>
<td>0.227</td>
<td>0.264</td>
</tr>
<tr>
<td>3.41</td>
<td>38</td>
<td>18</td>
<td>3</td>
<td>0.203</td>
<td>0.230</td>
</tr>
<tr>
<td>3.82</td>
<td>20</td>
<td>10</td>
<td>2</td>
<td>0.335</td>
<td>0.325</td>
</tr>
<tr>
<td>3.36</td>
<td>55</td>
<td>25</td>
<td>2</td>
<td>0.241</td>
<td>0.204</td>
</tr>
<tr>
<td>3.21</td>
<td>55</td>
<td>25</td>
<td>6</td>
<td>0.145</td>
<td>0.127</td>
</tr>
<tr>
<td>5.92</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.359</td>
<td>0.359</td>
</tr>
</tbody>
</table>

Figure 5. Predicted effect of pH on the specific growth rate of *L. plantarum*: (a) variable concentrations of undissociated lactic acid, 20 mM undissociated acetic acid, 4% NaCl, 50 mM hexose; (b) variable concentrations of undissociated acetic acid, 20 mM undissociated lactic acid, 4% NaCl, and 50 mM hexose; (c) variable NaCl concentrations, 20 mM undissociated lactic and acetic acids, and 50 mM hexose; (d) variable concentrations of hexose, 20 mM undissociated lactic and acetic acids, and 4% NaCl.
pH was demonstrated for different NaCl concentrations. In Fig. 5d, undissociated lactic acid, undissociated acetic acid, and NaCl concentrations were kept constant, and the effect of pH was demonstrated for different hexose concentrations.

**Product formation**

Batch growth experiments were conducted for evaluation of effects of variables on product yield. Glucose and fructose were degraded simultaneously, but at different rates, with the rate of glucose utilization higher than for fructose. *Saccharomyces rosei* did not utilize malate and glycerol was produced. Table 2 summarizes the main yield coefficients. The molar yield of ethanol was 1.75 (±0.07) mM ethanol per mM hексозе. When the molar product yield was calculated, combining the ethanol and glycerol production, the value was 1.93 (±0.05) mM product per mM hексозе.

The apparent biomass yield under anaerobic conditions was estimated as 12.2 (±1.3) g cells/mol hексозе. Aerobically, a higher biomass yield was observed, 30.7 g cells/mol hексозе. Figure 6 presents growth curves when N<sub>2</sub> or air was released into the headspace of the fermentor. Similar specific growth rates were observed for anaerobic (0.358 h<sup>-1</sup>) and aerobic (0.352 h<sup>-1</sup>) conditions.

**Discussion**

A mathematical model was developed to describe the anaerobic growth of *S. rosei* in

| Table 2. Final product concentrations, molar yield of ethanol produced from hексозе (Y<sub>ES</sub>), apparent biomass yield (Y<sub>XS</sub>), and product molar yield (ethanol+glycerol) (Y<sub>PX</sub>) from different batch fermentation runs of *S. rosei* in cucumber juice. All the sugar was fermented (30.5 mM glucose, 32 mM fructose) after 30 h. The last row are data from a batch growth under aerobic conditions. |
|-----------------|--------|--------|--------|--------|--------|--------|
| Cell mass (g/l) | Ethanol (mM) | Glycerol (mM) | Y<sub>ES</sub> | Y<sub>XS</sub> (g/M) | Y<sub>PX</sub> |
| 0.753           | 107.3  | 7.3    | 1.72   | 12.0   | 1.83   |
| 0.725           | 105.2  | 14.9   | 1.68   | 11.6   | 1.92   |
| 0.761           | 105.9  | 12.2   | 1.69   | 12.2   | 1.89   |
| 0.881           | 106.6  | 13.6   | 1.71   | 14.1   | 1.92   |
| 0.675           | 106.9  | 13.1   | 1.71   | 10.8   | 1.92   |
| 0.714           | 107.8  | 13.0   | 1.72   | 11.4   | 1.93   |
| 0.803           | 107.9  | 11.9   | 1.73   | 12.8   | 1.92   |
| 0.745           | 108.5  | 12.9   | 1.74   | 11.9   | 1.94   |
| 0.710           | 108.2  | 12.2   | 1.73   | 11.4   | 1.93   |
| 0.683           | 118.4  | 8.6    | 1.89   | 10.9   | 2.03   |
| 0.944           | 118.9  | 6.6    | 1.90   | 15.1   | 2.01   |
| Mean            |        |        | 1.75   | 12.2   | 1.93   |
| STD             |        |        | 0.07   | 1.3    | 0.05   |
| 1.919           | 91.2   | 5.9    | 1.46   | 30.7   | 1.55   |
cucumber juice at 30°C as influenced by various concentrations of acids (lactic and acetic), NaCl, and pH. The model can be used to predict the growth of *S. rosei* in the fermentation of cucumbers since the concentration range of the variables studied are those found normally in that process.

Aiba et al. (1968) used the Monod model to describe specific growth rate of a strain respiration-dependent mutant of baker's yeast and its dependence on glucose concentration, and obtained a $K_m$ of 1.22 mM. Abulesz and Lyberatos (1989) working with *S. cerevisiae* obtained a $K_m$ of 11.4 mM. These results demonstrate the possible large variations of rate coefficients from one strain to another. In this study a value of $K_m$ of 0.86 mM was measured for *S. rosei*.

When hydrochloric acid was used to lower the pH the effect on cell growth could be directly attributed to $[H^+]$ in the medium. *Saccharomyces rosei* was shown to be an acid-tolerant organism, especially when compared with *L. plantarum* (Passos et al. 1993). At pH 3.2 (the lowest value studied) the specific growth rate was reduced to 0.189 h$^{-1}$, 53% of the maximum value observed, 0.358 h$^{-1}$, at pH 4.8.

Acidification of cucumber juice with lactic or acetic acids resulted in an inhibitory effect on growth beyond the hydrogen ion effect. Growth inhibition was attributed primarily to the protonated acid form. Undissociated acetic acid concentration resulted in an inhibitory effect on the specific growth rate of *S. rosei*, slightly smaller than that caused by undissociated lactic acid (Fig. 3). Daeschel et al. (1988) suggested a value of 56 mM total lactic acid as a limiting growth concentration for *S. rosei*, a claim that the observed values in the present study contradict. *S. rosei* grew at 70 mM total lactic acid with a specific growth rate 44% less of the maximum growth rate observed.

Cells grew vigorously in NaCl concentrations up to 4%. When added in low concentration (1 to 2%, Fig. 3), NaCl stimulated the growth of *S. rosei*. Similar results, probably due to the small decrease in the water activity value, were earlier obtained by McMeekin et al. (1987), working with *Staphylococcus xylosus*. The concentration of NaCl to achieve a water activity ($a_w$) of 0.976 added in the medium supported a higher specific growth rate compared with no NaCl (McMeekin et al. 1987). From 0.976 to 0.848 $a_w$, they found a linear inhibitory relationship. Values for $a_w$'s of 0.976 and 0.848 represent NaCl concentrations of approximately 3-5 and 23-55%, respectively in water (Scott 1957). Similar stimulatory-inhibitory effects of NaCl were observed for *L. plantarum* (Passos et al. 1993). Beyond the initial stimulatory effect, a further increase in the NaCl concentration resulted in an inhibitory effect on the specific growth rate of *S. rosei*. For the same strain used in the present study, Daeschel et al. (1988) estimated a specific growth rate of 0.282 h$^{-1}$ in cucumber juice containing 5% salt and 26.6 mM total acetic (no reference was made to the pH value). Using 5% salt and 26.6 mM total acetic and pH=4.5 in Eqn 2, an estimated specific growth rate of 0.13 h$^{-1}$ is obtained.

When fermentations were conducted using cucumber juice and different initial concentrations of lactic acid, acetic acid, and NaCl, a good agreement between the data and predicted values was observed. The mean absolute deviation between predicted and experimental values, from 8 independent validation tests, was 10%. The goodness-of-fit of the observed values against the predicted values was subjected to a $\chi^2$ test; the fit was significant at the 0.005 level.

According to Kappeli (1986), the biomass yield of *S. cerevisiae* under aerobic conditions is approximately 90 g of dry cells/mol glucose, and the specific growth rate is 0.3 h$^{-1}$. At high glucose concentrations, ethanol is also produced and the yield of biomass drops to approximately 27 g dry cells/mol glucose and the specific growth rate increases to 0.45 h$^{-1}$ (Kappeli 1986). Kappeli (1986) cited a yield of approximately 18 g dry cells/mol glucose and specific growth rate of 0.35 h$^{-1}$ under anaerobic growth conditions. Under these conditions, energy originates exclusively from glycolysis and a linear relationship between growth rate and ethanol production is observed (Kappeli 1986). For *S. rosei* we found a biomass yield of 12.2 g cells/moles hexose at anaerobic conditions and 30.7 g cells/mol hexose under aerobic conditions.
with an excess of hexose. According to Kappeli (1986), S. cerevisiae and related yeasts are grouped as glucose-sensitive yeasts, which produce ethanol aerobically in the presence of excess glucose. Under aerobic conditions, the catalyzed breakdown of glucose by S. cerevisiae occurs oxidatively to yield biomass and CO₂ in appreciable amounts. Under anaerobic conditions, energy originates exclusively from glycolysis, yielding a lower biomass yield. Figure 6 illustrates this behavior.

Based on the profile concentrations of the main components varying in a controlled cucumber fermentation, hexose (0–27 mM), pH (3.4–4.7), undissociated lactic acid (0–130 mM), undissociated acetic acid (50–65 mM), and NaCl (4.6–11%) (Fleming et al. 1988), and from the model (Eqn 2, Fig. 5), we can conclude that hexose concentration, pH, and lactic acid concentration are major factors limiting growth of S. rosei. The Kₘ calculated for S. rosei (0.86 mM) is higher than that reported for bacteria (0.056 mM; Yabannavar and Wang 1991) and the concentration of hexose in the brine during the fermentation of whole cucumbers is characteristically low (≤2 mM) during the primary fermentation. These two factors (Kₘ and hexose concentration) suggest the importance of mass transfer of solutes in mixed culture cucumber fermentation by yeast and lactic acid bacteria. Also, it is interesting to speculate that the lower Kₘ for sugars of bacteria compared to yeasts, contributes to the dominant growth of lactic acid bacteria over yeasts during the primary fermentation of cucumbers and other vegetables.

From the results obtained, the following set of equations are proposed to represent S. rosei fermentation in cucumber juice.

\[
\frac{d[X]}{dt} = \mu[X] \tag{15}
\]

\[
\frac{d[S]}{dt} = \frac{1}{Y_{x/s}} \frac{d[X]}{dt} \tag{16}
\]

\[
\frac{d[Eth]}{dt} = Y_{ES} \frac{d[S]}{dt} \tag{17}
\]

The specific growth rate of S. rosei growing during cucumber fermentation can be predicted by Eqn 2. If equations for mass transfer (Passos 1993) and growth of lactic acid bacteria (Passos et al. 1993) are combined with the above yeast growth model, the fermentation of brined, whole cucumbers by mixed cultures of yeasts and lactic acid bacteria can be predicted. Further research is intended to verify the value of such predictions. xxx hexose utilization xxx ethanol production

Acknowledgements

This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, IL.

Author F. V. Passos received a scholarship from CNPq (Brazilian government agency) during the time this study was conducted.

References


**Appendix A—Nomenclature**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ac⁻]</td>
<td>dissociated acetic acid concentration, mM</td>
</tr>
<tr>
<td>[Ac_c]</td>
<td>total acetic acid ([HAc]+[Ac⁻]) concentration, mM</td>
</tr>
<tr>
<td>α, β</td>
<td>coefficients determined by model fitting.</td>
</tr>
<tr>
<td>[C]</td>
<td>inhibitory component concentration, mM</td>
</tr>
<tr>
<td>[C]_{max}</td>
<td>concentration of the inhibitory component where the specific growth rate is zero, mM, determined by model fitting</td>
</tr>
<tr>
<td>[H⁺]</td>
<td>hydrogen ion concentration, mM</td>
</tr>
<tr>
<td>[HLa]</td>
<td>undissociated lactic acid concentration, mM</td>
</tr>
<tr>
<td>[HAc]</td>
<td>undissociated acetic acid concentration, mM</td>
</tr>
<tr>
<td>K_m</td>
<td>concentration of nutrient for which the specific growth rate has half its maximum value, mM</td>
</tr>
<tr>
<td>[La⁻]</td>
<td>dissociated lactic acid concentration, mM</td>
</tr>
<tr>
<td>[La_c]</td>
<td>total lactic acid ([HLa]+[La⁻]) concentration, mM</td>
</tr>
<tr>
<td>μ</td>
<td>specific growth rate, h⁻¹</td>
</tr>
<tr>
<td>μ_{max}</td>
<td>maximum specific growth rate, h⁻¹</td>
</tr>
<tr>
<td>[NaCl]</td>
<td>sodium chloride concentration, %, w/v</td>
</tr>
<tr>
<td>[X]</td>
<td>concentration of cell mos, g/l</td>
</tr>
</tbody>
</table>