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Research Paper

Buffer Models for pH and Acid Changes Occurring in Cucumber Juice Fermented with *Lactiplantibacillus pentosus* and *Leuconostoc mesenteroides*

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ABSTRACT

The pH changes that occur during the fermentation of vegetables by lactic acid bacteria depend on the production of weak acids and on the buffering of the fermentation medium. Undefined buffering components of fermentation media make estimates of pH from acid production difficult. The objective of this research was to develop buffer models for a model cucumber fermentation brine system linking pH changes to acid concentrations. A novel titration method was used to measure buffer capacity in cucumber juice medium made from three grades of pickling cucumbers based on diameter. Fermentation of juice made from cucumbers of different sizes resulted in differences in fermentation biochemistry. The results of modeling indicated that the pH of the medium after 24 and 48 h of fermentation by heterolactic *Leuconostoc mesenteroides* and homolactic *Lactiplantibacillus pentosus* could be predicted from acid concentrations based on the measured buffer capacity of the corresponding unfermented medium. The differences for all observed and predicted pH values of the fermentation samples, based on measured acid concentrations, had a root mean square error of 0.064 pH units. The buffer models included a quantitative measure of the effect on pH of the malolactic reaction caused by the lactic acid bacteria. These models may have application for assessing the influence of a variety of lactic acid bacteria buffering reactions on pH and fermentation ecology by linking pH to fermentation acid concentrations.

HIGHLIGHTS

- Buffer models of cucumber brines linking pH and acid concentrations were developed.
- Heterolactic and homolactic fermentations were used to validate the models.
- The pH effects of the malolactic reaction of lactic acid bacteria were quantified.
- These buffer models may be used for the study of fermentation ecology and safety.

Key words: Buffer capacity; Fermentation; Lactic acid bacteria; Modeling; pH

At the start of most vegetable fermentations, the pH and environmental conditions are permissive for the growth of many types of epiphytic bacteria, including bacterial pathogens such as *Escherichia coli, Salmonella enterica,* and *Listeria monocytogenes (4, 6, 9, 11)*. As acid accumulates with concomitant pH reduction, the epiphytic microbiota and bacterial pathogens die off, and the acid resistant lactic acid bacteria (LAB) continue to grow and dominate the fermentation. The predominance of acid-resistant LAB has been linked to the ability of these organisms to tolerate a lower internal pH than most competitors in fermented vegetables *(17, 18)*. Protonated weak acids are the major controlling factors influencing the internal pH of bacteria and therefore the microbial ecology of vegetable fermentations *(11, 25)*. The antimicrobial protonated form of weak acids can be calculated based on pH and acid concentration with the Henderson-Hasselbalch equation *(23)*. However, modeling of the effects of acid production and the corresponding pH changes during fermentation is confounded by the fact that pH changes depend on undefined buffering components in the fermentation medium.

Lactic and acetic acids are the main fermentation acids in most traditional vegetable fermentations, including pickled cucumbers, sauerkraut, and kimchi *(7)*. These acids have different pK values (3.86 for lactic acid and 4.76 for acetic acid) *(12)* and differ in their antimicrobial activity *(14)*. For combinations of these acids typical of heterolactic fermentations it remains unclear how the pH will change in the fermentation brine. Both pH and acid concentration may vary significantly during these fermentations based on the

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buffering of the fermentation medium. For commercial cucumber fermentations, the addition of a calcium acetate buffer has been recommended to help assure complete fermentation of available sugars *(8)*. To assess the relationship between pH and the concentration of fermentation acids, the buffering in the fermentation medium must be considered.

Lu et al. *(16)* defined buffer capacity for cucumber brines as the milliequivalents of HCl required to reduce a 100-g sample of cucumber juice from the initial pH to pH 3.5. They found that cucumber size was inversely correlated with buffering and that buffering influenced fermentation biochemistry. Smaller cucumbers (size 1, <27 mm in diameter) had greater buffering than did size 2 (27 to 38 mm) or size 3 (39 to 51 mm) cucumbers. Greater acid accumulation was observed for size 1 than for larger cucumbers, primarily attributed to the differences in buffering *(16)*. Malic acid, a diprotic acid present in cucumber juice (CJ) medium *(21),* may influence buffering due to the malolactic reaction of LAB, which is also commonly used to deacidify wine *(26)*. Malic acid can also influence the growth rates of LAB, potentially by supplying energy to the cell *(22)*. Malic acid metabolism has also been implicated in bloater defects in commercial cucumber fermentations *(20)* due to carbon dioxide produced from malolactic enzymes. The malolactic enzyme reaction may proceed by a single step reaction, liberating carbon dioxide as malic acid is converted to lactic acid *(3)*. This reaction also directly contributes to the buffering of cucumber fermentation brines because it effectively consumes a proton from the medium, increasing the medium pH:

$$\text{Malic acid} + H^+ \rightarrow \text{lactic acid} + CO_2$$

The change in medium pH resulting from malolactic enzyme activity has been used for the development of differential and selective media to identify LAB based on the malolactic phenotype *(1, 5)*.

A method to quantify the buffering of food ingredients has been developed *(24)*. This model system was used to measure the buffering of ingredients in common salad dressing formulations. Buffer capacity (BC) models were developed for each ingredient and for mixtures of ingredients *(13)*. The data were then used to estimate the influence of low-acid ingredients on product pH. These methods may also be applied to a CJ model fermentation system representative of vegetable fermentation brines. The objectives of the present study included developing BC models of CJ media made from three sizes of cucumbers (with different buffer capacity) and determining how BC models can be used to predict pH changes during fermentation. Comparison of BC models for unfermented and fermented CJ media may help to quantify the relationship between pH and the changes in acid concentrations that occur during fermentation. By linking pH to weak acid changes during CJ fermentation, BC modeling offers a novel method for the examination of fermentation biochemistry and ecology.

MATERIALS AND METHODS

Media and cell cultivation. CJ media made from commercially graded pickling cucumbers (*Cucumis sativus*) with diameters of <27 mm (size 1), 27 to 38 mm (size 2), and 39 to 51 mm (size 3) were designated CJ1, CJ2, and CJ3, respectively. Cucumbers were blended to a homogeneous slurry and frozen at –20°C for at least 18 h. The CJ medium was prepared as needed by thawing to room temperature and then removing pulp by filtration with cheesecloth (bleached, grade 90; DeRoyal Textiles, Camden, SC). The filtrate was then heated until boiling (to enhance removal of solids), rapidly cooled, and centrifuged in 250-mL aliquots at 9,000 rpm (13,789 × *g*; Sorvall Lynx centrifuge with F12-6 × 500 LEX rotor, Thermo Fisher Scientific, Newton, CT) at 15°C for 60 min in 500-mL bottles. The supernatant was decanted and refiltered with cheesecloth. The resulting CJ was diluted to 50% (representing an equal mixture of cucumbers and brine in a cucumber fermentation) with deionized water and sodium chloride (NaCl) so the final NaCl concentration was 20 g/L. The CJ media were then sterilized by filtration with a 0.2-μm-pore-size sterile bottle filter (VWR International, Radnor, PA) and stored under refrigeration (4°C) until used.

The bacterial strains used in this study were *Leuconostoc mesenteroides* LA81 and *Lactiplantibacillus pentosus* LA445 (formerly identified as *Lactobacillus plantarum* MOP3) obtained from the culture collection of the Food Science and Market Quality and Handling Research Unit (North Carolina State University, Raleigh). Cells were streaked for isolation on de Man Rogosa Sharpe (MRS; Difco, BD, Sparks, MD) agar and then grown statically at 30°C for 16 h in 5 mL of MRS broth. Cell cultures were centrifuged (Sorvall Lynx centrifuge with A21-24 × 15c rotor, Thermo Fisher Scientific) at 5,000 rpm (4,424 × *g*) for 10 min at 10°C, supernatants were removed, and the cells were resuspended in 0.1 volume of sterile saline (0.85% NaCl). The resulted suspensions contained ca. 10^9 (*L. mesenteroides*) or 10^{10} (*L. plantarum*) CFU/mL. For enumeration, cells were serially diluted as needed with sterile saline containing 1% morpholine–propanesulfonic acid buffer (pH 7), plated on MRS agar with a spiral plater (easySpiral, Interscience, Woburn, MA), and incubated for 24 h at 30°C or for 72 h at 23°C. Colonies were counted with an automated plate counter (Scan3000, Interscience), and the lower limit of detection for LAB was ca. 10^2 CFU/mL.

Biochemical analysis. Organic acids were quantified by using a modification of the method of McFeeters and Barish *(19)*. A high-performance liquid chromatography (HPLC) system (Ultra Fast Liquid Chromatograph, Shimadzu Scientific Instruments, Durham, NC) was used with the accompanying software (LabSolutions, Shimadzu). Analytes were separated with an HPLC column (300 by 7.8 mm; HPX-87H, Aminex, Bio-Rad Laboratories, Hercules, CA) at 65°C with 0.015 N sulfuric acid as the mobile phase (0.9 mL/min). UV and refractive index detectors (Shimadzu) were used in series (SPD-20A for acids and RID-10A for sugars) for analysis of malic, lactic, and acetic acids, glucose, and fructose, requiring a single injection to determine all analyte concentrations. Eight standards were prepared for each analyte with a range of 0.5 to 100 mM. Brine and medium pH values were determined with a standardized pH meter (Hanna Instruments, Smithfield, RI). Controls for all fermentation samples included uninoculated brine samples.

Titration and buffer capacity modeling. All titrations were done with an automated titrator (model 931, Hanna Instruments). Solutions of NaOH and HCl (2 N each) were used for the CJ

titrations. The NaOH solution was standardized in triplicate by conventional end-point titration with 0.3 g of potassium hydrogen phthalate in 50 mL of deionized water. HCl was then standardized in triplicate with the NaOH stock to titrate 5-mL aliquots of the HCl solution in 50 mL of water. The acid and base concentrations were calculated based on the volume needed to reach the equivalence point as calculated by the titrator software (Hanna Instruments). To develop BC models for the CJ media, titrations were done using the methods of Price et al. *(24)*. CJ media were titrated for pH 2 to pH 12 in two 50-mL aliquots, one for NaOH titration and one for HCl titration, with a custom dynamic dosing protocol, and the data were processed as described using the published Matlab algorithms *(24)*. The volume and pH data from the titrations were imported into Matlab as .RPT files from the titrator and transformed to BC curves using equation 1:

$$\beta = \Delta(\text{volume of acid or base})/\Delta\text{pH} \quad (1)$$

For each medium, a matrix of values for seven monoprotic buffers consisting of molar concentration (C_i) and equilibrium constant (K_i) (transformed to pK by calculating the negative logarithm) was then obtained using a constrained minimization algorithm to fit the observed BC curve (modeled from equation 1, titration data) with a BC model defined by equation 2:

$$\beta = 2.303 \times \Sigma\left[C_i K_i [\mathrm{H}^+]/([\mathrm{H}^+] + K_i)^2\right] + (K_w/[\mathrm{H}^+]) + [\mathrm{H}^+] \quad (2)$$

where K_w is the equilibrium constant of water and $[\mathrm{H}^+]$ is the hydrogen ion concentration over the pH range (2 to 12). By default, all buffers with K_i values $\geq 10^{-7}$ were defined as acids (K_a and corresponding C_a values), and the remaining buffers were defined as bases (K_b and C_b). The resulting buffer tables were then used to optimize adj$C$ values (salts of an acid or base) for the initial pH (as $[\mathrm{H}^+]$) of each medium by solving equation 3 using Newton's minimization method as suggested by Butler and Cogley *(2)*:

$$0 = \Sigma[C_a K_a/(K_a + [\mathrm{H}^+])] - \Sigma[C_b[\mathrm{H}^+]/([\mathrm{H}^+] + K_b)] + (K_w/[\mathrm{H}^+]) - [\mathrm{H}^+] + \text{adj}C \quad (3)$$

The adj$C$ values represent sums of the molar concentrations for the salts of an acid (negative value) or base (positive value) and the error in the model *(2, 24)*. BC matrices were reported using the estimated molar concentrations (C_a or C_b) and the corresponding pK values (negative log of the K_a or K_b values). To generate predicted pH values for fermented CJ samples, lactic and/or acetic acid buffers were added to the BC tables derived from the unfermented CJ media. The additional buffers consisted of acid concentrations (C_a values were determined by HPLC analysis of fermentation samples), and the corresponding pK values for lactic and acetic acids. The pK values were adjusted for the ionic strength of 2% NaCl ($I = 0.342$ M). Adjustments of pK values were done using the Davies equation with suggested constants from Butler and Cogley *(2)*:

$$\mathrm{pK_{adj}} = \mathrm{pK_a} - 1.02\left[\sqrt{I}/\left(1 + \sqrt{I}\right)\right] - 0.3I \quad (4)$$

The pH for the summed acid and base buffer values was estimated by solving equation 3 as described above. For some pH predictions from the BC models, the lactic acid concentrations from fermentation samples were adjusted by subtracting the molar concentration of malic acid in the corresponding unfermented CJ medium to account for pH changes due to the malolactic reaction (as described below). Acetic acid concentrations were not similarly adjusted.

Fermentation of CJ made from three sizes of cucumbers. LA81 and LA445 cell cultures were transferred to an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) and used to inoculate CJ media. Sterile plastic bottles (100 mL) containing 40 mL of CJ1, CJ2, or CJ3 were preincubated overnight in the anaerobic chamber with the caps loose to allow dissolved oxygen to dissipate and were then inoculated to ca. 10^6 CFU/mL with 10^{-2} to 10^{-3} dilutions of the overnight LAB cultures. These cultures were then incubated at 30°C in an incubator inside the anaerobic chamber, and 5-mL samples were collected at 24 and 48 h for cell counts, pH, and HPLC analysis. Samples from each noninoculated control CJ medium were also prepared for analysis. Cell counts were obtained by serial dilution. The remaining cell suspension samples were centrifuged (5 min, 3,400 × g) at room temperature (Lynx 4000 centrifuge with A21-24 × 15c rotor, Thermo Fisher Scientific) and filtered with a sterile syringe filter (0.2-μm pore size). A 1.5-mL aliquot of the supernatant was frozen for subsequent HPLC analysis, and the pH of the remaining sample was determined with a standardized pH meter (Hanna Instruments). To generate fermented CJ3 samples for titration experiments, 250 mL of CJ3 was used for fermentation in 500-mL plastic bottles. After 48 h, the bottles were removed from the anaerobic chamber and a cell-free supernatant was obtained by centrifugation at 13,765 × g (F12-6 × 500 LEX rotor, Thermo Fisher Scientific) in 500-mL centrifuge bottles. The supernatant was decanted through cheesecloth and then sterilized by filtration with a 0.45-μm-pore-size 250-mL bottle filter. Samples were stored at 4°C as needed until to titration.

Statistics and modeling. HPLC analyte and pH data were collected from independently replicated experiments with three or more trials. A one-way analysis of variance was used for analyte concentrations in CJ media made from cucumbers of different sizes at 24 and 48 h of fermentation using the Tukey-Kramer test for separation of means (JMP version 12.0.1, SAS Institute, Cary, NC). JMP software also was used for analysis of linear regression models including the calculation of the root mean square error values for observed and predicted pH values.

RESULTS AND DISCUSSION

BC of CJ media. The BC of CJ media prepared from cucumbers of different sizes (1, 2, and 3) was measured with buffer models developed from titration data by using methods described by Price et al. *(24)*. BC models for CJ1, CJ2, and CJ3 each included seven monoprotic buffers that would reproduce the buffering identified by titration (Fig. 1 and Table 1). The concentrations and pK values of these buffers are represented by the vertical lines in Figure 1 that originate at the pK of each buffer; the height of each line corresponds to the maximum BC value at that pK. Because the buffering effect from individual buffers in the medium may overlap (by ca. ±1 pH unit), the lines do not necessarily meet the model BC curve at a given pK value. Each of the buffers identified for a CJ model may be representative of the composite buffering of undefined weak acids and bases in solution, including molecules with amino and carboxyl groups. Because the BC models were developed from titration data for media containing 2% NaCl, the predicted pK values were defined for an ionic strength representative of that salt concentration (0.342 M). The NaCl concentration of the medium was 2%, which was

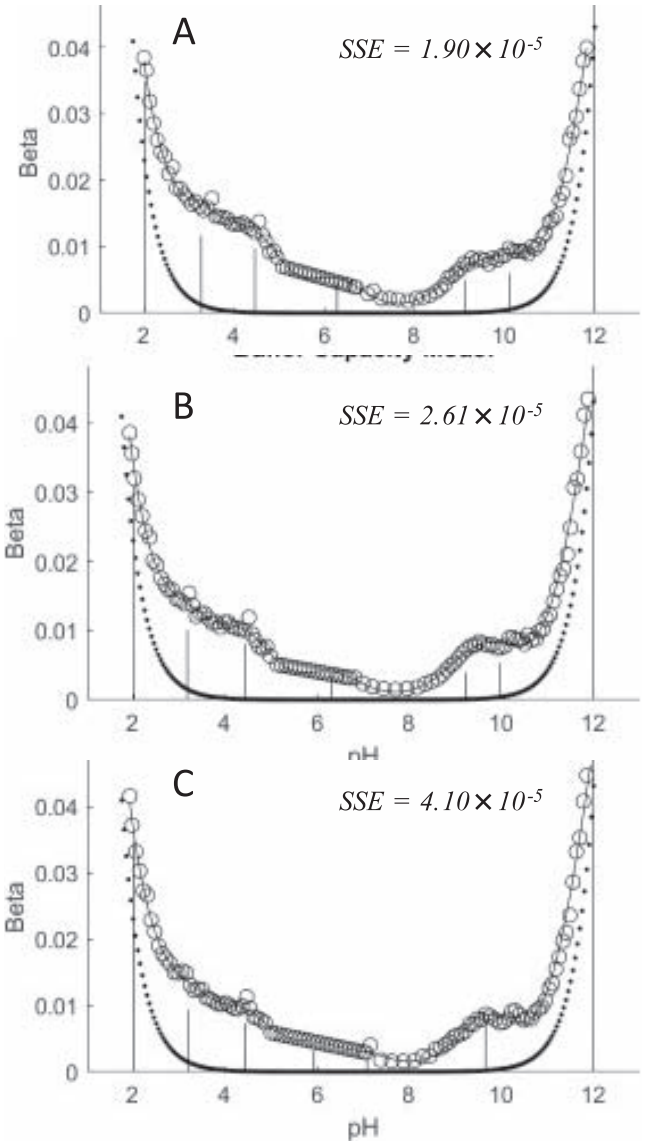

FIGURE 1. *Buffer capacity (BC) models for CJ1 (A), CJ2 (B), and CJ3 (C), showing the processed BC data from each titration (circles), the fitted model (solid line), and the BC of water (dotted line). Vertical lines represent the BC (β) at each pK value. The sum of squares error term for the fit of the model to the data is given for each medium.*

TABLE 1. *Monoprotic buffer components from BC models of CJ media*[a]

	CJ1		CJ2		CJ3	
Buffer	Concn (mM)	pK	Concn (mM)	pK	Concn (mM)	pK
1	21.58	2.01	15.76	2.02	20.42	2.00
2	18.15	3.27	14.85	3.18	13.68	3.20
3	16.70	4.46	13.94	4.43	12.50	4.42
4	8.06	6.28	6.32	6.32	5.53	5.93
5	8.61	9.11	6.85	9.23	3.43	7.08
6	9.93	10.12	8.91	9.95	13.98	9.65
7	44.07	12.00	43.84	12.00	46.32	12.00

[a] Concentrations and pK values for each buffer were estimated from the CJ models.

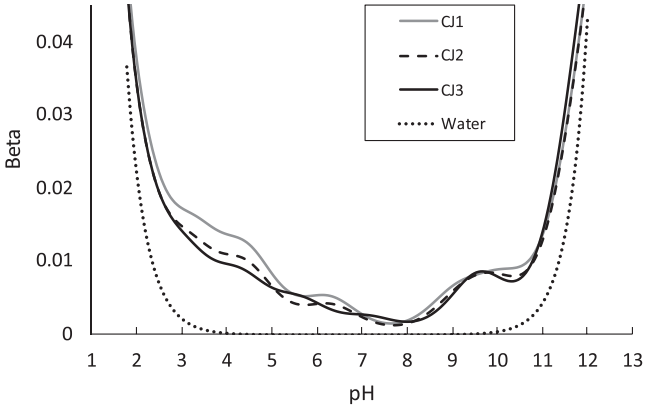

FIGURE 2. *Comparison of the BC models (β) for unfermented CJ1 (shaded line), CJ2 (dashed line), and CJ3 (solid line) and the BC of water (dotted line).*

chosen because it is representative of vegetable fermentations typical of home fermenters and of many fermented vegetable products consumed without further processing after fermentation (F.B., unpublished data). For the BC models, the sum of squared error values for the fit of the model to the BC data were 1.90×10^{-5}, 2.61×10^{-5}, and 4.10×10^{-5} for CJ1, CJ2, and CJ3, respectively (Fig. 1).

The concentrations and pK values for buffers identified at extremes of the pH ranges for each CJ medium were not precisely defined. These pK values were constrained to pH 2 and pH 12 (the extent of the titrations), and the estimated concentrations included buffering due to the buffer capacity of water. Other factors such as electrode calibration errors and the effect of weakly acidic hydroxyl molecules on glucose and fructose present in unfermented cucumber juice may complicate the measurement of buffering around pH extremes *(13)*. The effects of these confounding factors on the estimated pH of the CJ media from the model buffers may be compensated for by optimizing the adj*C* value (equation 3) for each model as described by Price et al. *(24)*. The adj*C* estimates may also be used to define the buffering effects of salts of an acid or a base. The optimized adj*C* values for the CJ models were 5.7, 14.4, and 15.5 mM for CJ1, CJ2, and CJ3, respectively. These adj*C* values were subsequently used for all pH estimations with the corresponding BC models for each CJ medium.

The pH changes observed during cucumber brine fermentations by LAB are primarily driven by the production of lactic and/or acetic acids *(7)* but also depended on buffering of the brine. The BC models for CJ media at pH 3 to 5 differed; CJ1 had greater buffering than did CJ2 and CJ3 in this range (Fig. 2). This observation supports the results of Lu et al. *(16),* who found that size 1 cucumbers had greater buffering effect on pH changes from lactic acid production than did size 2 or 3 cucumbers, resulting in complete sugar utilization during fermentation. The BC model for CJ1 (Fig. 1A and Table 1) had two buffers at pK values of 3.27 and 4.46 with estimated concentrations of 18.15 and 16.70 mM, respectively. Similar buffers were present in CJ2 and CJ3, but with approximately 4 mM lower concentrations (Table 1). Malic

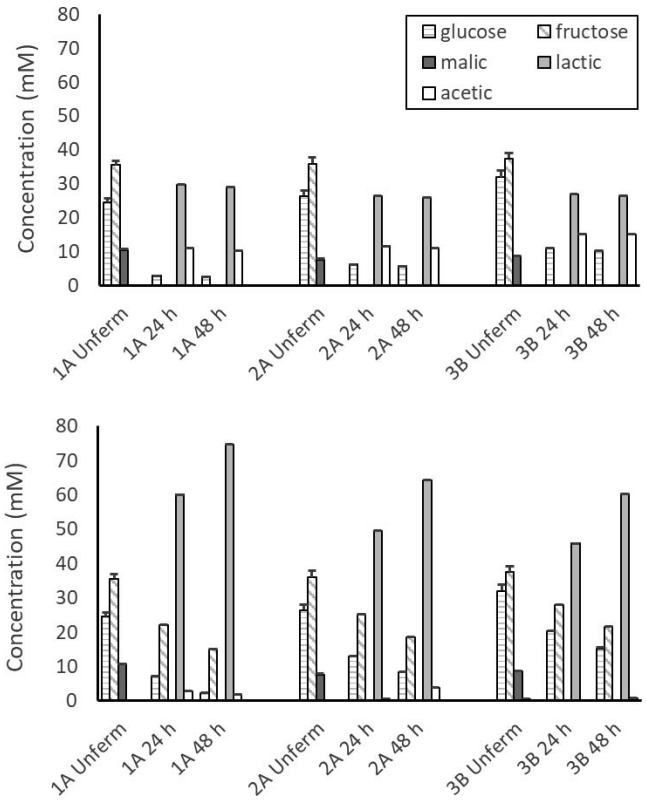

FIGURE 3. *Fermentation biochemistry of CJ by LA81 (A) and LA445 (B), with mean concentrations of glucose (bars with horizontal lines), fructose (bars with diagonal lines), malic acid (dark shaded bars), lactic acid (light shaded bars), and acetic acid (open bars). The CJ medium was made from size 1A, 2A, and 3B cucumbers, and results are given for unfermented media and media fermented for 24 and 48 h. Error bars indicate the standard deviation for three independent fermentations.*

acid, a constituent of cucumbers *(21),* decreases in concentration with cucumber size *(16)* and would buffer at pH 3 to 5. The theoretical pK values for diprotic malic acid in CJ media were estimated to be 3.13 and 4.93 after adjusting published pK values of 3.40 and 5.20 *(12)* for the ionic strength effects of 2% NaCl. Therefore, differences in malic acid concentration probably contributed to the differences in buffering of unfermented CJ1, CJ2, and CJ3 at pH 3 to 5 and contributed to the observed buffer peaks in Figure 1.

Fermentation biochemistry. Biochemical analysis of CJ1, CJ2, and CJ3 (Fig. 3) revealed that glucose and fructose concentrations in each medium were similar, except that CJ3 had significantly higher ($P < 0.05$) glucose concentrations (31.96 ± 0.1.96 mM) than did CJ1 (24.47 ± 1.28 mM) or CJ2 (26.40 ± 1.74 mM). Malic acid concentrations in unfermented CJ media were 10.56 ± 0.20, 7.65 ± 0.31, and 8.58 ± 26 mM for CJ1, CJ2, and CJ3, respectively (Fig. 3). These concentrations were 4 to 8 mM lower than the concentration estimates for the two BC model buffers (Table 1) at pH 3 to 5, which likely included other undefined buffer components. However, malic acid was not detected by HPLC in fermented CJ samples presumably because malolactic enzyme, which is active in LA81 and LA445, converted malic acid to lactic acid *(1, 10)*.

Analysis of the biochemistry of heterolactic LA81 fermentations at 24 and 48 h revealed no detectable fructose (Fig. 3), as expected. Fructose can be used as an electron acceptor by *L. mesenteroides* and directly reduced to mannitol *(27)*. However, the conversion of fructose to mannitol should not influence buffering. The principal fermentation acids produced by LA81 at 24 and 48 h were 25 to 30 mM lactic acid and 10 to 16 mM acetic acid (Fig. 3A). Residual glucose was found in CJ2 and CJ3 LA81 fermentations but not in CJ1 fermentation, similar to the observations of Lu et al. *(15)*. These authors concluded that the enhanced buffering of size 1 cucumbers allowed complete fermentation of sugars, whereas with larger cucumbers, fermentations were incomplete. Although small differences in acid concentrations were observed between 24 and 48 h for all LA81 CJ fermentations, catabolic activity had mostly ceased by 24 h.

Homolactic fermentation of CJ media by LA445 differed significantly ($P < 0.05$) in lactic acid production based on cucumber size and fermentation duration. All fermentations had residual sugars, 2 to 15 mM glucose and 15 to 21 mM fructose. CJ3 fermentations had the highest residual sugar and CJ1 had the lowest. CJ1 fermentations had higher lactic acid concentrations than did CJ2 or CJ3 fermentations, and all media had higher acid concentrations at 48 h than at 24 h (Fig. 3B). After 48 h of LA445 fermentation in CJ1, CJ2, and CJ3, the mean lactic acid concentrations were 74.66 ± 0.04, 64.18 ± 0.23, and 60.10 ± 1.9 mM, respectively. As expected, little or no acetic acid was produced (<4 mM for all media) by homolactic LA445 fermentation. Although undetermined nutrient differences between the three CJ media may influence the extent of fermentation by both LA81 and LA445, the data are consistent with the hypothesis that greater buffering in CJ1 compared with CJ2 and CJ3 explains the differences in sugar utilization and acid production.

BC models of fermented CJ3. To investigate the effect of acid production on buffering changes during fermentation, the BC models of CJ3 before and after fermentation with LA81 and LA445 for 48 h were compared (Figs. 4 and 5). The data for the BC models of CJ3 revealed two obvious differences with and without fermentation by LA81 (Fig. 4). At pH 3 to 5, buffering increased due to lactic and acetic acids produced during fermentation. The expected pK values for buffering of lactic and acetic acids were 3.59 and 4.49, respectively, considering the effects of the ionic strength of 2% NaCl (ionic strength of 0.342 M). The pK values reported for lactic and acetic acids in water at 25°C are 3.86 and 4.76 *(12)*. Temperature had a negligible effect on pK between 25 and 30°C and was therefore ignored (F.B., unpublished data). The BC model for CJ3 fermented with LA81 had two buffers in the pH 3 to 5 range, with estimated concentrations of 40.89 and 14.07 mM and pK values of 3.65 and 4.73, respectively (Table 2). The concentrations of lactic and

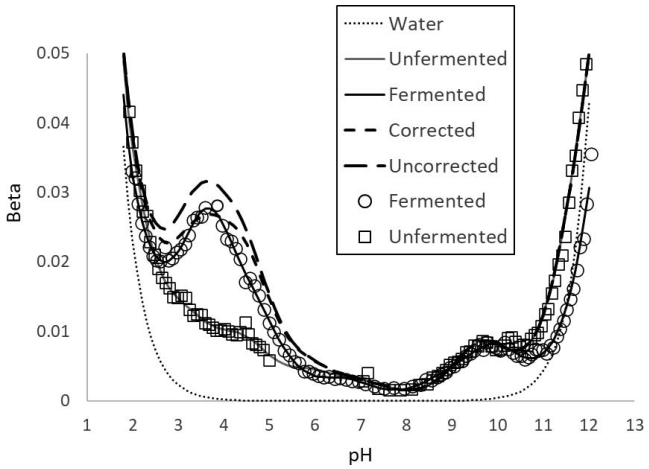

FIGURE 4. *Unfermented and LA81 fermented CJ3 at 48 h. BC model data include the BC of water (dotted line), unfermented CJ3 (shaded line), fermented CJ3 (solid line), model prediction for fermented CJ3 with the malic acid correction (LA81C; short-dashed line), and model prediction for fermented CJ3 without the malic acid correction (LA81UC; long-dashed line). The buffering from the titration data is shown for fermented (circles) and unfermented (squares) samples.*

acetic acids measured by HPLC (Fig. 3) were 26.50 ± 0.12 and 15.07 ± 0.24 mM, respectively. The predicted buffer for lactic acid had a higher acid concentration than expected (by 14.4 mM). This difference was likely due to additional buffering by undefined compounds in the CJ3 media (Fig. 1). The expected conversion of malic acid to lactic acid by the malolactic reaction of LA81 and LA445 would also influence buffering around the pK values of lactic and acetic acid.

A noticeable difference was found between the fermented and unfermented BC curves for the LA81

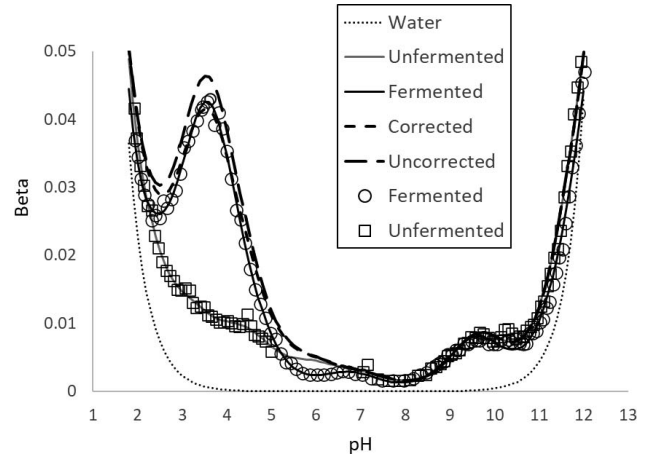

FIGURE 5. *Unfermented and LA445 fermented CJ3 at 48 h. BC model data include the BC of water (dotted line), unfermented CJ3 (shaded line), fermented CJ3 (solid line), model prediction for fermented CJ3 with the malic acid correction (LA445C; short-dashed line), and model prediction for fermented CJ3 without the malic acid correction (LA445UC; long-dashed line). The buffering from the titration data is shown for fermented (circles) and unfermented (squares) samples.*

TABLE 2. *BC model buffers for CJ3 fermented for 48 h with LA81 and LA445*

	LA81[a]		LA445[b]	
Buffer	Concn (mM)	pK	Concn (mM)	pK
1	13.84	2.27	13.08	2.31
2	40.89	3.65	66.51	3.56
3	14.07	4.73	8.02	4.36
4	4.76	6.65	4.45	6.67
5	3.84	8.84	10.70	9.75
6	12.39	9.93	3.55	8.93
7	12.62	12.00	36.24	12.00

[a] Buffers predicted from LA81 fermentation of CJ3.
[b] Buffers predicted from LA445 fermentation of CJ3.

fermentation shown in Figure 4 at pH 10 to 12. The buffering observed in the unfermented samples in this region was likely due to weakly acidic sugar hydroxyls *(13)*. At 48 h, the unfermented CJ3 had 31.96 ± 1.96 mM glucose and 35.97 ± 1.91 mM fructose, whereas the fermented CJ3 had 10.26 ± 0.08 mM glucose and no detectable fructose. These data support the assumption that the BC from small amounts of sugar remaining at 48 h of fermentation approximated the BC of water for pH values above 11. However, this change in buffering was unlikely to influence the pH of the medium because it represents acid buffering (although with a pK value above pH 11), not buffering due to a strong base. Similar results were found for the effects of acidic hydroxyls for BC models of sucrose and other sugars used in salad dressing products *(13)*.

For LA445 fermentation of CJ3 (Fig. 5), an acid BC peak was estimated with a pK of 3.56, corresponding to the adjusted pK for lactic acid of 3.59. The estimated concentration for this buffer was 66.51 mM, which roughly corresponded to the measured lactic acid concentration of 60.1 mM. The difference between the model and the measured acid concentration was likely due to additional buffers with similar pK values present in the CJ medium. Little change in buffering occurred above pH 10 (Fig. 6), unlike the change observed for LA81 (Fig. 5). However, the LA445 CJ3 fermentations had much higher residual sugar concentrations (Fig. 3), with 15.30 ± 0.14 mM glucose and 21.43 ± 0.19 mM fructose remaining at 48 h. The homolactic lactobacilli (including LA445) do not convert fructose to mannitol, and both the fructose and glucose were utilized for acid production, as is typical of cucumber fermentations *(7, 15, 16)*. The higher acid concentrations and lower pH of the LA445 fermentations compared with the LA81 fermentations presumably limited further sugar utilization by LA445. The similarities in buffering observed for the BC models above pH 10 for the unfermented CJ3 and the LA445 fermentation were therefore likely due to the residual sugar (>36 mM).

Modeling fermentation BC based on unfermented CJ3. The principal changes in BC between fermented and unfermented CJ3 were apparently due to the addition of lactic and/or acetic acids for LA81 and LA445 (Figs. 4 and

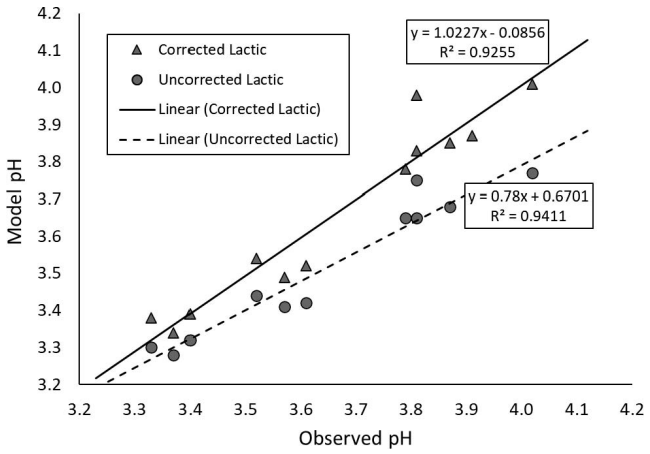

FIGURE 6. *Regression models for observed pH and pH estimated from the BC models for fermented CJ1, CJ2, and CJ3 with the malic acid correction (LA81C and LA445C, triangles) and without the malic acid correction (LA81UC and LA445UC, circles). Linear regression lines are shown for corrected pH estimates (solid line) and uncorrected estimates (dashed line) with the regression equation and corresponding* R^2 *values.*

5). These data suggest that BC models for fermented samples could be constructed based on the addition of acid buffers to the unfermented BC model. The addition of buffers based on the measured lactic acid and acetic acid concentrations from fermented CJ3 should approximate the fermented CJ3 BC models. However, the malolactic reaction quantitatively converts malic acid to lactic acid in a one-step reaction *(3),* which would therefore contribute to the lactic acid concentration measured in the fermented CJ at 24 and 48 h. This reaction also results in the loss of a proton from the medium, raising the pH. The effect on pH of the increased lactic acid concentration due to the decarboxylation of malic acid would therefore effectively be neutral with respect to the BC models.

To account for the effects of malolactic fermentation on the buffering of fermented CJ3, BC models of fermented CJ3 were constructed in two ways, with and without subtracting the malic acid contribution to the lactic acid concentration measured from fermented samples (Table 3). These BC models included LA81UC (uncorrected) with 28.94 mM lactic acid and 15.10 mM acetic acid (concentrations as shown in Fig. 3) and LA81C (corrected) with 20.57 mM lactic and 15.10 mM acetic acid. The LA81C lactic acid concentration was derived by subtraction of the molar concentration for the malic acid contribution to the measured lactic acid concentration in CJ3 at 48 h of fermentation. The pK values for the added lactic acid and acetic acid buffers were 3.59 and 4.49, corrected for ionic strength (Table 3). Similar buffer models (LA445UC and LA445C) were developed from the LA445 CJ3 fermentation biochemistry data. The buffer tables for each model were then used to generate predicted BC curves using equation 2, and the results depicted by the dashed lines in Figures 4 and 5.

The predicted BC curves from LA81UC overestimated the buffering predicted for CJ3 fermented with LA81 for 48 h (Fig. 4). The height of this peak likely represented BC contributions from the added lactic acid buffer and overlap from the existing buffers in unfermented CJ3, with estimated pK values of 3.20 and 4.42, and included the influence of malic acid buffering. However, after subtracting the malic acid contribution to lactic acid, the buffering (LA81C) and the model prediction more closely approximated the fermented CJ3 buffering (Fig. 4). Similar results were obtained for LA445 fermentation data with models LA445UC and LA445C (Fig. 5). The conclusion drawn was that the buffering contribution of the malic acid metabolized by malolactic enzyme was functionally equivalent to the buffering contribution of the lactic acid produced. Because buffering from the two pK values of malic acid (adjusted for ionic strength of 0.342, pK 3.13 and 4.93) would have overlapped with the adjusted pK for lactic acid of 3.59, a more precise analysis of buffer changes was not possible.

TABLE 3. *CJ3 buffer tables with added lactic and acetic acids*

	Buffer concn (mM)					
Buffer	CJ3[a]	LA81UC[b]	LA81C[c]	LA445UC[d]	LA445C[e]	pK
1	20.42	20.42	20.42	20.42	20.42	2.00
2	13.68	13.68	13.68	13.68	13.68	3.20
3	ND	28.94	20.57	60.10	51.73	3.59
4	12.50	12.50	12.50	12.50	12.50	4.42
5	ND	15.10	15.10	0.86	0.86	4.49
6	5.53	5.53	5.53	5.53	5.53	5.93
7	3.43	3.43	3.43	3.43	3.43	7.08
8	13.98	13.98	13.98	13.98	13.98	9.65
9	46.32	46.32	46.32	46.32	46.32	12.00

[a] Unfermented CJ3 (Fig. 1). ND, not detected.
[b] CJ3 with lactic acid (uncorrected) and acetic acid from LA81 fermentation.
[c] CJ3 with corrected lactic acid and acetic acid from LA81 fermentation.
[d] CJ3 with lactic acid (uncorrected) and acetic acid from LA445 fermentation.
[e] CJ3 with corrected lactic acid and acetic acid from LA445 fermentation.

Predicting fermentation pH with BC models. The BC models for CJ media represent the total buffering that would affect the pH of the medium *(24)*. BC models were therefore used to estimate pH by solving equation 2, using the sum of the individual buffers in a given BC model. Models with similar BC curves should have a similar pH. To estimate pH in fermented CJ media based on the analysis of the biochemistry of the fermentations, BC models were developed by supplementing the unfermented CJ1, CJ2, and CJ3 BC models with lactic and acetic acid buffers based on HPLC data from fermented media for 24 and 48 h of fermentation. The supplemented buffer models were prepared using lactic acid concentrations with and without the malic acid correction. The acid concentrations used were the measured lactic acid, the corrected lactic acid (subtracting the molar contribution from malic acid), and acetic acid (Table 4). The resulting pH estimates for all models were compared with the corresponding observed

TABLE 4. *Observed and predicted pH for fermented CJ media*

Sample[a]	Acid concn (mM)[b]			pH		
	Lactic	Lactic (adj)[c]	Acetic	Observed	Pred[d]	Diff[e]
LA81						
CJ1 24 h	29.82 ± 0.16	19.27	11.09 ± 0.07	3.81	3.98	−0.17
CJ1 48 h	28.94 ± 0.10	18.39	10.34 ± 0.11	4.02	4.01	0.01
CJ2 24 h	26.84 ± 0.05	19.05	11.66 ± 0.03	3.87	3.85	0.02
CJ2 48 h	25.88 ± 0.12	18.45	11.10 ± 0.07	3.91	3.87	0.04
CJ3 24 h	27.56 ± 1.11	18.64	15.03 ± 0.10	3.81	3.83	−0.02
CJ3 48 h	27.23 ± 1.44	20.57	15.11 ± 0.21	3.79	3.78	0.01
LA445						
CJ1 24 h	60.01 ± 0.10	49.46	2.66 ± 0.22	3.61	3.52	0.09
CJ1 48 h	74.66 ± 0.04	64.11	1.78 ± 0.01	3.4	3.39	0.01
CJ2 24 h	49.55 ± 0.13	42.12	0.55 ± 0.1	3.57	3.49	0.08
CJ2 48 h	64.18 ± 0.23	56.75	3.78 ± 0.14	3.37	3.34	0.03
CJ3 24 h	45.88 ± 0.11	37.47	ND	3.52	3.54	−0.02
CJ3 48 h	60.94 ± 1.70	51.73	0.89 ± 0.06	3.33	3.38	−0.05

[a] Samples from CJ1, CJ2, and CJ3 were fermented for 24 or 48 h.
[b] Values are means ± standard deviations. ND, not detected.
[c] Concentrations were adjusted by subtracting the malic acid concentration for each CJ medium.
[d] Predicted pH using the buffer matrix of the unfermented CJ medium supplemented with lactic and acetic acid.
[e] Mean observed pH minus the pH predicted from the model.

fermentation pH values. The predicted pH values estimated from corrected models had an excellent fit to the observed pH data (Fig. 6), with the slope for linear regression of 1.02, an intercept of 0.09, and an R^2 value of 0.93. The slope of the regression line for the uncorrected model estimates was 0.78, with an intercept of 0.67 and an R^2 of 0.94. All of the estimated pH values for the models with the uncorrected lactic acid concentrations (without subtracting the malic acid concentration of the corresponding unfermented medium) underestimated the observed values. Comparison of the data to a linear model with a slope of 1.0 and an intercept of 0 gave root mean square error values of 0.064 pH units for the corrected model data and 0.151 pH units for the uncorrected model. The predicted pH values for each corrected model are also shown in Table 4 with the difference from the observed pH for each fermentation sample. These data indicate that the principal factors influencing pH in fermenting CJ were the acid concentration changes (including malolactic fermentation) without other significant buffering changes.

These results indicate that BC models may be useful for quantifying the effects of other buffering reactions by LAB, such as the decarboxylation of amino acids, which can influence medium pH *(28, 29)*. The amino acid decarboxylation reactions did not have a noticeable effect on fermentation pH or buffering in our model fermentation systems with CJ1, CJ2, or CJ3. The pH changes observed were apparently predicted by changes in malic, acetic, and lactic acids during the fermentations, with little or no other buffering influences. As expected, the apparent differences in buffering from weakly acidic hydroxyls on glucose and fructose observed for pH values above pH 10 (for LA81 fermentations) also did not significantly affect estimated pH values.

The BC models described here effectively linked the measured acid concentrations with pH for the CJ model fermentation systems, so it may also be possible to estimate the acid concentrations in fermentations when the pH is known. The concentration of protonated acid, which may influence fermentation ecology *(17, 18, 25),* could also be calculated. Therefore, BC models may have application in mathematical modeling of bacterial competition in vegetable and related fermentations, where pH is usually modeled with empirical rather than mechanistic approaches. Microorganisms in actual cucumber fermentations may have growth characteristics different from those of the planktonic cells used in our studies. Further research will be needed to determine how BC models may be applied to modeling fermentation ecology and safety.

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