Influence of Microbial Growth on the Redox Potential of Fermented Cucumbers

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ABSTRACT: Commonly, pH measurements are used during the production of fermented cucumbers to indirectly monitor growth of lactic acid bacteria (LAB) and acid production. Redox potential (E_h) measurements, which are determined by the potential of an electron to reduce an acceptor, could serve as an alternative tool to monitor the progress of fermentation allowing the detection of the metabolic activity and/or growth of LAB and other microorganisms. Pasteurized and inoculated jars of cucumbers were observed to better understand how the E_h changes during the cucumber fermentation and how it could be used as a monitoring tool. Jars of diced, brined cucumbers were pasteurized and inoculated with microbes previously isolated from fermented cucumbers including *Lactobacillus plantarum*, *Zygosaccharomyces globiformis*, and *Enterobacter aerogenes*. Although an initial decrease in E_h was observed for all microorganisms, distinctive trends in E_h occurred when these organisms were inoculated. After a 2-wk fermentation period, the E_h (Ag/AgCl, 3 M KCl) in jars inoculated with *L. plantarum*, *Z. globiformis*, and *E. aerogenes* was at +453 ± 55, +104 ± 5, and −156 ± 73 mV, respectively. Cucumbers inoculated with a mixture of *L. plantarum* and *Z. globiformis* had a terminal E_h value of +202 ± 24 mV, which was between that found for the individual microorganisms. *L. plantarum* dominated the E_h trend when inoculated along with *E. aerogenes* with a final E_h of +411 ± 72 mV. The results showed that changes in E_h continued after pH measurements became stable. Thus E_h measurement can provide a tool to continuously monitor microbial growth during the course of cucumber fermentations.

Keywords: cucumber fermentation, enterobacter, lactobacilli, redox potential, *Zygosaccharomyces*

Introduction

Food fermentations have been used throughout the world for centuries as a means of preservation. Fermented foods typically develop unique sensory qualities and an increased shelf-life compared to the nonfermented raw ingredients. Pickles are a popular vegetable product that can be made through the acidification or fermentation of cucumbers. During the fermentation process, lactic acid bacteria (LAB) naturally present on the fruit grow and produce lactic acid as the major fermentation product from sugars. The resultant removal of sugar, increased acid levels, and low pH combine to preserve the product. Over the course of fermentations, pH is often used to indirectly monitor growth of LAB and completion of the fermentation.

The standard commercial cucumber fermentation is characterized by the predominant growth of lactic acid bacteria and concomitant production of lactic acid. However, growth of yeasts such as *Torulopsis* spp., *Zygosaccharomyces* spp., *Brettanomyces* spp., *Hansenula* spp., *Torulaspora* spp., and *Kloeckera* spp. (Etchells and Bell 1950) may occur postbrining (Etchells 1941), producing carbon dioxide that could cause cucumber bloating (Jones and others 1941). Additionally, *Enterobacter aerogenes* has been identified as an occasional and undesired participant in the early stage of cucumber fermentation due to its ability to produce hydrogen, which may contribute to the formation of bloated cucumbers (Etchells and Jones 1943). Although *E. aerogenes* is a part of the natural microflora of these fruits, it typically does not survive as fermentation proceeds and pH decreases.

Considering the chemical changes that may occur during cucumber fermentations, which are largely dictated by the natural microflora, and the consequences of these changes on the success of the manufacture of quality products, it is useful to develop tools that may aid in monitoring microbial growth throughout the fermentation process. In addition to pH, redox potential (E_h) may be used as a tool to monitor fermentations and predict spoilage. Redox potential is a measurement of the ability of compounds to be oxidized or reduced. During oxidation, electrons are transferred from an electron donor to an acceptor, which is reduced. While pH is a measurement of the concentration of free hydrogen ions in a system, redox potential is a measurement of the activity of electrons in a system (Kjaergaard 1977). This may provide a different, broader picture of the electron transfer during fermentation processes and may detect growth of microbes other than LAB.

The goal of this study was to observe changes in redox potential during pure culture cucumber fermentations to establish representative E_h trends for the environments generated from growth of lactic acid bacteria, yeasts, and enteric bacteria. Determination of such trends could aid in following the growth of beneficial or undesirable microorganisms during cucumber fermentations.

Materials and Methods

Microorganisms and media

Microbial cultures were obtained from the culture collection of the U.S. Dept. of Agriculture-Agricultural Research Service, Food

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Science Research Unit, located in Raleigh, N.C. Cultures of *Lactobacillus plantarum* LA0219; *Zygosaccharomyces globiformis* SPY 9, 15, 21, and 29; and *Enterobacter aerogenes* were prepared on deMan Rogosa and Sharpe agar (MRS), yeast mold, agar supplemented with 1 mM chlortetracycline and 1.5 mM chloramphenicol (YM), and Tryptic Soy Agar (TSA), respectively. All culture media were obtained from Becton, Dickinson and Co. (Sparks, Md., U.S.A.). *L. plantarum*, *Z. globiformis*, and *E. aerogenes* were originally isolated from fermented cucumbers (Daeschel and other 1984), spoiled sweet pickles (Bell and Etchells 1952), and bloated cucumbers, respectively.

Cucumber fermentation

Size 3B (44 to 51 mm dia) cucumbers were diced into 10 mm cubes with a Hobart FP150 Food Processor (Hobart Corp., Troy, Ohio, U.S.A.). Diced cucumbers (1892 g) were covered with 1892 mL of cover solution in 3.84-L glass jars (50:50 cucumber:brine pack out ratio). Cover brine solution contained acetic acid from commercial vinegar, sodium chloride (Morton Canning and Pickling Salt; Morton Salt, Chicago, Ill., U.S.A.) and calcium chloride (Dow Chemical, Midland, Mich., U.S.A.) such that after equilibration with the cucumbers the final concentrations were 8.33 mM (0.05%), 1.03 M (6%) and 40 mM (0.44%), respectively. Jars were closed with commercial lug caps fitted with a rubber septum to allow for inoculation and sampling of the jars with sterile syringes and a metal fitting to hold a redox probe in place. The lids were heated in boiling water for at least 30 s to soften the sealing compound and immediately applied to the filled jars. Redox probes were calibrated, rinsed thoroughly with 70% ethanol and secured into the fittings on the jar lids. The tip of the probe was placed in the center of the jar and remained in contact with the brine cover solution. The sealed jars were heated to a temperature of 74 °C for 15 min at the slowest heating point to kill vegetative microorganisms and then cooled to 30 °C with cold tap water. Triplicate jars were then inoculated with *L. plantarum* LA0219 (10^6 CFU/mL), *Zygosaccharomyces globiformis* cocktail (10^3 CFU/mL), *L. plantarum* LA0219 plus *Zygosaccharomyces globiformis* cocktail, *E. aerogenes* (10^6 CFU/mL), or with *L. plantarum* LA0219 plus *E. aerogenes* and stored in a 30 °C incubator during fermentation. Six noninoculated control jars were stored at 4 °C to prevent germination and growth of spores, which were not killed by the heat treatment. Samples for chemical and microbiological analyses and redox measurements were collected at the start of the fermentations and at 24 h intervals for a period of 12 d.

Evaluation of microbial growth

Jars were visually monitored for the development of turbidity and/or pressure on the lids. Additionally, brine samples were collected using aseptic techniques for microbiological analysis. Samples were plated on MRS, YM, plate count agar (PCA), and violet red bile agar (VRBG) (Becton, Dickinson and Co.). Plating was conducted using a spiral plater (Autoplate 4000, Spiral Biotech Inc., Norwood, Mass., U.S.A.). An automated colony counter (QCount, Spiral Biotech Inc.) was used to determine plate counts. MRS plates containing a mixed microbial population were incubated under anaerobic conditions to prevent growth of yeasts. The occurrence of microbial growth was also evaluated by determination of changes in sugars, organic acids, and ethanol during incubation of the jars at 30 °C. Brine and cucumber cube samples (50:50 [w/v] ratio) were collected daily using aseptic techniques and frozen at –80 °C. Prior to analysis, samples were thawed, macerated as required, and centrifuged at 12000 rpm using an Eppendorf 5810R Centrifuge (Eppendorf North America, Inc., Westbury, N.Y., U.S.A.). Analyses were done by HPLC on a 30 cm HPX-87H column (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) (McFeeters and Barish 2003). The column was heated to 65 °C and eluted with 0.03 N sulfuric acid at a flow rate of 1.0 mL/min. Samples were analyzed for malic acid, lactic acid, acetic acid, propionic acid, and butyric acid using a UV6000 LP detector (Thermo Separation Products, Inc., San Jose, Calif., U.S.A.). A Waters model 410 refractive index detector (Waters, Milford, Mass., U.S.A.) connected in series was used to measure glucose, fructose, and ethanol.

Determination of redox potential (E_h) and pH

Redox potential and pH measurements were taken every 24 h from each jar. pH was measured using an AccuFet solid-state pH combination electrode (Fisher Scientific, Pittsburgh, Pa., U.S.A.) and Accumet AR25 pH meter (Fisher Scientific) from aliquots of the equilibrated brine. Redox potential was measured using ORP combination electrodes (InLab 501/170, Mettler-Toledo, Bedford, Mass., U.S.A.) connected to a dual channel pH/ion meter (Accumet AR25, Fisher Scientific) to obtain readings in millivolts. The adequate functionality of the ORP electrodes was verified by using both pH 7 and pH 4 calibration buffers with added quinhydrone before each experiment. Alumina powder (1 micron; Precision Surfaces Intl., Houston, Tex., U.S.A.) was used to polish the metal tip of the electrodes periodically. The measured redox potentials were converted to the redox potential against the standard hydrogen electrode (E_h) by simply adding 203.4 mV (30 °C) as suggested by the manufacturer. Measurements obtained from samples incubated at 4 °C were adjusted by adding 222.5 mV instead.

Statistical analysis for the calculated E_h values was performed using a mixed model with a repeated measure design and an autoregressive structure in which intra-jar neighbors that are *d*-half days apart have a correlation of ρ^d and 2 measurements from different jars are independent. Fixed effects for treatments, time (d), and their interaction was considered. The models were fitted using the statistical analysis software mixed procedure (SAS Inst. Inc., Cary, N.C., U.S.A.). Tests for equality of E_h means over time were individually conducted for each treatment. Pairwise comparisons were made for each treatment. Pairwise differences were declared significant based on the unadjusted *P* values and those modified using the Tukey and Bonferroni adjustments for the family of all pairwise comparisons.

Results and Discussion

Commercial cucumber fermentations are typically dominated by growth of acid resistant lactic acid bacteria, primarily *Lactobacillus plantarum*. However, the natural cucumber microflora may vary with the production geographic area, climate, and soil conditions, the harvesting season, and distribution channels. As a result, atypical fermentations occasionally occur with undesirable consequences. The approach taken here was to remove the naturally occurring vegetative cells of microorganism from the cucumbers, inoculate the brined cucumber cubes with known cultures of bacteria or yeasts, and then observe changes in E_h along with measurements of pH and fermentation substrates and products. In this way, E_h was correlated with parameters that have been previously used to follow the course of cucumber fermentations.

Different trends in redox potential were observed in pasteurized cucumber jars inoculated with *L. plantarum*, *Z. globiformis*, and *E. aerogenes* (Figure 1A). As expected, fermentation by *L. plantarum* induced an initial decline in redox potential as lactic acid was produced (74.0 ± 12.2 mM), glucose and fructose (45.7 ± 6.2 mM) were

consumed (Figure 1D), and the pH decreased during fermentation (van Dijk and others 2000; Ouvry and others 2001; Ouvry and others 2002). E_h measurements unexpectedly increased as *L. plantarum* entered stationary phase of growth (Figure 1A and 1C). Although, this steady increase in the E_h may have been caused by induction of *L. plantarum* autolysis (Gasson 1996; Weinrichter and others 2003), further research is required to explain this observation. Changes in redox potential as a result of *Z. globiformis* growth were minimal and within experimental error not different from the control in which cucumbers were not inoculated. *Z. globiformis* consumed less sugar (38.5 ± 1.9 mM) than *L. plantarum* (Figure 1D) and produced ethanol as the primary fermentation product. As expected, growth of *E. aerogenes* caused a decline in E_h, which remained stable after the culture reached stationary phase (Figure 1A and 1C). *E. aerogenes* generates hydrogen gas as a metabolic end product, which is a strong reductant capable of creating a highly reduced environment (Johansen and others 1975). *E. aerogenes* is not well adapted to the acidic fermentation environment developed in the cucumber jars, so even in the absence of competitive microorganisms only 63.4% (29.2 ± 1.3 mM) of the sugars (glucose + fructose) were utilized (Figure 1D).

Trends of E_h measurements from cucumbers fermented with mixed cultures of *L. plantarum* and *Z. globiformis* (Figure 2) and *L. plantarum* and *E. aerogenes* (Figure 3) were compared with the trends of pure culture fermentations and with changes in pH and the sugars utilized. E_h measurements (Figure 4A) indicated that jars inoculated with both *L. plantarum* and *Z. globiformis* were more reduced than jars inoculated with only *L. plantarum*, and more oxidized than jars inoculated with only *Z. globiformis* (Figure 1A) such that the E_h changes were intermediate between the trends observed with the individual organisms. Statistical analysis revealed that there is a significant difference between the E_h trend observed for *L. plantarum* and that of *L. plantarum* and *Z. globiformis* after terminal values were reached ($\alpha = 0.05$). Change in pH was similar to the pH change observed with the pure culture *L. plantarum* fermentation (Figure 1B). Sugar utilization was greater with the mixed culture than with either of the pure culture fermentations with these 2 organisms (Figure 4C). The amount of lactic acid produced in the mixed culture fermentations (77.0 ± 10.5 mM) was similar to that obtained with *L. plantarum* alone, while the amount of ethanol produced was about half of that observed (22.7 ± 6.3 mM) for the *Z. globiformis* fermentations (49.6 ± 3.1).

The E_h trend resulting from mixed culture fermentations of *L. plantarum* and *E. aerogenes* (Figure 4A) was similar to the E_h changes observed during fermentation of cucumbers by *L. plantarum* only (Figure 1A). No significant difference between these

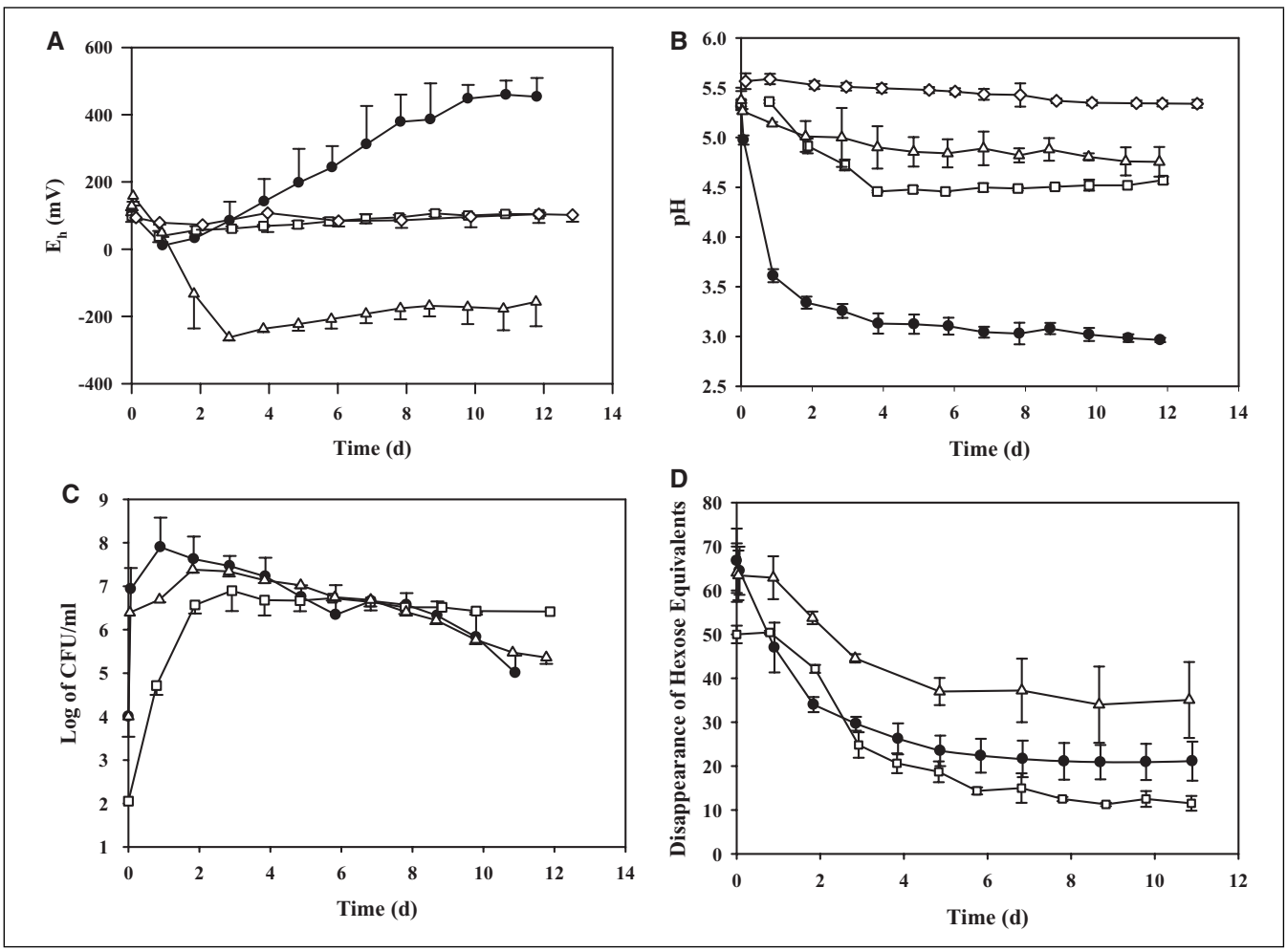

Figure 1—Comparison of the change in redox potential in brined cucumbers with growth of the inoculated microorganisms, pH, and utilization of sugars. (A) E_h, (B) pH, (C) microbial growth, and (D) utilization of sugars (glucose + fructose). Brined and pasteurized cucumbers were not inoculated (◊), inoculated with *L. plantarum* (•), *Z. globiformis* (□), or *E. aerogenes* (Δ). Statistical analysis of the E_h trends from the treatments revealed a significant difference between them at a level of $\alpha = 0.05$ after the terminal E_h values were reached. No significant difference was declared for the comparison between the noninoculated jars and those inoculated with *Z. globiformis*.

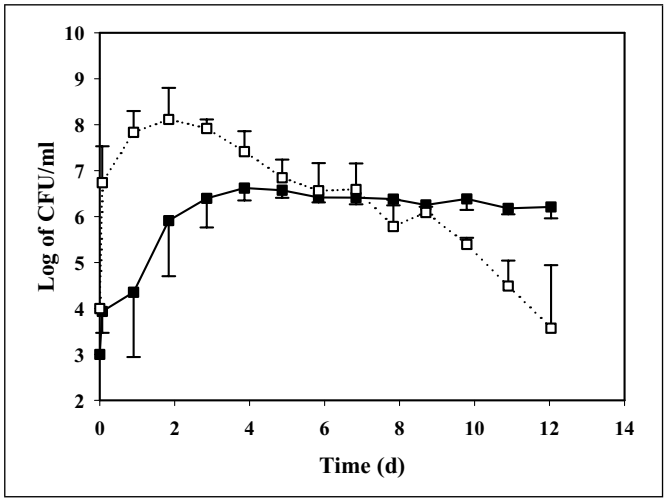

Figure 2—Changes in microbial counts during fermentation of cucumbers with a mixed culture of *L. plantarum* and *Z. globiformis*. Logs of CFU/mL of *L. plantarum* and *Z. globiformis* are depicted with the open (□) and closed (■) square symbols, respectively.

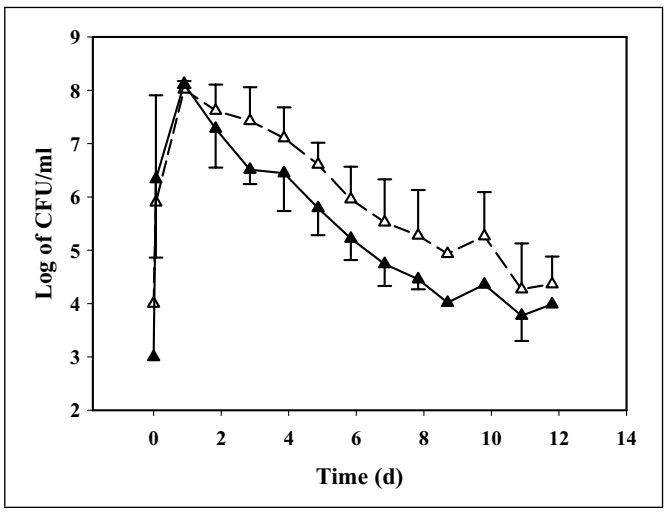

Figure 3—Changes in bacterial counts during fermentation of cucumbers with a mixed culture of *L. plantarum* and *E. aerogenes*. Logs of CFU/mL of *L. plantarum* and *E. aerogenes* are depicted with the open (Δ) and closed (▲) triangle symbols, respectively.

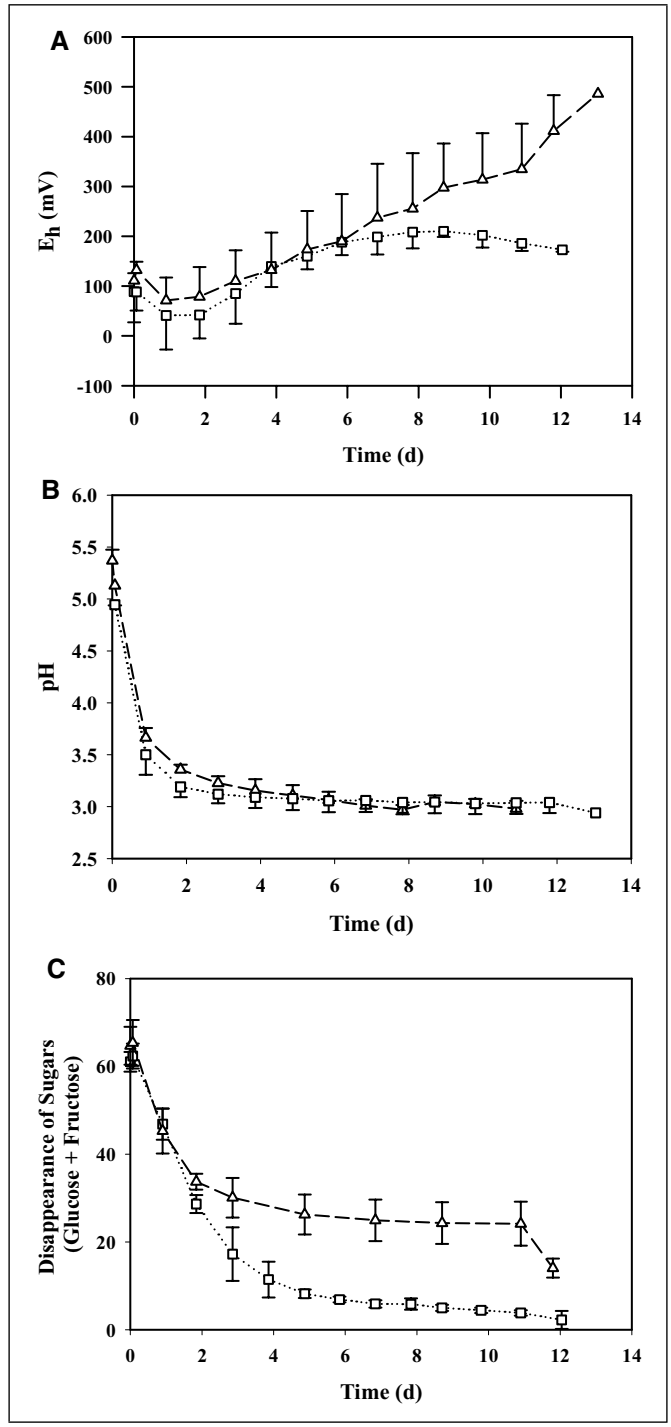

Figure 4—Changes in E_h (A), pH (B), and sugar utilization (C) during mixed culture fermentations of cucumbers with *L. plantarum* / *Z. globiformis* (□) and *L. plantarum* / *E. aerogenes* (Δ). Statistical analysis to compare the E_h trends among the treatments revealed a significant difference between most of them at a level of $\alpha = 0.05$ after terminal values were reached. No significant difference was declared for the comparison between the jars inoculated with *L. plantarum* and those inoculated with *L. plantarum* and *E. aerogenes*.

treatments was declared by the statistical analysis ($\alpha = 0.05$). The increase in E_h (Figure 4A), decrease in pH (Figure 4B), and utilization of sugars (Figure 4C) in the mixed culture situation demonstrate the typical dominant role of *L. plantarum* during cucumber fermentations where the increasingly acidic conditions result in rapid die off of *E. aerogenes* (Etchells and Jones 1943).

Conclusions

Redox potential measurements showed a pattern of changes in cucumber fermentations that did not parallel viable cell counts, pH change, or sugar utilization. Thus, E_h provided a different indicator of the course of cucumber fermentations than other methods that have been used to follow the progress of vegetable fermentations. Distinctive and significantly different ($\alpha = 0.05$) trends in redox potential during fermentation of brined, pasteurized cucumbers were observed when the jars were inoculated with *L. plantarum, Z. globiformis,* or *E. aerogenes.* Thus, it may be possible to develop and use E_h measurements in conjunction with pH as an early indicator of whether a fermentation is proceeding in a normal or abnormal course so that corrective actions could be taken to avoid spoilage.

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