CHANGES IN DISSOLVED OXYGEN AND MICROFLORA DURING FERMENTATION OF AERATED, BRINED CUCUMBERS

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ABSTRACT

Dissolved oxygen (DO) and oxygen uptake rates (OUR) were measured in cucumbers that were brined in 5-gal pails according to the controlled fermentation process. Saturation levels of oxygen in the brines were 5–6 ppm depending on salt concentration and temperature. DO was expressed as % saturation (% Cs). Either air or nitrogen was used to purge CO_2 from the brines to prevent bloater formation. In continuously aerated (air-purged) brines, DO increased before the onset of microbial growth (ca 2 days) and then decreased, converse to OUR. Thus, there were two distinct stages of oxygen levels and uptakes. In nitrogen-purged brines, DO was negligible throughout the period of purging; OUR of the brine samples was low (1% Cs/min maximum during the microbial fermentation stage) and lactic acid bacteria predominated the microbial flora. In air-purged brines, DO varied with rate of aeration; OUR of brine samples was high and populations of film yeasts were high. High aeration rates (100 ml/min/gal cucumbers) resulted in lower DO, higher OUR (40% Cs/min maximum) and greater populations of film yeasts than lower aeration rates (2-5 ml/min/gal). Tests in commercial tanks confirmed that appreciable levels of oxygen are incorporated into brines using a side-arm purger.

INTRODUCTION

THE SERIOUS PROBLEM of bloater damage (hollow cucumbers) in large pickling cucumbers during brine fermentation can be greatly reduced by purging dissolved CO_2 from the brine with nitrogen (Fleming et al., 1973, 1975; Etchells et al., 1973; Costilow et al., 1977). Purging CO_2 from brines with air also prevents bloater formation, but has not been recommended because of potential adverse effects on quality factors of the cucumbers such as texture and appearance (Etchells et al., 1973; Fleming et al., 1975).

Because air is readily available and inexpensive as compared to nitrogen, commercial briners have inquired about ways for safe use of air for purging brines. Levels of dissolved oxygen and rates of oxygen uptake in air-purged, brined cucumbers should be determined for the evaluation of methods used for purging.

We, therefore, measured levels of dissolved oxygen (DO) and determined factors that are responsible for oxygen uptake in aerated (air-purged), fermenting cucumbers. Laboratory- and commercial-scale fermentations were used in the study.

MATERIALS & METHODS

Brining

For laboratory studies, size no. 3 pickling cucumbers (3.8–5.1 cm diam) were obtained from a nearby pickle company, and stored at ca 95% RH and 10°C until used, usually within 3 days. Only cucumbers free of visible disease and mechanical injury were brined. Cucumbers were brined to equalize at 20° salometer (5.3% NaCl) by modifications of the controlled fermentation process of Etchells et al. (1973); using adaptations for small containers (Etchells et al., 1975), in 5-gal, plastic pails at a pack-out ratio of 50/50, cucumbers/brine and omission of the chlorine treatments. Fermentations were incubated indoors at 23–28°C. Air-purged fermentations were in open pails with ultraviolet lamps ca 2 ft above the brine surface to prevent surface growth (Etchells et al., 1975). Nitrogen-purged fermentations were in sealed pails containing a fermentation lock in the cap. Data reported represent single fermentations for each purging treatment.

Through the cooperation of a commercial cucumber briner in eastern North Carolina, cucumbers were brined in large, wooden tanks (ca 10,000 gal) to equalize at 20–25° salometer according to the controlled fermentation procedure of Etchells et al. (1973). The brine surface was exposed to sunlight.

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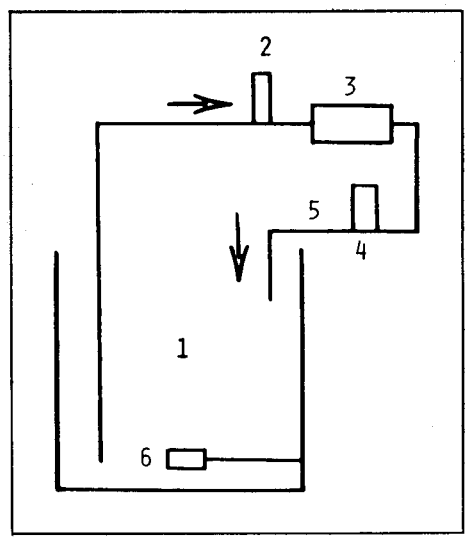

Fig. 1—Apparatus for measuring dissolved oxygen and oxygen uptake rate in brined fermenting cucumbers: (1) Plastic pail of fermenting cucumbers, 5-gal capacity; (2) Clark electrode; (3) Vibrostaltic pump; (4) Flowmeter; (5) Rubber tubing, 5/16 inch diam; (6) Sparger.

Purging

Five-gal, plastic pails were fitted with bottom spargers made of 5–8 cm sections of porous plastic tubes (Fig. 1), average pore size 10–20μ (Porex Materials Corp., Fairburn, GA). Purging gas was bottled nitrogen or air, with gas flow regulated by flowmeters (Air Products and Chemicals Co., Allentown, PA). Dissolved oxygen was measured in pails which were purged continuously at gas flow rates of 5 or 100 ml/min/gal (MMG). Oxygen uptake rate (OUR) was measured in pails intermittently purged with air at a rate of 100 MMG for 30 min duration, once in the morning and once in the evening. Nitrogen purging was continuous at a rate of 5 MMG. All purging was begun on the day of brining. The above purging rates are higher than the 20–50 standard cubic feet per hour (scfh) recommended for continuous purging of commercial cucumber tanks of 5–10,000 gal capacity (Etchells et al., 1973; Costilow et al., 1977); 5 and 100 MMG represent ca 106 and 2120 scfh, respectively. The rates used, however, were convenient for laboratory equipment available and served to illustrate effects of widely varying conditions. The high aeration rate (100 MMG) was extreme and would not be expected to occur under normal commercial conditions.

Commercial tanks were purged with a "side arm" apparatus designed and fabricated by company personnel and similiar in princi-

ple to the side arm device described by Costilow et al. (1977). The company has successfully used the device for several years. The device consists of a 4-inch diam, plastic pipe inserted in the salt box located on the inside of the tank with a sparger, made from Porex porous tubing placed near the bottom of the tank inside the pipe. Gas flow, controlled by flowmeters, causes the brine to circulate, entering the bottom of the pipe and exiting through an elbow placed just below the brine surface. Air purging (30–100 scfh) was begun 3 days after brining according to an intermittent schedule: air purging by day and no purging by night. By this time, a lactic acid fermentation was established. Prior to this time, purging was with nitrogen (30 scfh) on a continuous schedule. Nitrogen-purged tanks were purged continuously (35 scfh) for the entire fermentation.

Units of measurement and concentration of DO at saturation

The instruments used in this study measured DO as percent of the concentration at saturation (% Cs). Thus, OUR was recorded as percent saturation per minute (% Cs/min). DO values calculated from the data of Truesdale et al. (1955), as graphed in Figure 2, were within 0.2 ppm of values that we determined by the Winkler method up to 30° salometer (7.93% NaCl w/w). For higher NaCl concentrations, experimental DO values became increasingly higher than calculated values. This occurred because the formula of Truesdale et al. (1955) relies upon the linear decrease of DO with increasing salt concentration, which is a good estimation below 30° salometer, but becomes increasingly inaccurate at higher salt levels.

Measurement of DO and OUR in laboratory-scale fermentations

Dissolved oxygen and oxygen uptake rate in laboratory studies were measured polarographically with a YSI 4004 Clark electrode (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). The oxygen electrode was chosen because of its accuracy in the presence of NaCl and its ease of use and because small quantities of glucose and other organic material that are present in cucumber brines do not interfere with the electrode but do interfere with the standard Winkler method (APHA, 1971) of DO measurement (Theirault and McNamee, 1932). A 0.4-cm, round hole was bored in 5/16-inch diam, rubber tubing and stretched to accomodate the head of the Clark electrode. This provided a tight, nonleaking seal. With ca 150 cm of rubber tubing and a Vibrostaltic pump (Chemical Rubber Co., Cleveland, OH), DO and OUR were measured directly by circulating the brine past the electrode head and back into the container (Fig. 1). Brine flow through the tubing was 600 ml/min. DO was read directly from the strip chart recorder in continuously air-purged brines.

OUR was determined by measuring the initial slope of the oxygen consumption curve, obtained after air flow was stopped during intermittent purging. At the time air flow was stopped, DO in the brine exceeded 90% Cs. Oxygen electrodes were calibrated in air-saturated NaCl solutions of equal temperature and of ca equal salometer reading as the fermentations being measured.

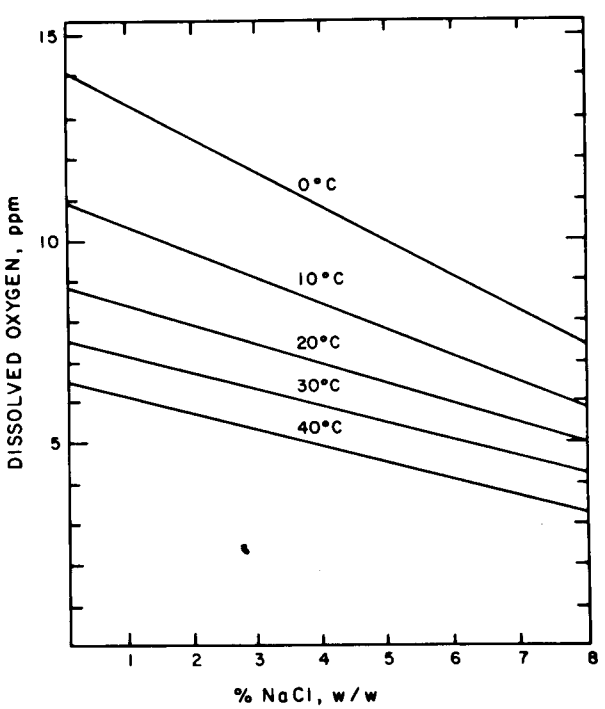

Fig. 2–Solubility of oxygen in aqueous NaCl solutions, equilibrated with air, at various temperatures. Based on data of Truesdale et al. (1955).

Measurement of DO in air-purged, commercially brined cucumbers

DO was measured in commercial tanks with a portable oxygen electrode (model 2110, multi-range analyzer for DO, BOD and temperature, Delta Scientific Corp., Lindenhurst, NY). DO measurements were made on the 2nd and 7th days of aeration at three positions in the tank. Position one was at the mouth of the side arm as the brine exited, about 6 inches below the brine surface and above the headboards. Position two was in the brine circulating above the headboards at a 270° arc from the side arm. Position three was ca. 5 ft beneath the brine surface inside the salt box, such that the brine was not being aerated directly or being circulated rapidly. For measurement of oxygen consumption, the sparger was removed, thus aeration was stopped, and the decrease of DO with time at position one was noted. Nearby tanks of fermenting cucumbers, which had been brined on the same day and in a similar manner, except that they had been continuously purged with nitrogen since brining, served as controls.

Brine sampling

Brine samples, 25–30 ml, were taken from the 5-gal pails with sterile, disposable syringes through rubber septums, located mid-way on the side of the pails. Ten ml were used for microbiological studies, 10 ml were preserved with 2 drops of 1% Merthiolate at 5°C for chemical tests and the remainder used for determination of brine OUR. Brine samples from commercial tanks were stored on ice until transported to the laboratory (3–5 hr) where part was used for microbiological studies and the remainder preserved with Merthiolate as above.

Oxygen uptake rate of brine samples

Brine samples, which were taken from the 5-gal pails, were used to determine the OUR of the brine in the absence of cucumbers. Brine sample OUR was measured at 25°C in a water-jacketed, 1.5-ml electrode vessel (Gilson Medical Electronics, Inc., Middleton, WI). With a Pasteur pipette and bottled air, the brine sample was aerated to ≥90% Cs in 1–2 min, and OUR determined by measuring the initial slope of the oxygen consumption curve after air flow had ceased.

Microbiological and chemical tests on brine samples

Brine samples were diluted serially and pour plated with various media to determine numbers and types of microorganisms present. Total aerobes were estimated with trypticase soy agar + 1% glucose (TSA), lactic acid bacteria in the presence of high populations of yeasts with *Lactobacillus* selective agar + 200 ppm Actidione (cycloheximide) + 250 ppm potassium sorbate (LBS), yeasts with dextrose agar acidified with 5 ml of sterile 10% tartaric acid per 100 ml of melted, 45–50°C agar (DA), and coliforms with violet red bile agar (VRBA). VRBA plates were incubated at 37°C; all other plates at 30°C. Ten yeast colonies were picked from DA plates, purified by successive streaking and tested for bottom growth and/or film-forming growth in cucumber juice broth + 5% NaCl w/v. Cucumber juice broth was prepared according to Fleming and Etchells (1967, with 65 parts cucumber juice and 35 parts water.

Brines were analyzed for pH, for titratable acidity (calculated as lactic acid) with 0.111N NaOH to a pH 8.3 endpoint, for reducing sugar by the method of Sumner and Somers (1944) with glucose as a standard, and for NaCl with 0.171N $AgNO_3$ to the dichlorofluoresein endpoint (Etchells et al., 1964). Brines were analyzed for pectinolytic activity according to Bell et al. (1955).

RESULTS

Laboratory-scale fermentations

DO in the brine increased to a maximum of 68% Cs after 2 days of continuous aeration at a rate of 5 MMG (Fig. 3). DO maximized at 97% Cs during the same period at an

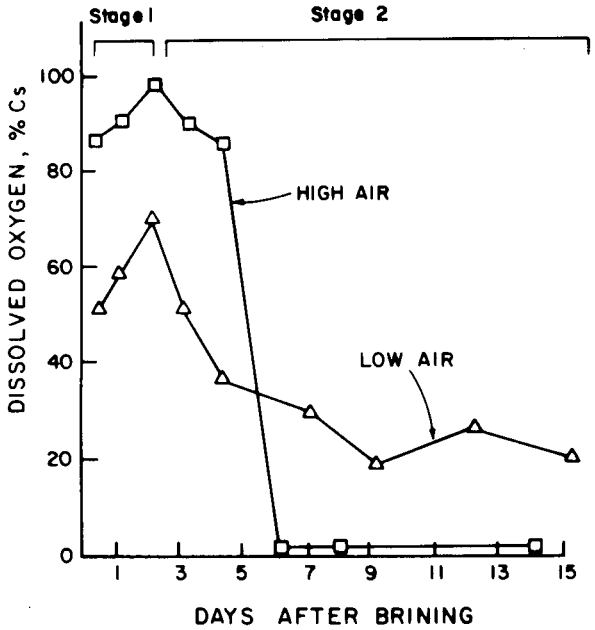

Fig. 3—Dissolved oxygen concentration in fermentations continuously aerated at 5 (Δ) and 100 (□) ml/min/gal.

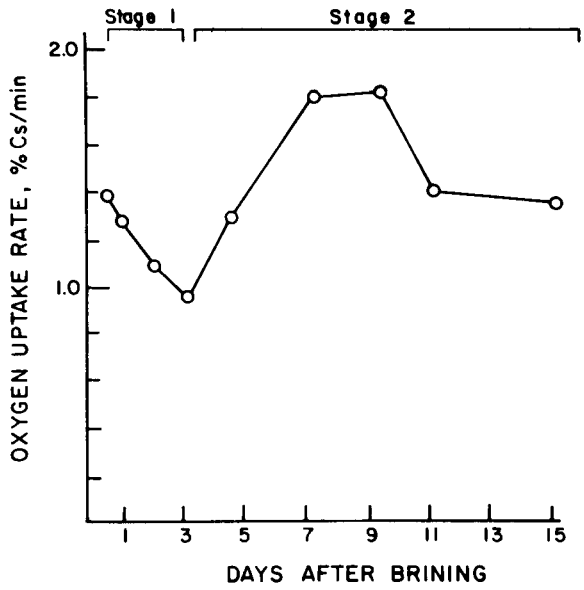

Fig. 4—Oxygen uptake rate of an intermittently air-purged fermentation. Air purged at 100 ml/min/gal for 30 min, twice daily.

aeration rate of 100 MMG. Brines were clear during this time, with no visual evidence of microbial growth. After the onset of microbial growth, DO in brine aerated at 5 MMG decreased steadily, reaching 20% Cs on day 9. DO remained at 20–27% Cs until day 15 when air purging was ceased. DO in brine aerated at 100 MMG slowly decreased to 83% Cs on day 4, and then fell rapidly to nearly 0% Cs by day 6. Nitrogen-purged and nonpurged brines contained no measurable DO. Thus, in air-purged cucumber brines, levels of DO were divided into two stages: stage one, the period of increasing DO before the onset of microbial growth; and stage two, the period of decreasing DO after the growth and establishment of microflora.

In intermittently aerated brine, measurement of oxygen directly in the fermentation pail revealed that OUR decreased from 1.4% Cs/min to 0.98% Cs/min during the first 3 days after brining, and thereafter increased to a maximum of 1.82% Cs/min by day 10 (Fig. 4). OUR was divided into stages corresponding to the two stages of DO measurement, except that DO and OUR were inversely related, such that stage one was the period of decreasing OUR and stage two the period of increasing OUR.

Brine samples were removed from the brine-cucumber mass for determination of OUR of the brine in the absence of cucumbers. Regardless of purging gas, or rate of purging, OUR was negligible for brine samples taken during stage one of the fermentation (Fig. 5). Thus, the appreciable OUR of the fermentations during stage one (Fig. 4) was apparently due to cucumber respiration. During stage two, OUR of brine samples was affected directly by the rate of aeration. Brine sample OUR increased to 38% Cs/min at 100 MMG and 2.6% Cs/min at 5 MMG; OUR of brine samples was only 0.85% Cs/min with nitrogen-purged fermentations.

During stage two, yeast populations rose dramatically, and reached nearly 10^8/ml of brine when aerated at 100 MMG and 10^6/ml when aerated at 5 MMG, but never exceeded 10^2/ml with nitrogen purging (Fig. 6). With air purging at 5 MMG, 10 of 10 yeast colonies isolated from brine samples taken 3 days after brining exhibited film-forming growth normally associated with oxidative yeasts. With nitrogen purging, 10 of 10 yeast colonies similarly isolated exhibited bottom growth in cucumber juice broth.

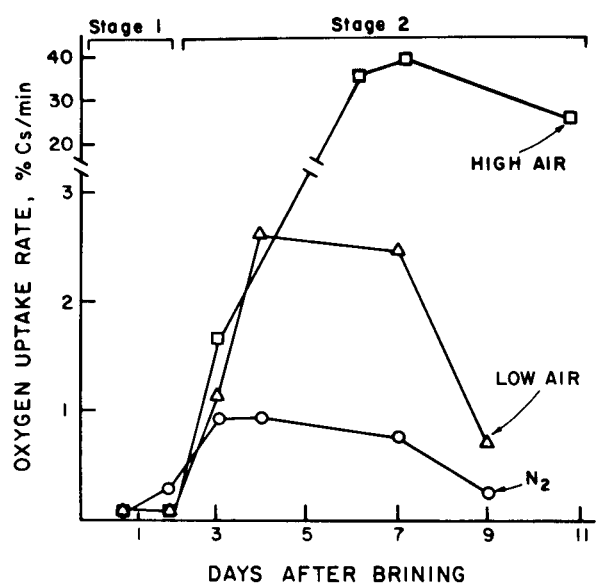

Fig. 5—Oxygen uptake rate of brine samples from continuously purged, cucumber fermentations: ○ = Nitrogen purged at 5 ml/min/gal; Δ = Air purged at 5 ml/min/gal; □ = Air purged at 100 ml/min/gal.

The rate of growth and subsequent maximal population of lactic acid bacteria were inversely related to rate of aeration (Fig. 7). Within 4 days after brining, lactic acid bacteria counts exceeded 10^9/ml with nitrogen purging, but were less than 10^8/ml with air purging at 5 MMG. After 8 days, brine air purged at 100 MMG contained less than 10^7/ml lactic acid bacteria. The number of total aerobes closely approximated the number of lactic acid bacteria throughout the fermentation. Less than 10 coliforms per ml were found throughout the fermentation, regardless of purging gas.

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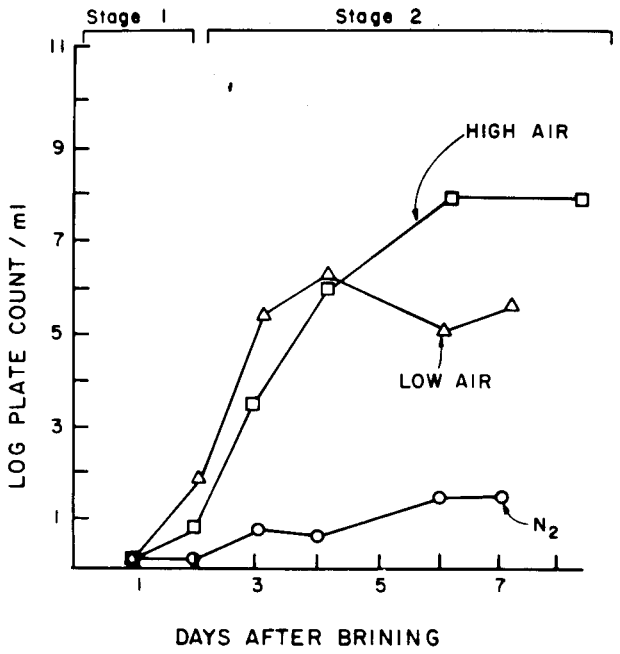

Fig. 6—Yeast populations in air- and nitrogen-purged, cucumber fermentations: ○ = N_2 purged at 5 ml/min/gal; Δ = Air purged at 5 ml/min/gal; □ = Air purged at 100 ml/min/gal.

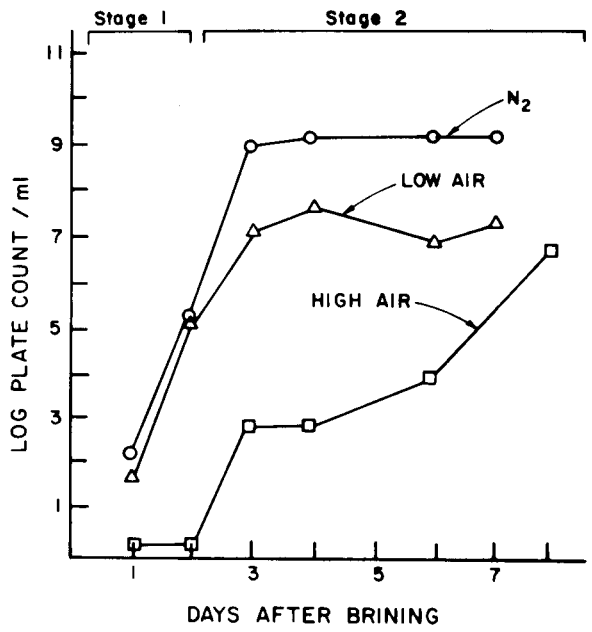

Fig. 7—Lactic acid bacteria populations in air- and nitrogen-purged, cucumber fermentations: ○ = N_2 purged at 5 ml/min/gal; Δ = Air purged at 5 ml/min/gal; □ = Air purged at 100 ml/min/gal.

Rate of acid development and titratable acidity were decreased by air purging (Fig. 8). By day 14, 1.1% titratable acidity (as lactic acid) had developed in nitrogen-purged brine, compared to 0.7% in brine that was air purged at 5 MMG. Air purging at 100 MMG resulted in consumption and disappearance of all titratable acidity as of 6 days after brining. By day 14 brine pH was 3.4 with nitrogen purging, 3.7 with air purging at 5 MMG, and above 7.0 with air purging at 100 MMG (Fig. 9). None of the brines contained pectinolytic enzyme activity. Brine stock was free of bloater damage.

Commercial fermentations

DO ranged from 20–75% Cs in air-purged, commercial brines (Table 1). DO was highest (50–75% Cs) at position one, directly in the flow of aerated brine from the side arm, somewhat less (40–68% Cs) at position two, at the top of the tank, 270° from the side arm, and least (20–45% Cs) at position three, ca 5 ft beneath the brine surface. Thus, substantial DO was distributed throughout the brine. Air purging was temporarily stopped and DO monitored continually for 30 min to obtain an indication of oxygen depletion rates. DO remained relatively high (58 and 12% Cs) in

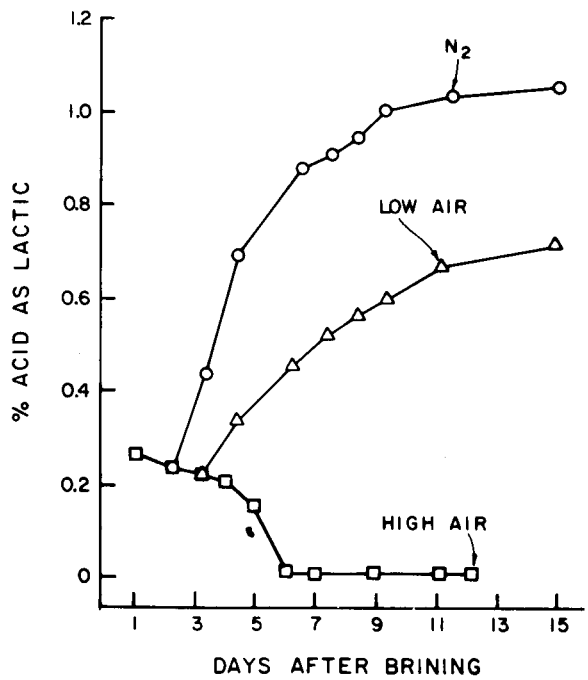

Fig. 8—Effect of purging treatment on acid production in cucumber fermentations: ○ = N_2 purged at 5 ml/min/gal; Δ = Air purged at 5 ml/min/gal; □ = Air purged at 100 ml/min/gal.

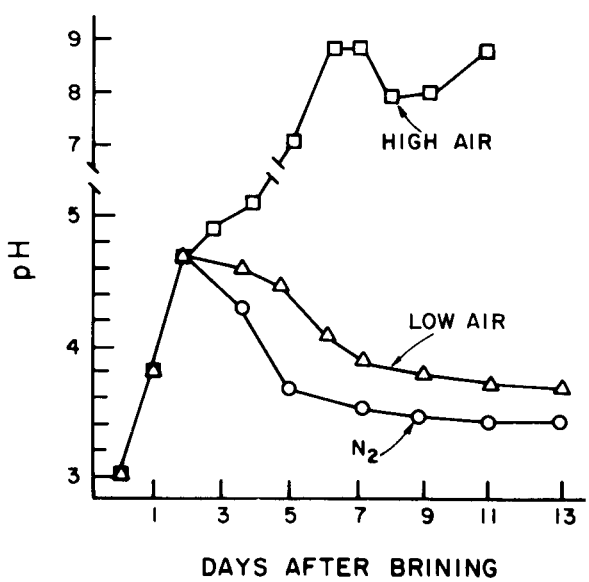

Fig. 9—Effect of purging treatment on the brine pH in cucumber fermentations: ○ = N_2 purging at 5 ml/min/gal; Δ = Air purged at 5 ml/min/gal; □ = Air purged at 100 ml/min/gal.

Table 1—Dissolved oxygen, brine analyses and microorganisms in air- and nitrogen-purged, commercial cucumber brines

Tank	Purging Gas	Purging Rate scfh	Purging Rate MMG[a]	Days after brining[b]	DO as % Cs at position:[c] 1 Initial	1 30 min	2 Initial	3 Initial	Brine analyses pH	Acid %	NaCl %	Microbes/ml brine Lactic acid bacteria	Yeasts
A	Air	30	1.42	4	50	12	40	20	3.9	0.41	6.3	5.0×10^8	9.9×10^1
A	Air	70	3.03	9	58	2	54	40	3.4	0.80	7.2	6.7×10^7	2.9×10^4
B	Air	30	1.42	4	75	58	68	45	3.6	0.52	7.1	1.1×10^8	4.9×10^1
B	Air	100	4.72	9	61	1	52	26	3.5	0.74	6.9	3.0×10^7	7.9×10^4
C,D	N_2	35	1.65	4	1	—	1	—	3.8[d]	0.48[d]	6.8[d]	1.7×10^8[d]	5.0×10^1[d]
C,D	N_2	35	1.65	9	—	—	—	—	3.4[d]	0.94[d]	7.3[d]	6.9×10^7[d]	5.4×10^2[d]

[a] Gas flow rate in standard cubic feet per hour (scfh) for 10,000 gal tanks converted to ml/min/gal (MMG).
[b] Cucumbers were brined on July 10, 1977; the brines were purged with nitrogen until July 13, then purged with air. Therefore, tanks A and B were sampled on the 2nd and 7th days of aeration, which corresponded to the 4th and 9th days, respectively, after brining.
[c] Dissolved oxygen was determined at the three tank positions noted in the text while the brine was being air purged. After purging was temporarily stopped, DO was monitored continually at position 1 for 30 min.
[d] Average of tanks C and D.

4-day old brines but decreased to near zero in 9-day old brines 30 min after purging was stopped (Table 1). DO was negligible in nitrogen-purged brines.

Air purging had little effect on the numbers of lactic acid bacteria in the brine of commercial cucumber fermentations (Table 1). However, the yeast population increased ca 1,000-fold after 5 days of air purging to between 10^4 and 10^5/ml, compared to only a 10-fold increase with nitrogen purging (Table 1). On the 4th day after brining, titratable acidity (as lactic acid) was ca equal, but after 5 days of aeration, aerated brines had attained only 0.74 and 0.80% acid as compared to an average of 0.94% (0.92 and 0.95) acid in the nitrogen-purged brines (Table 1).

DISCUSSION

TEMPERATURE AND SALT concentration are primary factors that influence the solubility of oxygen in cucumber brines. Within the usual range of salt (5–8%) and temperature (15–30°C) at which cucumbers are brined, dissolved oxygen at saturation is between 4.2 and 7.0 ppm (Truesdale et al., 1955). The amount of dissolved oxygen in air-purged, fermenting cucumbers depends primarily upon two opposing factors: the rate of oxygen dissolution and the rate of oxygen uptake by the cucumber-brine mass. Oxygen dissolution is affected by factors such as aeration rate, bubble size, and contact time, which are similar to the factors that influence CO_2 removal during purging. When air is used as a purging gas, systems that efficiently remove dissolved CO_2 would also efficiently incorporate dissolved oxygen.

Immediately after brining, before the onset of microbial growth (stage one), oxygen uptake is attributed primarily to the respiration of cucumbers. Oxygen consumption declines with time after brining, probably because of diffusion of salt and acid into the cucumbers. During stage one, increases in aeration rate lead to increases in the amount of dissolved oxygen present in the brine. On the other hand, after the onset of microbial growth (stage two), oxygen uptake is related to increased populations of yeasts in the brine, and DO no longer increases with aeration rate.

Because aeration increases the competitive advantage of yeasts, especially film-forming yeasts, beneath the brine surface, numbers of yeasts increase with aeration rate. The increased rate of oxygen dissolution produced at high aeration rates is compensated for by increased numbers of yeasts that elevate the rate of oxygen uptake. This relation explains the apparent contradiction, during stage two of the fermentation, of very low dissolved oxygen with high aeration, and high dissolved oxygen with low aeration (see Fig. 3). In aerated brines, yeasts are the major users of oxygen during stage two, although lactic acid bacteria do use a small amount.

Stimulation of yeast growth by air-purging cucumber brines was not unexpected. In fact, yeasts have been used to reduce the biological oxygen demand of waste cucumber brines (Hontz, 1975), and yeast cells have been produced in sauerkraut brines (Hang et al., 1972, 1975). Increased growth of yeasts in air-purged brines may be of little importance in some cases, but might lead to the growth of undesirable microorganisms in fermenting cucumbers. High populations of film-forming yeasts could result in oxidation of acid with a resultant rise in pH, and favor undesirable microbial growth. Also, air purging could stimulate the growth of aerobic microorganisms in the brine that produce cucumber-softening, pectinolytic enzymes. Many fungi produce these pectinases (Raymond et al., 1960). Certain species of yeasts also produce pectinase, but have not been isolated from cucumber fermentations (Bell and Etchells, 1956). Of the many species of bacteria that produce pectinases (Rombouts and Pilnik, 1972), some can be reasonably expected to populate pickling cucumbers. Although we have observed softening in air-purged, natural fermentations (cucumbers brined and fermented by microflora naturally present on cucumbers) (Fleming et al., 1973 and unpublished data), we have not encountered softening problems with air-purged, controlled fermentations (acidified with acetic acid, buffered and inoculated with *L. plantarum* according to Etchells et al., 1973) in the laboratory.

Reasons for the lower growth rate of lactic acid bacteria in air- as compared to nitrogen-purged, laboratory fermentations (Fig. 7) are not fully understood. Air may have retarded growth of the bacteria, or resulted in limited nutrients for the bacteria as a consequence of increased growth of yeasts (Fig. 6). No marked reduction of lactic acid bacteria was noted in the air- as compared to nitrogen-purged, commercial fermentations (Table 1), perhaps because these fermentations were allowed to progress for 3 days under nitrogen purging prior to initiating air purging. Thus, lactic acid bacteria were established prior to aeration.

It is now clear that oxygen can be incorporated into cucumber brines whether by a bottom sparger as demonstrated in laboratory fermentations, or a side arm sparger as demonstrated in commercial fermentations. Furthermore, aeration of brines affects the relative populations of yeasts and lactic acid bacteria. Laboratory fermentations demonstrated that the magnitude of these effects are dependent on the rate of aeration. The possibility that air may stimulate the growth of undesirable microorganisms in the brine should be considered. Therefore, users of air as a purging gas should minimize and carefully control the purging rate and extent of purging; and monitor brines for CO_2, pH, acidity, pectinase activity, and brine-stock quality.

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