Determination of Carbon Dioxide in Cucumber Brines

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A simple, accurate method for determining carbon dioxide in fermenting cucumber brines is described. The method involves distillation of carbon dioxide from the acidified brine into standardized sodium hydroxide inside a closed jar. The sample is injected by a syringe and needle through a rubber serum stopper placed in the jar cap, into an acid solution. A small vial of sodium hydroxide placed inside the jar traps the carbon dioxide as it distills from the acidified solution. After being held in the jar 24 hr at 37°C, the vial is removed; the remaining base is titrated to the phenolphthalein end point with standardized hydrochloric acid. Advantages of the method include a limited working time, minimized loss of carbon dioxide during analysis, and a relatively small sample size.

Carbon dioxide has been suggested as a cause of bloater damage in brined cucumbers (1). To study the problem, a method was sought for the routine analysis of CO_2 in the brine during fermentation and storage. Such brines differ in NaCl content (about 5–15%), acidity (about 0.1–1.0%, as lactic acid), and pH (about 3.1–5.8), depending on the brining treatment being used and the stage of fermentation when sampled.

Caputi (2) compared the 3 AOAC methods for determining CO_2 (3), cited their disadvantages, and developed an alternative method, which employed a modification of the AOAC enzymatic method. The AOAC manometric (11.053–11.057) and volumetric (11.058–11.060) methods require considerable working time and are rather cumbersome. The third method (11.061–11.062), and its modification by Caputi (2), seemed impractical, since it involves enzymatic activity and could be influenced by the NaCl content of the brine samples. Also, this method requires the titration of a degassed sample to serve as a blank to correct for the presence of acids in the sample.

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The microdiffusion principle (4) has been used to determine CO_2, but the equipment used requires delicate handling, and loss of CO_2 during manipulation could occur. To eliminate these problems, a study was undertaken to modify and adapt the microdiffusion principle for the analysis of CO_2 in cucumber brines. The method described herein in detail recently was used for this purpose, and the procedure was briefly described earlier (5).

METHOD

Reagents

(a) *Acid phosphate solution.*—Dissolve 200 g $NaH_2PO_4 \cdot H_2O$ and 30 ml 85% H_3PO_4 in ca 800 ml CO_2-free water. Then slowly add 63 ml concentrated H_2SO_4 and dilute to 1 L.

(b) *Indicator.*—Dissolve 1 g phenolphthalein in 100 ml ethanol.

(c) *CO_2 solution.*—100 mg CO_2/100 ml. Dilute 0.1909 g $NaHCO_3$ to 100 ml with ca 0.1*N* NaOH (carbonate-free).

Apparatus (*See* Fig. 1)

(a) *Diffusion jars.*—8 oz glass pickle jars with 58 mm diameter, 4 lug, "Twist-Off" caps (White Cap Co., Chicago, Ill.). Insert tapered rubber serum stopper, 14 mm diameter at small end, through hole made in cap as described by Etchells *et al.* (6).

(b) *Vials.*—27 mm diameter × 56 mm high.

(c) *Vacutainers.*—(Becton-Dickinson). 10 ml draw, containing 0.5 ml ca 5*N* NaOH (optional, used for taking and storing samples under field conditions).

(d) *Syringes.*—Plastic, disposable, 60 and 12 ml, with 22 gauge needles. For gas samples and brine samples under pressure, attach G.T.S. valve (Hamilton Co.) to male luer of syringe for sampling.

(e) *Device for sampling commercial-size tanks (50–1000 bushel capacity).*—*See* Fig. 2. Connect syringe plunger to retractable small stainless steel rod (5/32" diameter) by grooved plate. Mount 60 ml plastic syringe barrel in stationary position to large stainless steel rod (5/16" diameter) by curved brackets welded to rod and hold in place with

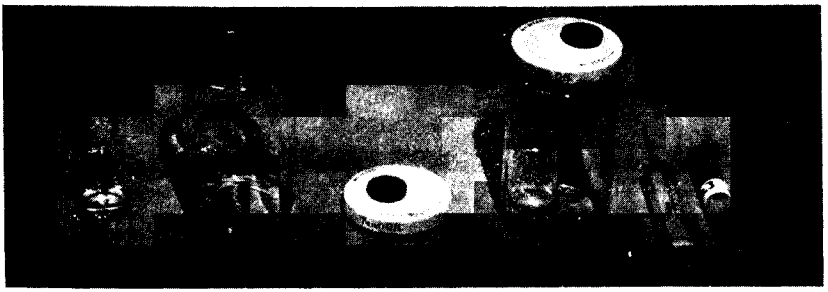

FIG. 1—Assay assembly for CO_2 (NaOH vial, jar, cap with rubber serum stopper, and complete assembly). Samples are introduced with syringe and needle. Sample tube (Vacutainer) is used for storing sample, if desired, before assaying.

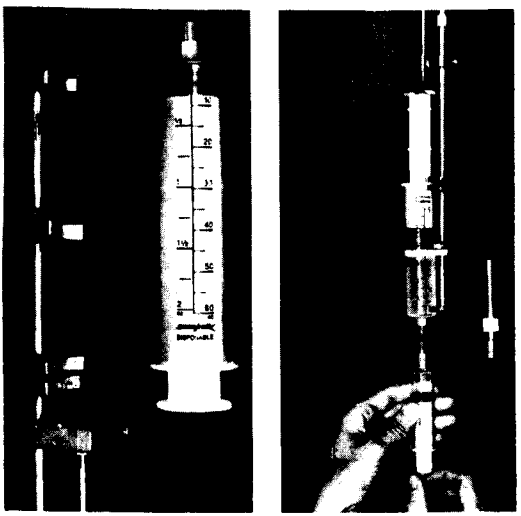

FIG. 2—Brine sampling device for large cucumber brining tanks. Left, 60 ml syringe and rod assembly prior to coupling. Right, coupled assembly, illustrating how subsamples are taken.

strong rubber band. (Rods are in 4′ sections and may be lengthened by adding other sections for sampling at greater brine depths. Sampling assembly can also accommodate 12 ml syringe for taking smaller samples.)

Sampling of Brine

(Sampling techniques vary, depending on type of container used for fermentation.) Place rubber serum stopper in cap of glass jar or in side of plastic or metal container for sampling with syringe and needle (6). Withdraw samples slowly to prevent loss of CO_2 from liquid during operation.

For sampling commercial-sized tanks, lower assembly (Fig. 2) into sampling slot of tank to desired depth. Fill syringe by withdrawing plunger rod to predetermined stop mounted on syringe support rod (Fig. 2). After bringing assembly to surface, immediately place small rubber stopper (sleeve type with 3.5 mm id hollow plug) over male luer of 60 ml syringe. Remove samples for CO_2 analysis through this stopper by 12 ml syringe and needle, and then inject into CO_2 assay jars or into Vacutainers containing NaOH if sample is to be stored. Take samples for other chemical and bacteriological analyses from same 60 ml syringe.

Assay Procedure

Add 10 ml acid phosphate solution to 8 oz jar. Place vial containing 5 ml 0.200*N* NaOH inside jar. Close jar with cap that has been held in ca 80°C water for 2–3 min to soften liner and thereby obtain hermetic seal. Add 10 ml brine sample with syringe and needle by injection through rubber serum stopper in cap and into acid phosphate solution; avoid spillage or splashing into NaOH vial. Include control jar(s), containing only acid phosphate solution and vial of NaOH, in each set of samples. Incubate jars 24 hr at 37°C. Remove vials from jars, add 5 ml 0.2*M* $BaCl_2$ and 1 drop of indicator, and titrate to phenolphthalein end point with 0.100*N* HCl, using Teflon-coated magnetic bar for stirring.

When it is not convenient to inject brine sample directly into assay jar, e.g., under field conditions, store by injecting exactly 8.0 ml brine into Vacutainer containing 0.5 ml 3*N* NaOH. Remove 8.0 ml from Vacutainer for assay and inject into assay jar.

Calculation

Generalized equation for calculating CO_2 is same as that used for AOAC volumetric method (**11.058–11.060**). When exact amounts and concentrations of acid and base specified in method are adhered to, following simplified equation may be used:

$$\text{mg } CO_2/100 \text{ ml} = (1.000 - \text{ml } 0.1N \text{ HCl}) \times 220$$

Subtract quantity of CO_2 determined for control jar from above value.

When samples are stored in Vacutainers, correct calculation to account for volume of NaOH in vial

and use of 8 instead of 10 ml sample by multiplying meq. base neutralized (value in parentheses above) by 292 instead of 220.

Results and Discussion

The effect of temperature on rate of recovery of CO_2 is illustrated in Fig. 3. When the jars were incubated at 90°C, the recovery was nearly complete after 6 hr. However, the resulting high pressure in the jar made gas leakage through the stopper and around the cap liner more of a problem and minimized reuse of the cap. At 37°C, the recovery was complete within 16–24 hr, and the caps could be reused several times. Incubation for 24 hr at room temperature (24°C) resulted in about 5% lower recovery than at 37°C.

Initially, the acid phosphate solution used was the same as that of the AOAC volumetric method (11.058–11.060), which resulted in a pH of about 2.5. Occasionally, yeasts present in cucumber brines continued to metabolize in the acid solution, giving erroneously high CO_2 values. The problem increased when the assay jars were allowed to incubate for longer periods, e.g., over weekends. This problem was overcome by the addition of H_2SO_4 to the acid phosphate solution to lower the pH to ≤ 1. Recovery rates of CO_2 were not appreciably different at pH 2.5 or 1.

The recovery of CO_2 from pure aqueous solutions (added as $NaHCO_3$) averaged 99.0% and from cucumber brines, 100.7% (Table 1). The brine used was obtained from a cucumber fermentation (about pH 3.3, 1% titratable acidity, 7% NaCl). It was degassed by sweeping with nitrogen for 2 hr. Then it was adjusted to pH 7.2 with NaOH, and the desired amount of $NaHCO_3$ was added. The degassed brine contained 3.6 mg CO_2/100 ml; this value was subtracted as a correction in determining the recovery in Table 1.

Although lactic acid is the predominant acid in brine-fermented cucumbers, acetic acid may be present in varying amounts, depending on the type of lactic acid bacteria or other microbes responsible for the fermentation. Due to the volatility of acetic acid, the effect of this acid on CO_2 analysis was determined. The test solutions contained 50 mg CO_2/100 ml. The per cent error in recovery was the increase in the calculated amount of CO_2 after holding the assay jars 24 hr at 37°C. At 0.5, 1.0, and 2.0% acetic acid, the errors in CO_2 analysis were 1.6, 3.2, and 7.4%, respectively (average of triplicate assays). Maximal titratable acidity reached during fermentation is usually <1%; therefore, the 0.5% acetic acid concentration is higher than would normally occur in brine-fermented cucumbers.

The method described is now used routinely in our laboratory. Comparatively little working time is required for an analysis. An experienced technician can easily analyze ≥100 samples per day, depending on the degree of automation for the pipetting and buretting operations. Headspace gas in jars of pickles also has been analyzed for CO_2 by the method. The method should be applicable for CO_2 analysis in other products or for research purposes when the results need not be known until the next day.

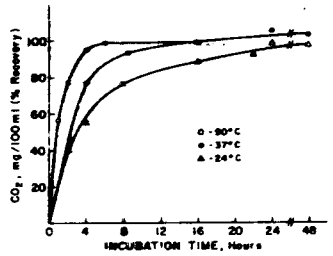

FIG. 3—Rate of CO_2 recovery in NaOH vials at 3 temperatures. Each assay jar initially contained 10 ml CO_2 standard solution, 100 mg/100 ml.

Table 1. Recovery of added CO_2 from pure aqueous solutions and spent cucumber brines

CO_2 added, mg/100 ml	CO_2 found,[a] mg/100 ml	Rec., %
Aqueous Solutions		
25.0	25.2	100.8
50.0	50.0	100.0
75.0	74.2	98.9
100.0	98.2	98.2
150.0	147.8	98.5
200.0	195.2	97.6
		Av. 99.0
Degassed Cucumber Brines		
25.0	25.9	103.6
50.0	49.0	98.0
75.0	77.9	103.9
100.0	57.2	97.2
		Av. 100.7

[a] Values are averages of quadruplicate determinations. Standard deviation for these 10 quadruplicate determinations averaged 1.9 mg/100 ml/determination.

Mention of a proprietary product does not necessarily imply endorsement by the U.S. Department of Agriculture.

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