APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1984, p. 884–885
0099-2240/84/040884-02$02.00/0

Vol. 47, No. 4

Quantitative Method for Colorimetric Determination of Formate in Fermentation Media

ROBERT SLEAT AND ROBERT A. MAH*

Division of Environmental and Occupational Health Sciences, School of Public Health, University of California at Los Angeles, Los Angeles, California 90024

Received 25 October 1983/Accepted 24 January 1984

The effects of fermentation products and media supplements on a colorimetric assay for formate were evaluated. Formate was detected at concentrations as low as 0.5 mM in fermentation media.

Until the early 1960s, formate in biological fluids was determined mainly by chemical means (1, 10). These methods depended on relatively high concentrations of formate and were not suitable for many samples. The quantitative separation of silage fermentation acids by acetone-hexane elution from celite columns (9) was also used for formate determination (5). This method was time-consuming and difficult to perform. However, later advances in chromatography and enzymology permitted the development of more sophisticated methods of determination at lower formate concentrations.

Gas-liquid chromatography is routinely used for the identification and quantification of volatile fatty acids (including formate) in culture fluid (2). Extraction of the acids into an organic solvent (usually ether) is required, and the volatile fatty acids are detected by thermal conductivity after separation on an appropriate column. A flame ionization detector cannot be used for the detection of formate unless formate is first derivatized (8).

Two enzymatic methods for the determination of formate have been reported (3, 6). Formyltetrahydrofolate synthetase (EC 6.3.4.3) catalyzes the formylation of tetrahydrofolate in the presence of ATP to N^{10}-formyltetrahydrofolate, which is converted to 5,10-methenyltetrahydrofolate at low pH. The increase in absorbance at 350 nm corresponds stoichiometrically with formate concentration (6). Formyltetrahydrofolate synthetase is not commercially available and must be isolated and purified from an appropriate bacterium (7). The second enzymatic method relies on the reduction of NAD to NADH + H^+ and is catalyzed by formate dehydrogenase (EC 1.2.1.2), using formate as substrate and hydrogen donor (3). The reaction is monitored by an increase in absorption at 340 nm which is due to the formation of NADH + H^+. Formate dehydrogenase exhibits both formate and NADH + H^+ oxidase activity (3); consequently, formation of NADH + H^+ is proportional to formate removal (3). Formate dehydrogenase is not commercially available.

A nonenzymatic specific reaction forming a red color with an absorption maximum at 510 to 520 nm (4) was used for the identification of formate. This reaction takes place when formate and citric acid are mixed in the presence of acetic anhydride, an acetamide-isopropanol solution, and a small quantity of alkali (4). We examined this method for the quantification of formate and report here the feasibility of using this reaction for quantitatively detecting formate in fermentation media.

An 0.5-ml standard formate solution or a culture sample was added to a disposable culture tube (18 by 150 mm; VWR Scientific Inc., Norwalk, Calif.). The standard formate solution was prepared by dissolving sodium formate in water purified by reverse osmosis. The culture sample was first centrifuged at 11,600 × *g* for 15 min in a Microfuge 11 (Beckman Instruments, Inc., Palo Alto, Calif.) to remove bacterial cells. Then 1 ml of a solution containing 0.5 g of citric acid and 10 g of acetamide dissolved in 100 ml of reagent grade 2-propanol (Scientific Products, Irvine, Calif.), 0.05 ml of 30% (wt/vol) sodium acetate, and 3.5 ml of acetic anhydride (Sigma Chemical Co., St. Louis, Mo.) were added and the tubes were stoppered with no. 1 butyl rubber stoppers. The tubes (optical length, 18 mm) were briefly mixed on a Vortex mixer and incubated at room temperature. The absorbance at 510 nm was then determined by using a Bausch & Lomb Spectronic 710 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). A control tube without added formate was used to set the spectrophotometer at zero.

Maximum absorbance was attained between 1.5 and 2 h (Fig. 1A). A plot of maximum absorbance against formate concentration was linear up to 15 mM ($r^2 = 0.997$, $P < 0.01$) (Fig. 1B). If the absorbances of the empty tubes are accounted for and the appropriate corrections are made, an accurate determination of formate at an 0.5 mM concentration is possible. The solution containing citric acid, acetamide, and

TABLE 1. Effect of possible fermentation products on the maximum absorbance produced by a 2 mM formate standard solution

Addition[a]	Concn (mM)	% Control absorbance[b]
Propionate	20	100
	10	101
Butyrate	20	93
	10	99
Isobutyrate	20	93
	10	100
Lactate	20	85
	10	94
Succinate	20	93
	10	95
Ethanol	20	91
	10	103

[a] Either 50 or 100 μl of a 0.1 M stock solution of one of the above compounds was added to 2 mM formate to give the final concentrations shown.

[b] Relative to absorbance of standard formate solution supplemented with 50 or 100 μl of distilled water.

* Corresponding author.

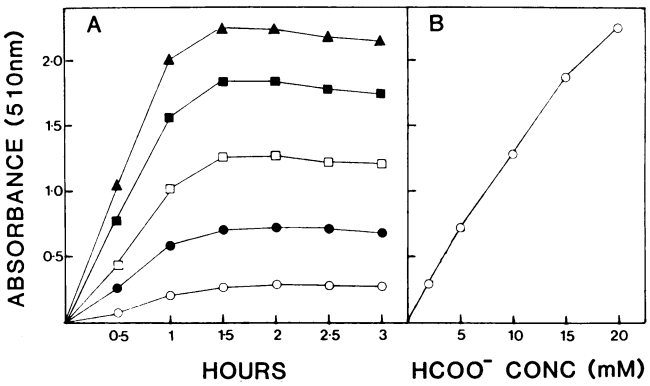

FIG. 1. Color formation as a function of formate concentration. (A) Time course of color development. Symbols: ○, 2 mM; ●, 5 mM; □, 10 mM; ■, 15 mM; ▲, 20 mM. (B) Plot of maximum absorbance against formate concentration.

2-propanol was prepared freshly each time. Storage of this solution at 4°C led to decreased sensitivity of the assay. Prior acidification of the standard solution with mineral acids abolished the color formation.

Methanol, formaldehyde, ethyl formate, ethanol, acetaldehyde, acetone, glycerol, oxalate, malonate, succinate, tartrate, glucose, and fructose reportedly did not interfere with color formation (4). The effect of possible fermentation products on the maximum absorbance, using a 2 mM standard formate solution, was examined (Table 1). This formate concentration was chosen as the standard because it is an intermediate concentration when formate occurs as a fermentation product. Propionate, butyrate, isobutyrate, succinate, and ethanol had no effect on color development. High concentrations of lactate did significantly decrease the maximum absorbance (Table 1). At higher concentrations of formate this effect was not significant (data not shown).

Yeast extract (Difco Laboratories, Detroit, Mich.), Trypticase (BBL Microbiology Systems, Cockeysville, Md.), rumen fluid, and various digester supernatant fluids, at concentrations routinely used in our laboratory as medium supplements (ca. 5%), all had some effect on maximum color formation (Table 2). However, the percent recovery of 2 mM formate was high, ranging from 83 to 96%. Thus, to determine formate accurately in culture media, the formate standards must be prepared in uninoculated media. Plots of maximum absorbance against standard formate concentrations (data not shown) were linear up to 15 mM when the formate was prepared in the various complex media (as described in Table 2, footnote *a*). Correlation coefficients were greater than 0.99, and a formate concentration of 0.5 mM was detected in each case. More than 90% of added formate (1 to 10 mM final concentration) was detected in the fermentation broth of a previously unreported (manuscript in preparation), non-formate-producing, cellulolytic isolate.

TABLE 2. Effect of complex organic compounds on maximum absorbance of a 2 mM formate standard solution

Addition[a]	% Concn (wt/vol or vol/vol)	% Control absorbance[b]
Yeast extract	0.1	90
	0.05	88
Trypticase	0.1	89
	0.05	96
Rumen fluid	5	91
Sewage digestor supernatant	5	95
Kelp digestor supernatant	5	83
Woody digestor supernatant	5	84

[a] Experimental formate solution was made up in the presence of yeast extract or Trypticase, as indicated, or supplemented with 50 μl of 50% stock solutions of rumen or digestor supernatant fluid.

[b] Relative to absorbance of standard formate solution supplemented with 50 μl of distilled water as appropriate.

Determination of formate in culture media containing high concentrations of organic buffers (>20 mM) resulted in precipitation of the buffer, which interfered with the measurement of low formate concentrations. Centrifugation of the precipitated sample, followed by transfer of the supernatant to a fresh tube before measurement of absorbance, overcame this problem.

Formyltetrahydrofolate synthetase and formate dehydrogenase can be used to detect formate concentrations as low as 10 to 30 μM (3, 6). The minimum concentration of formate detected by gas chromatography, after butylation, ranged between 1 and 2 mM (8). The present colorimetric method was at least as sensitive as the gas chromatographic method. However, it had the advantage of not requiring extensive sample pretreatment, involved enzyme preparation, or the use of expensive gas chromatographs. We have successfully used the colorimetric method to determine the amount of formate produced by a previously unreported, formate-producing, anaerobic cellulolytic isolate during cellulose fermentation (manuscript in preparation).

We thank H. Wang for technical assistance and B. Ollivier for bringing this method to our attention.

This research was supported by research grant DE-AT03-80ER10684 from the U.S. Department of Energy and by research grant 5080-323-0423 from the Gas Research Institute, Chicago, Ill.

LITERATURE CITED

1. **Gabriel, O.** 1966. Determination of formate. Methods Enzymol. **9**:29–32.
2. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va.
3. **Höpner, T., and J. Knappe.** 1974. Formate. Determination with formate dehydrogenase, p. 1551–1554. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis, vol. 3. Academic Press, Inc., New York.
4. **Lang, E., and H. Lang.** 1972. Spezifische Farbreaktion zum direkten Nachweis der Ameisensäure. Z. Anal. Chem. **260**:8–10.
5. **Mah, R. A., and R. E. Hungate.** 1965. Physiological studies of the rumen ciliate, *Ophrysocolex purkynei* Stein. J. Protozool. **12**:131–136.
6. **Rabinowitz, J. C.** 1974. Formate. Determination with formyltetrahydrofolate synthetase, p. 1546–1550. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis, vol. 3. Academic Press, Inc., New York.
7. **Rabinowitz, J. C., and W. E. Pricer.** 1962. Formyltetrahydrofolate synthetase. 1. Isolation and crystallization of the enzyme. J. Biol. Chem. **237**:2898–2902.
8. **Salanitro, J. P., and P. A. Muirhead.** 1975. Quantitative method for the gas chromatographic analysis of short-chain monocarboxylic and dicarboxylic acids in fermentation media. Appl. Microbiol. **29**:374–381.
9. **Wiseman, H. G., and H. M. Irvin.** 1957. Determination of organic acids in silage. J. Agric. Food Chem. **5**:213–215.
10. **Wood, H. G., and H. Geist.** 1957. Determination of formate. Methods Enzymol. **3**:285–292.