NOTE

A New Method for Ethanol Measurement Utilizing an Immobilized Enzyme[1]

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ABSTRACT

Ethanol in 10 different beer samples was determined, comparing an immobilized enzyme system with the AOAC distillation/pycnometer procedure. In the enzymatic system, alcohol oxidase is immobilized in a thin microporous membrane. This membrane is mounted on an amperometric electrode. When ethanol enters the membrane, it is oxidized, producing H_2O_2 and acetaldehyde. The H_2O_2 is measured at the electrode. Precision for the enzymatic method was excellent, ±0.05% (v/v) ethanol. Agreement with the AOAC procedure was good.

Key words: *Alcohol oxidase, Beer, Ethanol, Immobilized enzyme*

In industrial processes and quality control, rapid, accurate, and precise chemical determinations have always been needed. This is certainly true for ethanol determinations in the brewing industry, in which traditional rapid methods (specific gravity and refractive index) have been reported as difficult to perform with precision (2).

The number of enzymatic methods of analysis has grown considerably over the last few years. Enzymatic methods have several advantages; they are generally rapid, specific, and highly sensitive. Sample treatment can be minimal.

We previously demonstrated (4) the ability to determine certain carbohydrates with an immobilized enzyme system,[2] which can be used to determine ethanol.

EXPERIMENTAL

Ethanol determination in a model 27 Industrial Analyzer is based on immobilizing alcohol oxidase in a membrane as shown in Fig. 1. This is accomplished by mixing the enzyme with dithioerythritol and glutaraldehyde and then constructing the membrane shown in Fig. 1 using a patented process (3). The membrane is made in three layers. The outer layer is a Nuclepore polycarbonate membrane about 5 μ thick with pores ~0.015 μ in diameter. This outer membrane excludes most unwanted large molecules from the middle enzyme layer. The middle layer contains immobilized alcohol oxidase and is 1–2 μ thick. When ethanol enters this layer, it is oxidized to acetaldehyde and H_2O_2. The H_2O_2 diffuses through a third layer, of cellulose acetate, to a platinum anode where oxidation continues. This oxidation produces an electrical current directly proportional to the H_2O_2 produced and hence to ethanol concentration. The platinum anode is at +700 mV, with respect to a silver/silver chloride reference electrode. This electrode would be capable of oxidizing substances other than H_2O_2 if they were allowed to reach it. The cellulose acetate layer, with a molecular weight cut-off at ~100, prevents most potential interferences from reaching the electrode.

After the membrane is assembled, it is mounted on the instrument's electrode assembly, as shown in Fig. 1. The electrode/membrane is then mounted in a small (350-μl) sample chamber. The instrument is calibrated (5) by injecting 5 μl of a 0.100% (v/v) ethanol solution. Sixty seconds after the injection is made, the readout is adjusted to the calibration value. The sample chamber is cleared with ~3.5 ml of buffer, which takes ~35 sec. Typical working life for an enzyme membrane of this type is from 10 days to two weeks.

Reagents

(a) *Ethanol standard,* 0.100% v/v, aqueous solution.
(b) *Buffer,* pH 7.5, 0.25*M* phosphate.
(c) *Enzyme membrane,* as described above.

Apparatus

(a) *Immobilized Enzyme Analyzer,* Yellow Springs Industrial Analyzer, model 27.
(b) *Volumetric glassware,* Class A, as required.

Method

Decarbonate the beer by a 15-min treatment in an ultrasonic bath. Pipet a 2-ml sample into a 100-ml volumetric flask and dilute it to the mark with reagent distilled water. Calibrate the instrument as described above. Inject the sample, record the reading, clear the sample chamber, and repeat the cycle for all samples. Ethanol concentration in the diluted sample must be multiplied by the appropriate dilution factor to determine actual ethanol concentration. AOAC procedure 10.023 (1) for ethanol in beer was used as a reference procedure. All samples were assayed once using the AOAC procedure and six times on the model 27 analyzer.

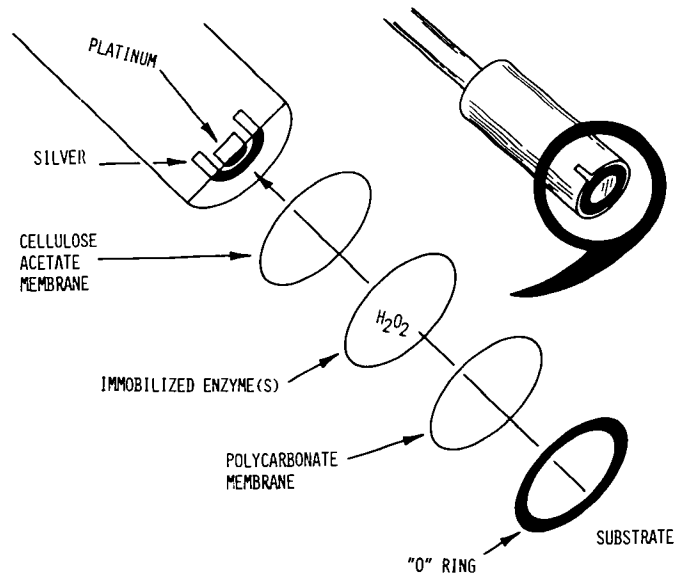

Fig. 1. Model 27 electrode/membrane-immobilized enzyme assembly.

[1]Presented in major part at the 47th Annual Meeting, Miami, FL, May 1981.
[2]B. Li. 1981. Unpublished data.

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TABLE I
Comparison of Ethanol (% v/v) in Beer Determined by Distillation/Pycnometer and Immobilized Enzyme

Sample	Enzymatic ±S. D. (E)	Distillation (D)	Difference (E-D)
A	4.30 ± 0.04	4.26	0.04
B	4.35 ± 0.03	4.26	0.09
C	4.50 ± 0.02	4.48	0.02
D	4.50 ± 0.05	4.40	0.10
E	4.70 ± 0.03	4.77	−0.07
F	4.75 ± 0.04	4.69	0.06
G	4.76 ± 0.03	4.77	−0.01
H	4.90 ± 0.06	4.84	0.06
I	5.40 ± 0.06	5.51	−0.11
J	5.55 ± 0.05	5.51	0.04

RESULTS AND DISCUSSION

Results (Table I) show that the precision of the enzymatic assay was excellent. The pooled standard deviation for the enzymatic method was ±0.05 (v/v) ethanol. Agreement with the AOAC procedure was good.[3]

This method of enzymatic determination of ethanol has several advantages. Sample preparation is easy because no distillation is required. The procedure is fast; after the sample has been diluted, analysis can be accomplished in 2–3 min. Color and turbidity interferences are not factors. A skilled operator is not required. The procedure is inexpensive, costing less than $0.40 per test. Accuracy and precision are acceptable.

[3]Lack of familiarity with the distillation procedure may be a factor in the minor differences observed between the two methods.

LITERATURE CITED

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