STUDIES ABOUT THE ALCOHOL DEHYDROGENASE ACTIVITY IN ETHANOL FERMENTATION USING OPTICAL FIBRE

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Abstract

It was studied the enzymatic activity of alcohol dehydrogenase (ADH) over ethanol solution using spectrophotometer method and optical fiber sensor

Key words:optical fibre, ethanol fermentation, ADH

INTRODUCTION

Ethanol is involved in different application including biotechnologies, food industry, medicine, pharmaceutical and many other new fields. Every kind of these applicationsneed to detect and quantify ethanol with high accuracy, but all of them have different requirements, such as detection limit, sensitivities and assay time. There are many methods usedto determine the ethanol concentration in different samples, such as spectrometry, chromatography, electrochemistry, polarography, Raman and mass spectroscopies, enzymatic assay, etc (Beutler, 1984), but most of these methods are usuallytime consumersand require expensive instrumentation. Enzymatic determinationsuse simple and widely available spectroscopic or electrochemical methods in order to study enzyme-catalyzed reactions (Bernt., Gutman, 1974).

Sensors involving light measurements associated with fiber-optics are particularly attractive (Azevedo et al, 2005; Ahuja et al, 2007)

The different configurations for fiber optic based sensors, their characteristics and their potential applications have been recently studied (Toselli et al., 2007; Fabbri et al., 2008; Lin, Brown, 1997).

In the reaction involving alcohol dehydrogenase (ADH) ethanol is converted to acetaldehyde with the reduction of the nicotinamide adenine dinucleotide cofactor (NAD^+) to NADH.

$$Et\text{-}OH + NAD^+ + ADH \rightarrow acetaldehyde + NADH + H^+$$

According to this enzyme reaction, ADH enzymatic activity (ethanol concentration) can be determined by measuring the absorption of NADH at a wavelength of 340 nm (Bernt, Gutman, 1974)

Changes in alcohol concentration affects the amount of NADH produced, resulting in a change of absorbance at 340 nm.

MATERIAL AND METHOD

Materials

All materials used for determinations were purchased from Sigma-Aldrich Company (Germany).

- Ethanol solution (5%);
- Sodium phosphate buffer solution 10mM, pH 7,5;
- β-nicotinamide-adenine-dinucleotide (NAD^+) solution 15mM;
- Alcohol dehydrogenase enzyme ADH (EC 1.1.1.1) 60 U/ml in sodium phosphate buffer solution pH 7,5;

Equipment

For the experiments were used the following equipment:

- Portable laser spectrophotometer, Jazz from Ocean Optics, with two channels, one channel of spectrometric type "master" for UV-VIS range (1025-200 nm) and a "slave" channel for the VIS-NIR (360-1100 nm). The spectrophotometer is equipped with a built-in microprocessor and OLED display for independent operation (without PC), with 2-channel and source excitation deuterium-halogen-tungsten, 210-1100nm, 7W, with integrated power supply lithium-ion battery, included in the platform(figure 1).

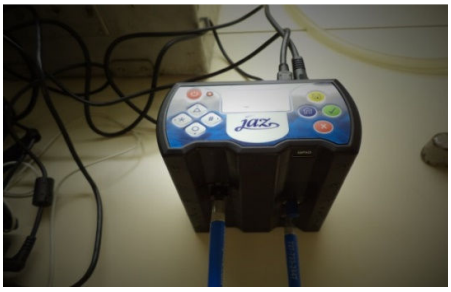

Fig.1. Portable laser spectrophotometer, Jazz from Ocean Optics

- Optical Fiber - for the 210-1100 nm, fiber length 2 m, SMA 905 connectors, (figure 2)
- Fiber type: UV-VIS;

- 300um fiber core diameter;
- Jacket: Zip Tube;
- Length: 2m

Fig. 2. Optical Fiber

- Software Spectrasuite for acquisition and real time analysis of data, as well as for data processing (figure 3).

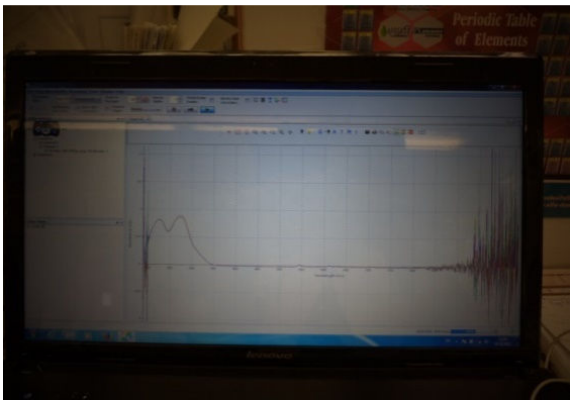

Fig. 3. Spectra acquisition by optical sensor

- PC that runs software Spectrasuite for acquisition and real time analysis of data(figure 4).

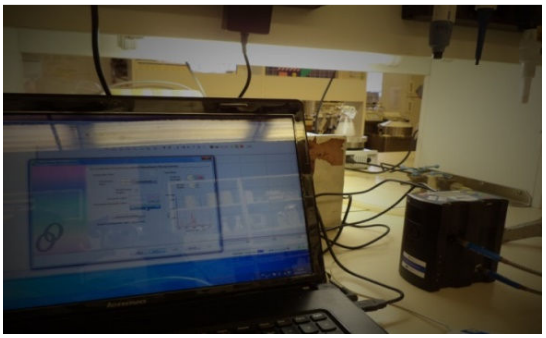

Fig.4. PC that runs software Spectrasuit

Method

The working principle is based on a classic colorimetric approach. The interrogator light is scattered and absorbed by the sensing system that include ADH and NAD^+. The NADH produced in biochemical reaction induces a change of the optical spectrum of the outgoing light collected by the fiber. Analyzing the spectrum of this optical signal the ADH activity on ethanol can be measured.

In a glass tube it was added 0,6 ml of phosphate buffer solution pH 7,5, 0,6 ml of ethanol solution 5%, 0,7 ml of NAD^+ solution andthen, the fiber optic was immersed into the tube and measured the UV-VIS spectra of the mixture (control). After that, it was 0,1 ml of ADH solution in the tube and it were measured the test UV-VIS spectra acquired at an integration time of 2000 ms, scan average 10, time of data acquisition 1000 seconds.
All the measurements were performed at room temperature.

RESULTS AND DISCUSSION

The UV-VIS control spectrum (buffer, ethanol and NAD^+) (figure 5) is compared with test spectrum (buffer, ethanol, NAD^+ and ADH) (figure 6).

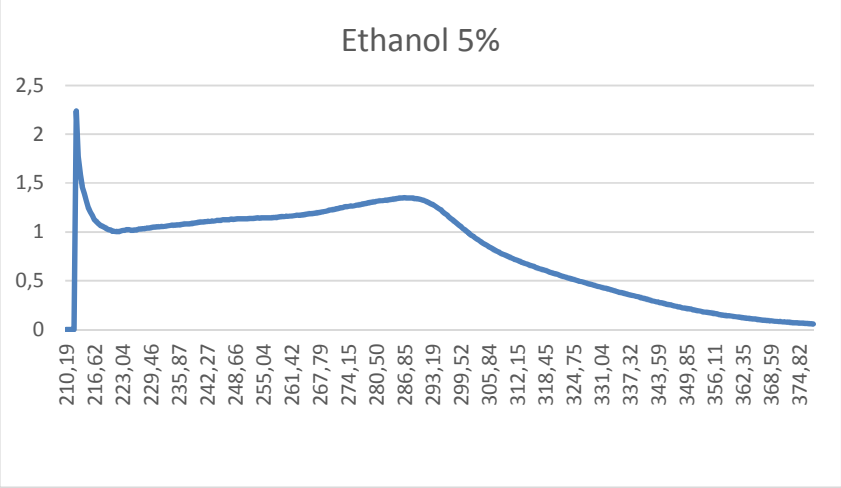

Fig. 5. UV-VIS control spectrum

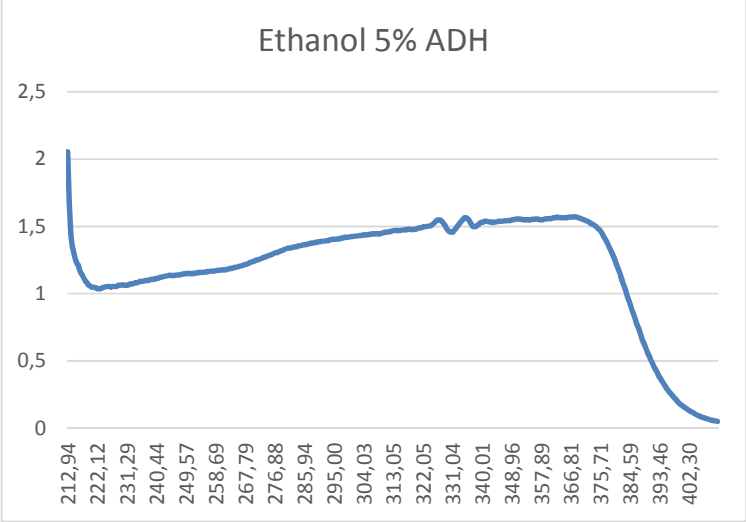

Fig. 6. UV-VIS test spectrum

The presence of ADH in ethanol system determined the increasing of NADH content in the biochemical system. The maximum of absorbance at 340 nm is corresponding to NADH obtained in fermentation reaction for ethanol detection.

The ADH activity over ethanol 5% solution in the test biochemical system is represented in figure 7. During the analysis the test spectra are acquired in real time.

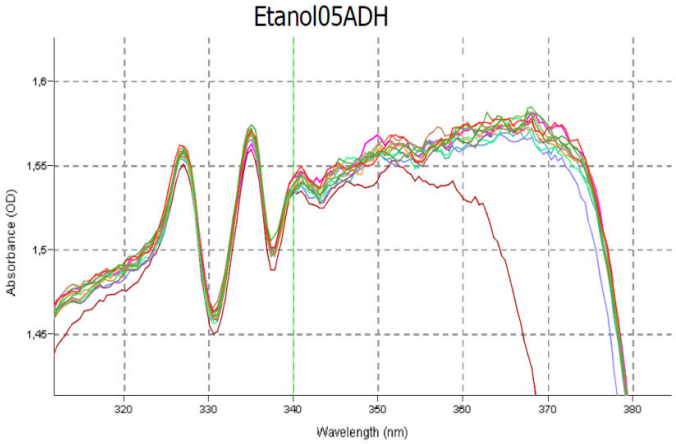

Fig. 7. The ADH activity in ethanol 5% solution

All the obtained test spectra show the increasing of NADH content during the enzymatic reaction of ADH over ethanol solution, in correspondence with the ADH kinetic on ethanol. These experimental results are obtained in real time and the method can be used for optical fiber applications in monitoring of ethanol content and ADH activity during the ethanol fermentation process.

CONCLUSIONS

The alcohol dehydrogenase (ADH) enzymatic activity (respective the ethanol content) can be experimental determined in real time and in biochemical systems, using spectrophotometer method and optical fibers.

ACKNOWLEDGMENT

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