PUBLIKASI ARTIKELILMIAH PADA JURNAL INTERNASIONAL

A Simple Visual Ethanol Biosensor Based on Alcohol Oxidase Immobilized onto Polyaniline Film for Halal Verification of Fermented Beverage Samples

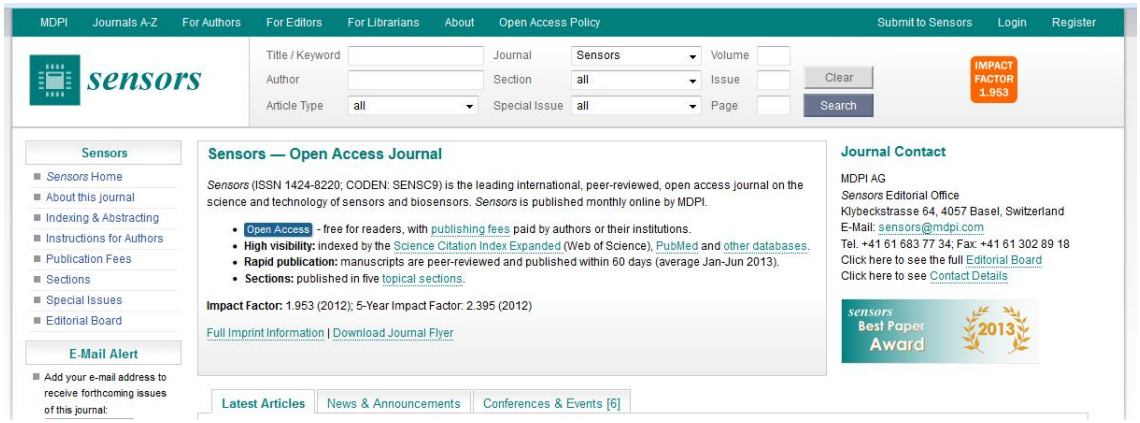
Sensors 2014, 14: 2135-2149

Oleh :

Prof. Drs. Bambang Kuswandi, M.Sc., Ph.D.
Moch. Amrun Hidayat, S.Si., Apt., M.Farm.

FAKULTAS FARMASI
UNIVERSITAS JEMBER

2014



Latest Articles | News & Announcements | Conferences & Events [6]

Open Access
Article: **Development of Real-Time Dual-Display Handheld and Bench-Top Hybrid-Mode SD-OCTs**
by Nam Hyun Cho, Kibeom Park, Ruchire Eranga Wijesinghe, Yong Seung Shin, Woonggyu Jung and Jeehyun Kim
Sensors **2014**, *14*(2), 2171-2181; doi:10.3390/s140202171 (doi registration under processing) - published online 27 January 2014
Show/Hide Abstract

Open Access
Article: **Assessing Inter-Sensor Variability and Sensible Heat Flux Derivation Accuracy for a Large Aperture Scintillometer**
by Evan H. Rambikur and José L. Chávez
Sensors **2014**, *14*(2), 2150-2170; doi:10.3390/s140202150 (doi registration under processing) - published online 27 January 2014
Show/Hide Abstract

Open Access
Article: **A Simple Visual Ethanol Biosensor Based on Alcohol Oxidase Immobilized onto Polyaniline Film for Halal Verification of Fermented Beverage Samples**
by Bambang Kuswandi, Titi Irmawati, Moch Amrun Hidayat, Jayus and Musa Ahmad
Sensors **2014**, *14*(2), 2135-2149; doi:10.3390/s140202135 (doi registration under processing) - published online 27 January 2014
Show/Hide Abstract

Open Access
Article: **Gaze Tracking System for User Wearing Glasses**
by Su Yeong Gwon, Chul Woo Cho, Hyeon Chang Lee, Won Oh Lee and Kang Ryoung Park
Sensors **2014**, *14*(2), 2110-2134; doi:10.3390/s140202110 (doi registration under processing) - published online 27 January 2014
Show/Hide Abstract

Open Access
Article: **Mobile Robot Self-Localization System Using Single Webcam Distance Measurement Technology in Indoor Environments**
by I-Hsum Li, Ming-Chang Chen, Wei-Yen Wang, Shun-Feng Su and To-Wen Lai
Sensors **2014**, *14*(2), 2089-2109; doi:10.3390/s140202089 (doi registration under processing) - published online 27 January 2014
Show/Hide Abstract

Open Access
Article: **A Wavelet Bicoherence-Based Quadratic Nonlinearity Feature for Translational Axis Condition Monitoring**
by Yong Li, Xiufeng Wang, Jing Lin and Shengyu Shi
Sensors **2014**, *14*(2), 2071-2088; doi:10.3390/s140202071 (doi registration under processing) - published online 27 January 2014

Sensors **2014**, *14*, 2135-2149; doi:10.3390/s140202135

OPEN ACCESS

sensors

ISSN 1424-8220

www.mdpi.com/journal/sensors

Article

A Simple Visual Ethanol Biosensor Based on Alcohol Oxidase Immobilized onto Polyaniline Film for Halal Verification of Fermented Beverage Samples

Bambang Kuswandi [1,2,*], Titi Irmawati [1], Moch Amrun Hidayat [1], Jayus [1] and Musa Ahmad [2]

[1] Chemo and Biosensors Group, Faculty of Pharmacy, University of Jember, Jl. Kalimantan 37, Jember 68121, Indonesia; E-Mails: t.irmawati@yahoo.com (T.I.); amrun.farmasi@unej.ac.id (M.A.H.); jayus.ftp@unej.ac.id (J.)

[2] Halal Science & Technology Research Group, Faculty of Science & Technology, USIM, Bandar Baru Nilai, Nilai 71800, Negeri Sembilan, Malaysia; E-Mail: andong@usim.edu.my

***** Author to whom correspondence should be addressed; E-Mail: b_kuswandi.farmasi@unej.ac.id.

Received: 29 October 2013; in revised form: 20 November 2013 / Accept: 28 November 2013 / Published: 27 January 2014

Abstract: A simple visual ethanol biosensor based on alcohol oxidase (AOX) immobilised onto polyaniline (PANI) film for halal verification of fermented beverage samples is described. This biosensor responds to ethanol via a colour change from green to blue, due to the enzymatic reaction of ethanol that produces acetaldehyde and hydrogen peroxide, when the latter oxidizes the PANI film. The procedure to obtain this biosensor consists of the immobilization of AOX onto PANI film by adsorption. For the immobilisation, an AOX solution is deposited on the PANI film and left at room temperature until dried (30 min). The biosensor was constructed as a dip stick for visual and simple use. The colour changes of the films have been scanned and analysed using image analysis software (*i.e.*, ImageJ) to study the characteristics of the biosensor's response toward ethanol. The biosensor has a linear response in an ethanol concentration range of 0.01%–0.8%, with a correlation coefficient (r) of 0.996. The limit detection of the biosensor was 0.001%, with reproducibility (RSD) of 1.6% and a life time up to seven weeks when stored at 4 ℃. The biosensor provides accurate results for ethanol determination in fermented drinks and was in good agreement with the standard method (gas chromatography) results. Thus, the biosensor could be used as a simple visual method for ethanol determination in fermented beverage samples that can be useful for Muslim community for halal verification.

Keywords: halal; polyaniline; alcohol oxidase ethanol; biosensor; fermented beverage

1. Introduction

Halal verification and authentication of food products are an issue of major concern and one of these issues is related to the as halal verification of the alcohol content in foods, particularly in fermented beverages. From an Islamic point of view, alcohol is a serious matter and totally prohibited in food products. As food products are part of our daily life, Islamic Laws give a special significance to this issue. In Islam, foods containing alcohol are *haram* (prohibited or unlawful) for Muslim consumption [1]. Ethanol is the main constituent found in alcoholic beverages and other products that undergo fermentation. Alcoholic drinks are totally prohibited in Islam, and even a small amount of the drink added into foods or drinks will render the products *haram* [2], but trace amounts of ethanol (naturally present as in fermented beverage) are allowed if the amount is insufficient to cause intoxication, usually less than 1% [3].

Hence, developing analytical methods for halal verification is very important, especially for the Muslim consumers to protect them from prohibited or *haram* products and also to ensure product safety and quality. Conventional methods, such as HPLC, GC-MS & FTIR have been used for food analysis, where the food samples have to be sent to laboratories to analyze for the presence of alcohol. The process takes days and is very tedious. In addition, such methods are time consuming, are subject to sources of errors and discrepancies between laboratories, and need skilled personnel for operation of those expensive instruments [4]. Therefore, the development of alternative methods for ethanol determination which simplify the analysis is needed. If one could easily detect the presence of alcohol within minutes this would be very useful to the Muslim community for enforcement in determining the safe consumption of food products in terms of their halalness.

In this context there is therefore a need to explore alternative methods of ethanol detection for halal verification using a tool that is accurate, simple, low-cost, rapid, reliable and consumer-friendly. A biosensor is an excellent candidate for this purpose. Biosensors are versatile analytical tools, offering an attractive alternative for ethanol detection [5]. The use of enzyme-based biosensors for the detection of ethanol in complex samples offers better specificity and therefore, a simpler sample treatment. Alcohol oxidase (AOX) [6,7], NAD^+-dependent alcohol dehydrogenase (ADH) [8,9] and PQQ-dependent alcohol dehydrogenases [10,11] have all been used as bioselective elements in ethanol biosensors.

Alcohol oxidase-based biosensors have an advantage over alcohol dehydrogenase biosensors, due to the fact the latter need the cofactor to be added to the sample or to be immobilised on the sensor surface, while AOX-based biosensors are simpler because they use only molecular oxygen (O_2) for co-factor regeneration. The enzyme requires O_2 to oxidize the ethanol and the products formed are acetaldehyde and hydrogen peroxide. Since, AOX enzymatically converts all primary alcohols and formaldehyde [12], it suffers from a lack of selectivity to ethanol. However, this should not be a problem in the use of such a biosensor for analysis of ethanol in fermented beverage samples, since ethanol is present at much higher levels. The main problem of AOX-based biosensors is their limited