

Microbial Consortium of *Saccharomyces cerevisiae* and *Bacillus megaterium* as Alcohol Dehydrogenase (ADH) Production for Ethanol Biosensor

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Abstract: Determining ethanol levels is very important in the food and beverage industry, especially in relation to health and permissible limits. Biosensors are one alternative for measuring ethanol levels because they are inexpensive, easy to use, fast, and sensitive in measurement. Previous research results from researchers have shown that ethanol biosensors using microbial consortia as a substitute for enzymes can detect ethanol in the range of 0.2-6% with 80% stability during 7 weeks of storage. However, the ethanol detection range is still not wide enough, so further research is needed to expand the ethanol detection range. The objective of this study is to produce an ethanol biosensor based on a microbial consortium with a wider detection range, high linearity, and stability equivalent to ADH as an enzyme for detecting ethanol. The methods used to achieve this objective were to optimize the formation of a biofilm consortium of *S. cerevisiae* and *B. megaterium* as ADH producers, compare the quality of ethanol biosensors using microbial consortia with pure ADH, and determine analytical performance. Pure ADH at a concentration of 3 µg/mL was tested on 10% ethanol, producing an oxidation current of 45 µA. Ethanol concentrations of 0.1-10% tested using the ethanol biosensor produced an optimal oxidation current of approximately 60 µA, which was linear with increasing ethanol concentration and had a storage stability of 80% over 10 weeks of storage.

Keywords: Ethanol, biosensor, Microbe, Alcohol Dehydrogenase (ADH)

INTRODUCTION

Ethanol is a type of alcohol that is widely used in various sectors, including the food industry as a fermentation product, additional solvent, and product quality control agent (Zhang *et al.* 2021). Monitoring ethanol levels in products is very important to ensure quality, safety, and regulatory compliance. According to MUI Fatwa No. 10 of 2018, the permissible ethanol limit in beverage products is less than 0.5%. Various analytical methods have been used to detect ethanol, such as HPLC, Raman spectroscopy, NIR, and refractometry (Nguyen *et al.* 2019; Liu *et al.* 2017). Although accurate, these methods are generally expensive, time-consuming, and require trained operators (Zhang *et al.* 2021). Therefore, an alternative, more efficient approach is needed, one of which is a biosensor.

A biosensor is an analytical device that uses biological components such as enzymes, cells, or tissues as the sensing element and a physico-chemical transducer to detect or measure specific substances (Alzeer *et al.* 2016).

Research on ethanol biosensors based on ADH enzymes includes Anisimova *et al.* (2023), who developed a conductometric biosensor using gold nanoparticle and chitin composites, which increased sensitivity by up to 11 times compared to conventional methods. Additionally, Liu *et al.* (2017) developed an electrochemical biosensor with

AuNPs/ERGO/PAH nanocomposites, achieving a detection limit of 10 μM with stability up to six weeks. Zhang *et al.* (2021) designed a wearable biosensor based on ADH and carbon nanotubes to detect ethanol in sweat in real-time with high sensitivity and a detection limit of 3 μM . Shah *et al.* (2020) also reported an amperometric biosensor using Au–Ag/P(L-Cys)/ERGO nanocomposites, achieving a detection limit of 5 μM . The use of microorganisms such as *S. cerevisiae* and *B. megaterium* as sensing elements is considered effective for detecting ethanol (Singh *et al.* 2019). A recent approach using a microbial consortium enables ethanol detection over a broader concentration range, where *S. cerevisiae* is sensitive to low ethanol concentrations and *B. megaterium* to high concentrations (Nurdiani *et al.* 2023).

The goal of this study is to produce a microbial consortium-based ethanol biosensor with a wider range detection and high linearity and stability equivalent to ADH as an enzyme for detecting ethanol. Microbial consortium research indicates that the use of microbial biofilms in biosensors enhances detection performance (Nurdiani *et al.* 2023). However, parameters such as biofilm age, suspension volume, and incubation conditions still need to be optimized to produce a reliable and industrially applicable ethanol detection system. The results of previous research by Nurdiani *et al.* 2023 showed an ethanol biosensor using a microbial consortium as a substitute for enzymes, which can detect ethanol in the range of 0.2-6% with 80% stability over 7 weeks of storage. However, this range is still not broad enough, so further research is needed to improve the detection range and stability of the ethanol biosensor to produce a reliable ethanol detection system for testing ethanol in a wider detection range.

RESEARCH METHODS

Materials and Tools

The materials used were screen printed electrode (SPCE) refs. 100 (DropSens, Asturias, Spain), distilled water, 96% ethanol (Sigma Aldrich, Saint Louis, United States), phosphate buffer solution or Phosphate Buffer Solution (PBS), synthetic zeolite, glutaraldehyde, bovine serum albumin (BSA), aluminum foil, crystal violet, lugol and safranin (Sigma Aldrich), solid heterotrophic media, liquid media, and isolates of *S. cerevisiae* and *B. megaterium* (derived from the microbial collection of the Health Microbiology Laboratory, National Research and Innovation Agency in Cibinong).

The tools used in this study were a Sanyo MIR-162 incubator, a Hiramaya HVE 50 autoclave, a CVB 1300M laminar air flow, a GR-202 analytical balance (A&D, Tokyo, Japan), a 100 μL micropipette, a TOA DK HM-250 pH meter, a 5415C centrifuge, an Eppendorf tube, a SPCE DRPAC71190 Metrohm connector (DropSens, Asturias, Spain), an EDAQ potentiostat/galvanostat (ADIstruments Pty Ltd, New South Wales, Australia) with a 3-electrode system from eDAQ (Newzeland, Australia), a computer equipped with the Echem v.2.1.0 and Origin Pro 7.0 data processing programs (Originlab Corporation, Norhampton, United States), a BIORAD iMark microplate absorbance reader (BIORAD, California, United States), a UV-Vis spectrophotometer, Nikon YS100 microscope, SEM JSM-6360LA (JEOL, Michigan, United States), oven, gas chromatography, microwave and other laboratory glassware.

Methods

Cultivation, Characterization, and Measurement of Optical Density (OD) Absorbance of ADH-Producing Microbes

Microorganism cultures of *B. megaterium* and *S. cerevisiae* from the BRIN collection were rejuvenated and obtained using the streak technique on sterile solid media. *B. megaterium* was cultured on heterotrophic agar (HTR) medium and *S. cerevisiae* on YM medium. After incubation for 16–18 hours at 37 °C, the isolates were grown in 10 mL of liquid medium and incubated for 24 hours at 30 °C in a shaker. The cultures were centrifuged, and the pellets were washed three times using PBS pH 7 to obtain ready-to-use biomass (Mira *et al.* 2022).

Microbial growth was monitored by measuring absorbance at a wavelength of 595 nm (absorbance OD₅₉₅) using a spectrophotometer. Microbial characterization was performed using Gram staining with the Hucker method: microbial preparations were fixed, stained with crystal violet, iodine solution, decolorized with 95% ethanol, and counterstained with safranin before being observed under a microscope at 100–1000× magnification (Liu *et al.* 2017).

Production and Optimization of Microbial Consortium

The consortium biofilm was created from a suspension of harvested microbes immobilized on a Screen Printed Carbon Electrode (SPCE) working electrode (Nurdiani *et al.* 2024). The suspension consists of a mixture of *S. cerevisiae* and *B. megaterium* in a 1:1 ratio, with a consortium volume of 100 µL added gradually (Shah *et al.* 2020). The electrode was stored in a sealed container at room temperature until dry. Optimal conditions were determined based on the peak oxidation current from cyclic voltammetry results. Tests were conducted on ethanol concentrations of 0.1–10% at an incubation temperature of 30°C. Optimizations included biofilm age (6–10 days), volume ratio (100 µL) of each microorganism, and (absorbance OD₅₉₅ = 0.5–0.7).

Determination of Ethanol Biosensor Analytical Performance and Data Analysis

The performance evaluation of the biosensor included linearity testing, limit of detection (LOD), limit of quantification (LOQ), sensitivity, accuracy, and stability. Current data were obtained from voltammetry curves and analyzed using Echem v2.1.0, OriginPro 7.0, and Microsoft Excel (Iswantini *et al.* 2017). Measurements were performed using cyclic voltammetry with an eDAQ potentiostat (Ecoder 410) equipped with Echem v2.1.0 software. Current measurements were performed using the following parameters:

Mode	: DPV	Step W	: 20 ms
Initial E	: +0 mV	Range	: 1 V
Final E	: + 800 mV	Rate	: 100 mV/s

Testing Alcoholic Beverage Samples with Ethanol Biosensors and Gas Chromatography

The test was conducted on commercial alcoholic beverage samples with ethanol concentrations of 2%, 4.9%, 11%, and 13.5%. A total of 0.5–1 µL of the sample was dropped onto the surface of the SPCE electrode immobilized with a consortium of *S. cerevisiae* and *B. megaterium* (Nguyen *et al.* 2019). For validation, 0.5–1 µL of standard ethanol solution was injected into a GC with an isothermal oven at 115 °C, an auto-sampler, and an auto-diluter at 20 ± 0.5 °C. Analysis was performed using an Rtx R-5MS column (30 m, ID 0.25 mm, stationary phase 5% diphenyl/95% dimethylpolysiloxane).

RESULTS AND DISCUSSION

Microbial Culture of *S. cerevisiae* and *B. megaterium* ADH Production

The cultivation was carried out to culture microorganisms in suitable environmental conditions (media, temperature, and pH) so that ADH production by the cells reached maximum levels (Liu *et al.* 2017). ADH is an enzyme that plays a role in alcohol metabolism and is commonly found in microorganisms such as *S. cerevisiae* and *B. megaterium* (Singh *et al.* 2019). *S. cerevisiae* cultures were obtained through isolation from tape and fermipan. These microorganisms were incubated at 37°C for 24 hours in suitable media until pure colonies formed. Their morphological characteristics include round cells, white to cream-colored, and a smooth texture, as shown in Figure 1a.

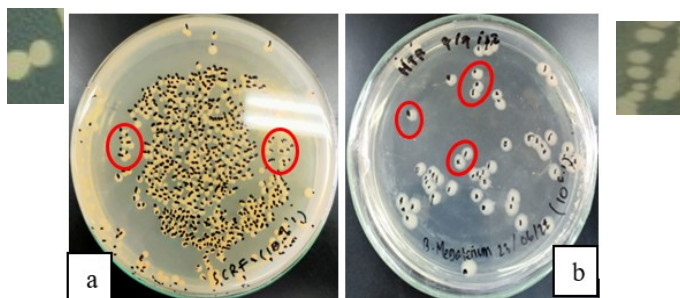


Figure 1. Microbial a) *S. cerevisiae* and b) *B. megaterium* in solid media HTR

This microorganism is known as an effective source of ADH due to the stability of its enzyme under various pH and temperature conditions (Park *et al.* 2016). *B. megaterium* is cultured on heterotrophic media and incubated under similar conditions. This microorganism is gram-positive and produces ADH in the form of large, white to cream-colored rod-shaped cells with a dry-looking colony surface (Figure 1b). The advantage of *B. megaterium* lies in its ability to produce ADH in high quantities (Iswantini *et al.* 2020).

Relationship between Absorbance Value and Incubation Time of Microbial Consortium

Optical density or OD₅₉₅ absorbance is used to monitor the growth of microorganisms based on the OD₅₉₅ absorbance of cells in liquid culture. Absorbance measurements are performed at a wavelength of 595 nm to evaluate the growth of two ADH enzyme-producing microorganisms. This wavelength corresponds to the maximum absorbance of microbial cells in complex medium and is often used in standard cell growth protocols (Yousefi *et al.* 2020).

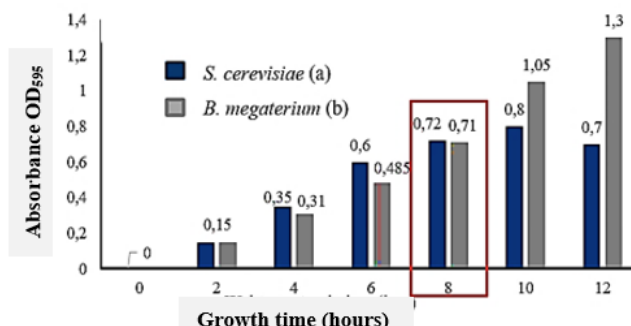


Figure 2. Graphic showing the relationship between absorbance and incubation time of the microbial consortium (a) *S. cerevisiae* and (b) *B. megaterium*

The OD₅₉₅ absorbance values of *S. cerevisiae* and *B. megaterium*, respectively, during growth in the mid-logarithmic phase indicated that optimal conditions were achieved at 8 hours, with values of 0.720 (Figure 2a) and 0.710 (Figure 2b). Both microorganisms were in the mid-logarithmic growth phase, which is the optimal phase for ADH enzyme production, characterized by high metabolic activity and cell division (Wang *et al.* 2022). The same or nearly the same OD₅₉₅ absorbance value is required for stable biofilm structure formation, balanced microbial proportions, and synergistic microbial interactions so that the microbial consortium results are reliable. Imbalances in cell density can disrupt biofilm formation due to substrate competition, dominance of certain species, and loss of metabolic synergy (Sukma *et al.* 2023). Harvesting cells during the logarithmic phase offers advantages in terms of the stability of the intracellularly produced ADH enzyme. In previous studies, maximum enzyme activity was achieved when microbial cells were harvested when the OD₅₉₅ absorbance approached 0.7–0.8, which directly correlates with the highest expression of enzymatic genes (Zhang *et al.* 2021; Vigneshvar *et al.* 2016).

Results of Microbial Characterization of *S. cerevisiae* and *B. megaterium*

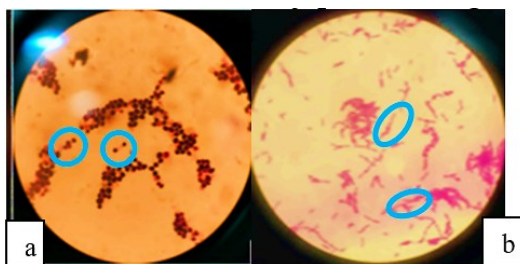


Figure 3. 100x magnification microscope of (a) *S. cerevisiae* and (b) *B. megaterium* bacteria

Gram staining is performed to quickly identify the structure of microbes and determine the type of microbe. *S. cerevisiae* is a yeast microbe with Gram-negative staining properties, having a round or oval cell shape (Figure 3a) with a size of 5–10 μm . Optimal growth occurs at a temperature of 28–30 °C and pH 4–6. *S. cerevisiae* is a facultative anaerobe and can efficiently ferment various carbohydrates, particularly glucose (Liu *et al.* 2017). *B. megaterium* is a Gram-positive rod-shaped microorganism, appearing purple in color (Figure 3b) and with a diameter of 2–5 μm . Morphologically, these microbes are rod-shaped and can grow in both aerobic and anaerobic conditions. *B. megaterium* spores are heat-resistant, and these microbes are capable of degrading carbohydrates (Anisimova *et al.* 2023).

Optimization of ethanol biosensors using a consortium of *S. cerevisiae* and *B. megaterium* microbes

Biofilm formation was carried out using two methods: physical adsorption and entrapment using 0.1% agarose as an entrapment matrix on the enzyme. Physical adsorption plays a role in the initial placement of microorganisms on the surface, while entrapment with agarose maintains the position of the microbes while still allowing substrate (ethanol) to diffuse in and products (acetaldehyde) to diffuse out. Optimization of the *S. cerevisiae* and *B. megaterium* biofilm consortium was performed to improve the performance of the ethanol biosensor in terms of linearity and response stability.

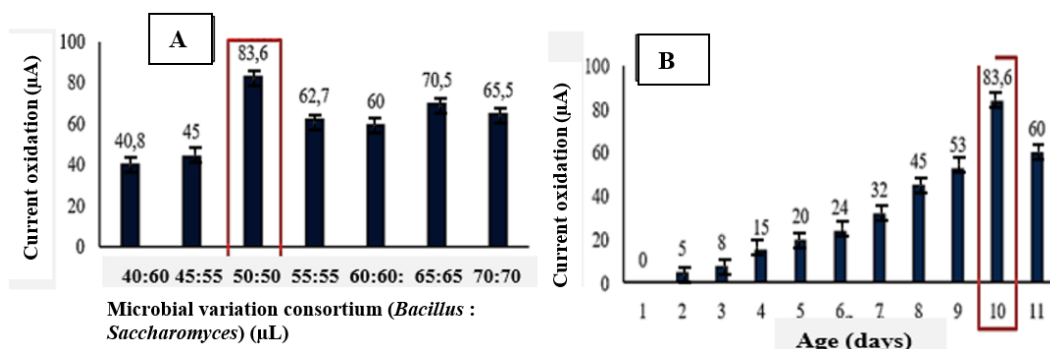


Figure 4. Graph A) results of microbial consortium age variation on oxidation flow obtained B) results of microbial consortium variation on oxidation flow obtained

Measurements using cyclic voltammetry showed that the consortium biofilm reached optimal conditions after 10 days of incubation (Figure 4A), producing the highest oxidation current of 83.6 μA , reflecting sufficient time for synergistic interactions between microorganisms and the formation of a well-structured biofilm (Mira *et al.* 2022). A drop volume of 100 μL (Figure 4B) yielded the highest oxidation current of 83.6 μA , which was deemed optimal as it produced a uniform and not overly thick biofilm layer, thereby maintaining optimal diffusion of ethanol substrate to the ADH enzyme within the biofilm.

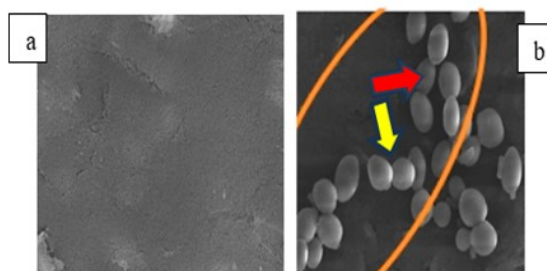


Figure 5. Morphology (a) SPCE; (b) biofilm from microbe *S. cerevisiae* (yellow arrow) and *B. megaterium* (red arrow) on SPCE using Scanning Electron Microscope (SEM)

Excessive biofilm thickness can inhibit mass transfer, while a layer that is too thin causes unstable microbial immobilization (Shah *et al.* 2020). Based on SEM analysis results (Figure 5A), SPCE has an uneven surface, while (Figure 5B) Screen Printed Carbon Electrode (SPCE) modified with a microbial consortium biofilm results in good adhesion between one microbe and another (Singh *et al.* 2019). *S. cerevisiae* (indicated by the yellow arrow) is spherical in shape, while *B. megaterium* is rod-shaped (indicated by the red arrow).

Optimization was carried out by considering three main parameters, namely incubation duration, microbial inoculum ratio, and cell density (OD_{595} absorbance). Biofilm formation by the consortium under optimal conditions resulted in an oxidation current of 60 μA (Figure 6) during testing at a 10% ethanol concentration. *B. megaterium*, a gram-positive microbe, produces exopolysaccharides that support the biofilm structure, while *S. cerevisiae* contributes to biofilm matrix formation and microenvironment stability. The study by Yousefi *et al.* (2020) evaluated the effect of inoculum volume on biofilm formation by microbial consortia. Increasing the inoculum volume to 3 mL increased the population, but

maximum biofilm mass was achieved at an inoculum volume of 2 mL. This indicates an optimal inoculum volume for stable biofilm formation.

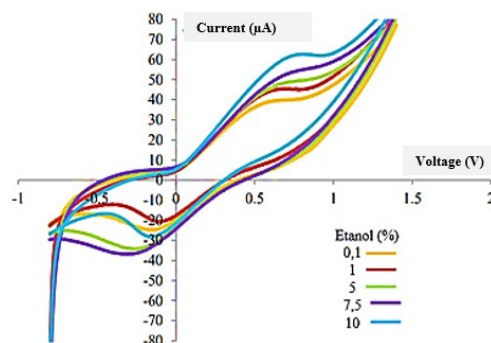


Figure 6. The ethanol voltamogram with varying concentrations was tested using a biofilm-modified SPCE with optimized microbial consortium age and volume

Electrochemical response of pure ADH enzyme

The testing of pure ADH enzyme on ethanol aims to accurately determine enzyme activity free from contaminants. Testing with pure ADH at a concentration of 3 µg/mL produced a maximum current of 45 µA at an ethanol concentration of 10% (Figure 7), indicating the presence of ADH catalytic activity toward the ethanol substrate, detected electrochemically through current signal transduction. This low response correlates with the concentration of pure ADH used, limiting the number of active enzyme sites available to catalyze the substrate (Yousefi *et al.* 2020). As reported by Shah *et al.* (2020), the efficiency of ADH-based biosensors is highly dependent on the amount of enzyme effectively immobilized on the electrode surface, where increasing the enzyme concentration to 10 µg/mL can more than double the current response.

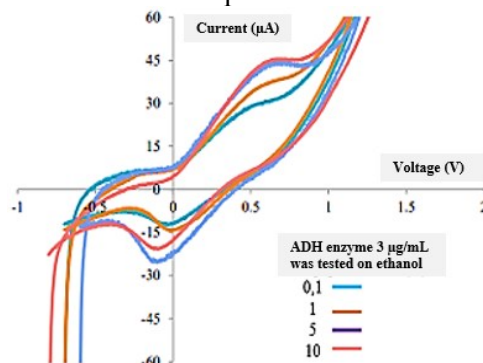


Figure 7. Voltammogram of pure ADH enzyme tested on ethanol with various concentrations

The performance of pure enzymes is greatly influenced by immobilization conditions and enzyme stability in the operational environment (Li *et al.* 2023). The development of biosensors and increased ADH concentrations have the potential to increase the number of interactions between enzymes and substrates, thereby increasing the redox reaction current (Park *et al.* 2016). The use of support materials such as carbon-based nanocomposites (e.g., graphene oxide or carbon nanotubes) can enhance the stability and conductivity of biosensors (Liu *et al.* 2017).

Analytical performance of ethanol biosensors

The determination of analytical performance aims to evaluate the ability of biosensors to accurately detect ethanol. The biosensor developed in this study showed good linearity with an R^2 value of 0.9946 (Figure 9), indicating a linear relationship between electric current and ethanol concentration in the range of 0.1–10%. These results expand the ethanol testing range, where Nurdiani *et al.* (2023) conducted their study within the 0.2–6% testing range. Zhang *et al.* (2021) developed an ADH-based biosensor using gold nanostructure electrodes, showing a detection range of 0.05–5% ethanol with linearity $R^2 = 0.991$. Yousefi *et al.* (2020) reported that an ADH-based biosensor could detect ethanol in the range of 0.01–8% with high sensitivity, but experienced a decrease in stability after 7 days of storage. Research by Li *et al.* (2023) demonstrated linear performance over the range of 0.2–12%, but faced challenges with current fluctuations at concentrations above 10% due to enzyme active site saturation.

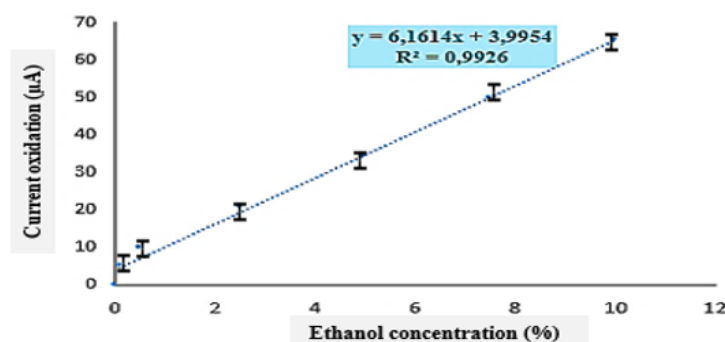


Figure 8. Linearity of the ethanol biosensor tested at various ethanol concentrations

The biosensor developed in this study showed good linearity with an R^2 value of 0.9946 (Figure 8), indicating a linear relationship between electric current and ethanol concentration in the range of 0.1–10%. These results expand the ethanol testing range, whereas the study by Nurdiani *et al.* (2023) tested the range of 0.2–6%. Zhang *et al.* (2021) developed an ADH-based biosensor using gold nanostructure electrodes, showing a detection range of 0.05–5% ethanol with linearity $R^2 = 0.991$. Yousefi *et al.* (2020) reported that an ADH-based biosensor could detect ethanol in the range of 0.01–8% with high sensitivity, but experienced a decrease in stability after 7 days of storage. Research by Li *et al.* (2023) demonstrated linear performance over the range of 0.2–12%, but faced challenges with current fluctuations at concentrations above 10% due to enzyme active site saturation.

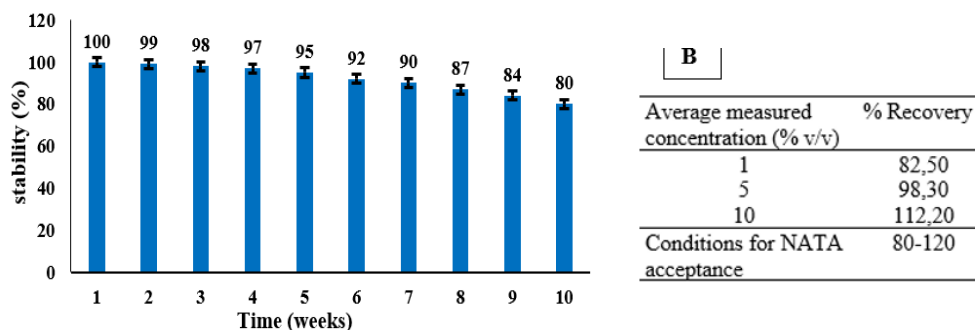


Figure 9. a) Stability of the ethanol biosensor over 10 weeks b) Accuracy of the ethanol biosensor

The stability of the ethanol biosensor for up to 10 weeks with a 20% decrease in performance (Figure 9a) at room temperature demonstrates the good biological and mechanical durability of the biosensor system. Research by Istrate *et al.* (2021) reported an ADH-based ethanol biosensor that maintained 90% of its initial activity stability over 5 days with a 10% performance decline on the second day. Consortium-based biosensors are considered more stable than pure enzymes because the biofilm structure formed protects the enzymes from environmental damage, facilitates substrate diffusion, and extends the sensor's lifespan (Liu *et al.* 2017).

The results of accuracy by looking at the percentage obtained range of 82.50–112.20% (Figure 9b) show that the standard ethanol yield at low, medium, and high ethanol concentrations is within a sufficient range when compared to the yield value from NATA (National Association Testing Authority) which requires 80–120%. Nurdiani *et al.* (2023) developed an ethanol biosensor based on a consortium of *S. cerevisiae* and *B. megaterium* microorganisms, yielding recovery percentages in the range of 90.27% to 111.07%.

Analysis of alcoholic beverage samples using ethanol biosensors and gas chromatography (GC)

Analysis of ethanol content in various types of alcoholic beverages (concentration 2–13.5%) using an ADH enzyme-based biosensor produced an oxidation current of 65 μ A, which was proportional to the increase in ethanol concentration in the sample (Figure 10). Categories of beverages such as beer (2–5%), wine (8–12%), and wine (10–13.5%) showed a proportional current response to their ethanol content, reflecting the linearity and sensitivity of the biosensor within that concentration range. Comparison with a previous study by Liu *et al.* (2017) shows that the ADH biosensor they developed could detect ethanol in the range of 1–10% with a maximum current of 50 μ A. Li *et al.* (2023) detected ethanol in commercial beverages using a biosensor, yielding a correlation coefficient $R^2 > 0.98$ with GC results, but the maximum current was only 40 μ A at 10% ethanol.

Table 1. Testing ethanol beverage samples with microbial consortium-modified SPCE and gas chromatography

Measurement of alcoholic beverage samples	Repeatability & Ethanol Concentration measurement with ethanol biosensor	Accuracy of ethanol by Biosensor	Repeatability & Ethanol concentration measurement by gas chromatography	Accuracy of ethanol by GC
2 %	1. 2% 2. 2.10%	102.5 %	1. $2.17\% \pm 0.05$ 2. $2.54\% \pm 0.05$	117.75 %
4.9 %	1. 5% 2. 5%	102.04 %	1. $4.53\% \pm 0.05$ 2. $4.13\% \pm 0.05$	88.37 %
11.5 %	1. 11% 2. 11%	95.65 %	1. $10.58\% \pm 0.05$ 2. $11.84\% \pm 0.05$	97.48 %
13.5 %	1. 13% 2. 13%	96.30 %	1. $11.13\% \pm 0.05$ 2. $11.88\% \pm 0.05$	85.22 %
The average measurement		99.1225 %		97.205 %

The test results (Table 1) show that the ethanol biosensor is capable of providing accurate measurement results in the low concentration range (2–5%) with a consistently lower measured ethanol content compared to GC results. This indicates that GC has limitations in analytical resolution and the potential for sample volatilization during injection (Zhang *et al.* 2021). However, at higher ethanol concentrations (5–13%), the GC method

actually shows more accurate measurement results compared to the biosensor. This is due to biosensor response saturation at high substrate levels and potential enzymatic inhibition. The accuracy of the biosensor showed a higher accuracy of 99.1225%. The biosensor demonstrated that optimizing microbial consortium biofilm produces alcohol dehydrogenase (ADH) or a biocatalytic system that is specific to (Park *et al.* 2016).

CONCLUSIONS

The consortium of *S. cerevisiae* and *B. megaterium* microorganisms showed optimal growth at an OD₅₉₅ absorbance value of 0.7, indicating the logarithmic phase of biofilm formation. Optimal biofilm conditions were achieved at pH 7, an incubation period of 10 days, and an inoculum ratio of 1:1 with a volume of 100 µL. The ADH contained in this consortium exhibits good electrochemical response to ethanol and can detect concentrations ranging from 0.1–10%. The developed ethanol biosensor demonstrates excellent analytical performance with high linearity ($R^2 = 0.9926$), accuracy up to 100%, and operational stability of 80% over 10 weeks at room temperature.

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REFERENCES

- Alzeer J, Hadeed KA. 2016. Ethanol and its halal status in food industries. *Trends Food Sci Technol.* 58: 14-20.
- Anisimova, O. S. Pavlova, T. V. Umarhkanov, R. A. & Archakov, A. I. (2023). Conductometric ethanol biosensor based on alcohol dehydrogenase and gold nanoparticle–chitosan composite. *Nanomaterials*, 13(16).
- Istrate OM, Rotariu L, and Bala C. 2021. A novel amperimetric biosensor based on poly(allylamine hydrochloride) for determination of ethanol in beverages. *Sensors*. MDPI. 21, 6510.
- Iswantini D, Indahsari F, Maddu A, Nurhidayat N, Purwaningsih H, Sugiarti S. 2020. Activity and stability of the alcohol biosensor using *B. megaterium* biofilm on screen printed carbon electrode. *Hayati J of Biosciences*. 27(1).
- Iswantini D, Nurhidayat N, Ferit H. 2017. Alcohol dehydrogenase of *Bacillus* strain for measuring alcohol electrochemically. *IOP Conf. Series: Earth and Environmental Science*.
- Li, Y. Luo, Y. & Sun, J. (2023). Microbial consortia biofilms for biosensing applications: Challenges and opportunities. *Biosensors and Bioelectronics*, 229, 115244. <https://doi.org/10.1016/j.bios.2023.115244>.
- Liu Y, Mingzhu D, Wei L, Yun Y, Xiao Z, Bing ZL, Tao C, Yong N, Mi Xiaoxiao W, Boxuan Z. 2017. A tree species microbial consortium for power generation. *Energy and Environmental Science. Royal Society of Chemistry*.

- Majelis Ulama Indonesia. (2018). Fatwa MUI No. 10 Tahun 2018 tentang Produk Makanan dan Minuman yang Mengandung Alkohol/Etanol. Komisi Fatwa MUI.
- Mira Portia, Pamela Yeh, and Barry G. Halls. 2022. Estimating microbial population data from optical density. *PubMed*. 17(10).
- Nguyen HH, Lee SH, Lee UJ, Fermin CD. 2019. Immobilized enzymes in biosensor applications. *Materials*. 12(1): 121. doi: 10.3390/ma12010121.
- Nurdiani, Iswantini D, Nurhidayat N, Wahyuni WT, Kartono A. 2023. Screening and optimization technique of the bacillus megaterium and *S. cerevisiae* microbial consortium for ethanol detection. *J Kimia Valensi*. 9(1):109-122.
- Nurdiani, Iswantini D, Nurhidayat N, Wahyuni WT, Kartono A. 2024. The analytical performance of *S. cerevisiae* and *B. megaterium* microbial consortium as recognition element in ethanol biosensor. *Biodiversitas*. 24(11): 5928-5936. DOI: 10.13057/biodiv/d241111
- Park S, Kim JC, Lee HS, Jeong SW, Shim YS. 2016. Determination of five alcohol compounds in fermented Korean foods via simple liquid extraction with dimethylsulfoxide followed by gas chromatography-mass spectrometry for halal food certification. *LWT Food Sci Technol*. 74: 563-570.
- Shah, U. Kim, D. & Lee, J. (2020). Optimization of alcohol dehydrogenase concentration in biosensor development for ethanol detection. *Analytical Biochemistry*, 593, 113567. <https://doi.org/10.1016/j.ab.2020.113567>.
- Singh, R. Shukla, A. & Tiwari, S. (2019). Applications of yeast-derived enzymes in biosensing: A comprehensive review. *Enzyme and Microbial Technology*, 129, 109353. <https://doi.org/10.1016/j.enzmictec.2019.109353>.
- Sukma, R. M., Iswantini, D., Nurhidayat, N., Rafi, M., & Ariyanti, D. (2023). Antioxidant Determining Using Electrochemical Method. *Chemistry*, 5(3), 1921-1941. <https://doi.org/10.3390/chemistry5030131>
- Vigneshvar S, Sudhakumari CC, Senthilkumaran B, Prakash H. 2016. Recent advances in Biosensor technology for potential applications: an overview. *Bioengineering Biotechnology*. 4(11): 1-9.
- Wang, Z. Lin, Y. & Xie, J. (2022). Spectrophotometric monitoring of microbial biomass: Advances and standardization in OD measurements. *Journal of Microbiological Methods*, 200, 106580. <https://doi.org/10.1016/j.mimet.2022.106580>.
- Yousefi, H. Ali, M. M. & Wang, S. (2020). Enhanced performance of ethanol biosensors using nanomaterials for enzyme immobilization. *TrAC Trends in Analytical Chemistry*, 130, 115987. <https://doi.org/10.1016/j.trac.2020.115987>.
- Zhang, L. Huang, Y. & Chen, X. (2021). Advances in analytical techniques for ethanol detection: A review. *TrAC Trends in Analytical Chemistry*, 140, 116274. <https://doi.org/10.1016/j.trac.2021.116274>.