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STABILIZATION OF AN MQ-3 SENSOR FOR ETHANOL MEASUREMENT IN COWPEA SEEDS

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KEYWORDS

Vigna unguiculata L., storage, fermentation, ethanol.

ABSTRACT

The widespread adoption of sensor technology has made it a standard practice for obtaining precise and timely information during the harvest and post-harvest periods. One sensor that has gained popularity for post-harvest seed monitoring is the MQ-3, which identifies ethanol in the air as products undergo fermentation. However, these sensors typically require a stable operation. This study aimed to assess the stabilization time of an MQ-3 sensor when measuring ethanol levels in anaerobic bean seeds. We used six bean seed samples, each with an average moisture content of around 14%. We employed a completely randomized experimental design with nine repetitions for each sample. Every repetition consisted of 25 bean seeds placed in sealed flasks containing 70 mL of distilled water. This setup induced anoxic conditions within the flask, promoting anaerobic respiration in the seeds. After 24 hours, we exposed an air sample to the MQ-3 sensor and took readings at various time intervals (12-14, 19-21, 36-38, 68-70, 130-132, 192-194, 314-316, 616-618 seconds). The average stabilization time for the MQ-3 sensor while quantifying ethanol concentrations in the bean samples were approximately 23 seconds. The sensor demonstrated efficacy, convenience, and rapidity in assessing ethanol levels in anaerobic bean seeds.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.)] is a legume of great socioeconomic importance for various regions of the planet, mainly due to its high nutritional value, which makes this crop one of the main agricultural products that make up the human diet. Furthermore, because it has hardiness characteristics, it can adapt to conditions of less water availability. In Brazil, the North and Northeast regions are the largest producers. The first crop monitoring of 2022/23 indicates that in this cycle, the total area sown should be 387.2 thousand hectares and that the states of Piauí and Bahia together should represent 82% of the estimated area for planting (Freire Filho & Costa, 2020; CONAB, 2022). However, cowpea cultivation still lacks technologies that can maximize its productivity, mainly in producing superior quality seeds, which can benefit from using sensors in pre- and post-harvest to assist in decision-making.

The use of sensors in agriculture has become a routine and highly accepted activity in the market, aiming to provide accurate information during harvest and post-harvest and for soil use in determining temperature and humidity. In this context, agricultural management has used various sensors, including remote aerial detection, field weather stations, environmental greenhouse sensors, electrochemical sensors, electronic "noses," biofuels, and sophisticated wireless sensor networks (Pineda & Pérez, 2017).

Using these sensors in post-harvest processes has alerted producers to the depreciation of their product quality due to various factors, especially during storage under inappropriate conditions. According to Capilheira et al. (2019), seed producers have been adopting various

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techniques to guarantee the quality of seeds after harvest and processing since there are qualitative losses due to deterioration stimulated by respiratory and metabolic activity. Deterioration of stored products occurs due to the seed respiration process by consuming their reserve substances.

According to Ochandio et al. (2017), the respiratory rate of soybean seeds, a crop similar to beans, is not affected by O_2 partial pressure up to 2.0 kPa. Below this level, the respiratory rate decreases due to hypoxia. However, free oxygen is essential to provide cellular respiration and reduce the energy used to maintain anaerobic organisms' metabolism.

In this context, low oxygen levels during seed storage can provide an environment with similar hypoxic characteristics, directly interfering with their aerobic respiration. With anaerobic respiration, alcoholic fermentation begins, where the two enzymes pyruvate decarboxylase and alcohol dehydrogenase act on pyruvate, producing ethanol and CO_2 and oxidizing NADH in the process (Taiz & Zeiger, 2017). Therefore, there is a need to use a sensor capable of detecting the presence of ethanol in seeds stored at low levels of O_2.

Thus, this study aimed to evaluate the stabilization time of an MQ-3 sensor for measuring ethanol in beans under anaerobic conditions.

MATERIAL AND METHODS

The study was conducted at the Agrotechnology Laboratory of the Engineering Center Federal University of Pelotas, Pelotas-RS. Six samples of cowpea seeds from the cultivar BRS Amendoim, harvested in 2015, were used as research material, with an average moisture content determined according to the Seed Analysis Rules (Brasil, 2009), around 14% (Brasil, 2009) for all samples.

Nine repetitions of 25 visually classified bean seeds were chosen for the ethanol measurements, which did not present apparent physical damage. First, each sample was individually placed in glass bottles of known volume (150 mL) and sealed with systems in their cap, then a volume of 70 mL of distilled water was added, which was enough to keep the seeds covered and in anoxia (Cavalcante et al., 2019), to simulate an anaerobic, anoxic environment, going through a resting period of 24 hours, which was the minimum safe time for ethanol production to occur (Cavalcante et al., 2019) at room temperature around 25°C. After this soaking period, measurements were taken with the sensor.

The MQ-3 sensor from the Datasheet brand is an electrochemical sensor capable of measuring resistance variations (surrounding ethanol). It was integrated into a microcontroller (ATMega 328p), converting resistance and voltage variations. Further information about the equipment cannot be detailed because it is applying for a patent from the National Institute of Industrial Property (INPI) published in the Official Gazette on 09/29/2020 with process number BR 10 2019 003242 1, which can be accessed after it is released. However, before the conversion process, any possible noise from the external environment was eliminated through low-pass filters present in the converter itself. This converter then converted the voltage variation signals into digital values, in which the amount of ethanol was subsequently presented in percentage after readings taken at different time intervals (12-14, 19-21, 36-38, 68-70, 130-132, 192-194, 314-316, 616-618 seconds).

A completely randomized design with nine repetitions for each of the six samples was used. The data were subjected to variance analysis at a 5% significance level, and when significant, the exponential curve model was applied to determine the minimum stabilization time of the MQ-3 sensor. The results were evaluated using the statistical program R™ version 3.1.1. and the "ExpDes.pt" data package.

RESULTS AND DISCUSSION

Figure 1A shows that the MQ-3 sensor took at least 22 seconds to stabilize for sample 1. Readings taken from 23 seconds onwards showed no variations and remained constant until the last reading was taken at 618 seconds. On the other hand, sample 2 (Figure 2B) achieved stability in readings from 40 seconds onwards and remained stable until the last reading at 618 seconds, which is different from what was observed for sample 1.

These results demonstrate the practicality and speed at which sensors work, making them suitable for use in a portable and affordable commercial device capable of detecting volatile organic compounds (VOCs), especially ethanol, with high-sensitivity sensors (Mcginn et al., 2020). Hamid et al. (2018) commented that various analytical techniques such as liquid chromatography, infrared and mass spectrometry had been used to detect ethanol concentrations, but they are expensive and not portable. Therefore, it is necessary to develop ethanol sensors with high sensitivity, affordability, portability, and fast response due to the high cost and complex process of stabilizing the sensor before comparing it with other methods.

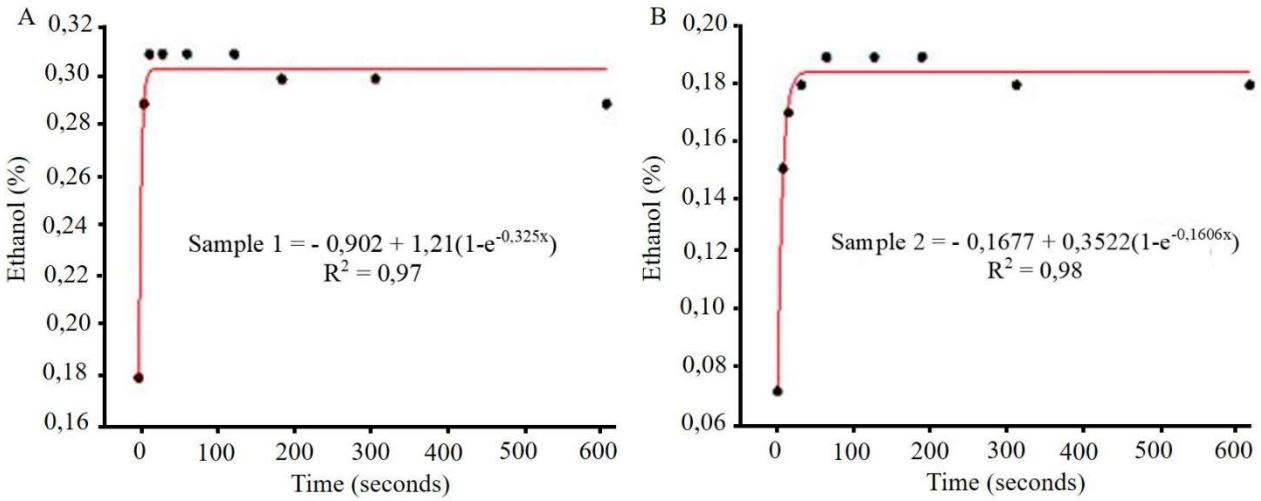

FIGURE 1. The average stabilization time of the MQ-3 sensor for measuring ethanol in different samples (1 and 2) of bean seeds subjected to an environment simulating hypoxia (anaerobiosis).

Both samples 3 and 4 (Figures 2A and 2B) showed similar stabilization times, 26 seconds for sample 3 and 29 seconds for sample 4. However, samples 5 and 6 (Figures 3A and 4B) showed the shortest stabilization time, corresponding to 16 and 13 seconds, respectively, demonstrating the efficiency of the MQ-3 sensor in determining the amount of ethanol released in a short time.

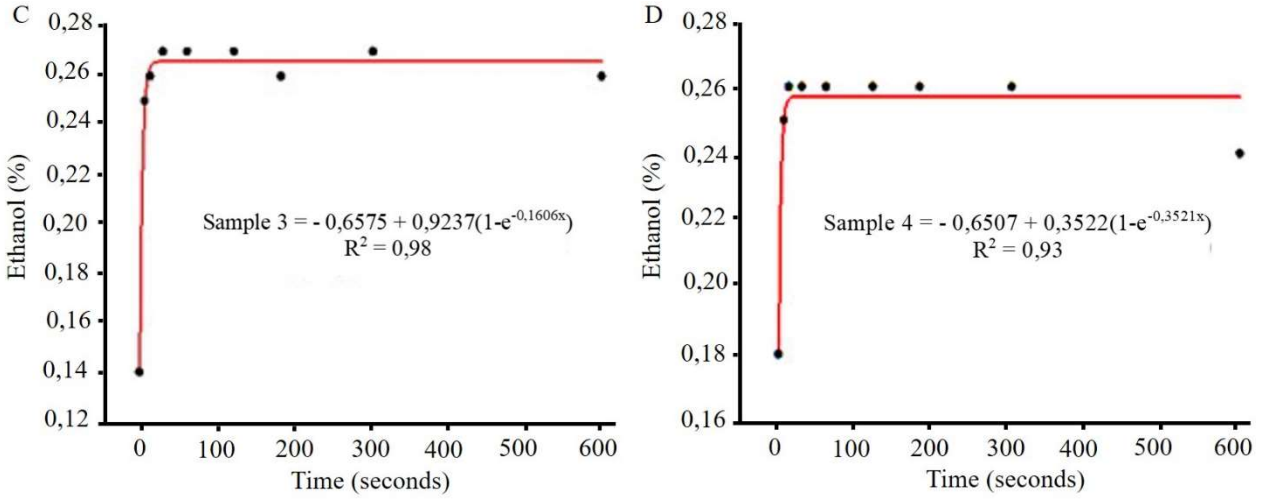

FIGURE 2. The average stabilization time of the MQ-3 sensor for measuring ethanol in different samples (3 and 4) of bean seeds subjected to an environment simulating hypoxia (anaerobiosis).

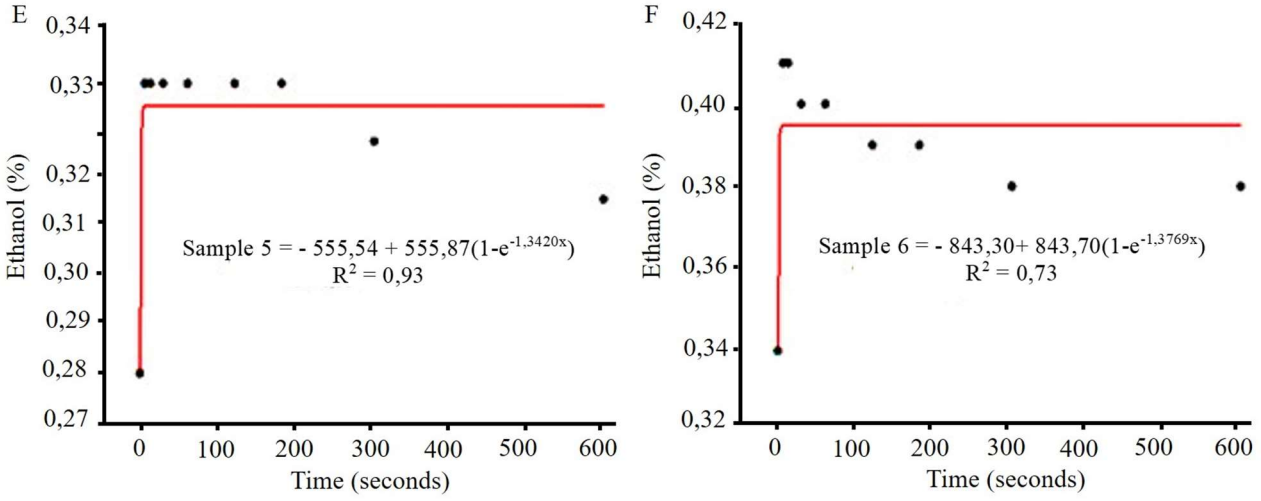

FIGURE 3. The average stabilization time of the MQ-3 sensor for measuring ethanol in different samples (5 and 6) of bean seeds subjected to an environment simulating hypoxia (anaerobiosis).

These variations in time between samples can be explained by differences in physiological quality, measured during internal quality control in seed production, mainly through germination tests, field emergence, tetrazolium tests, and accelerated aging. However, ethanol production by seeds is influenced by physiological factors inherent to the amount of reserve in them and the integrity of mitochondrial and cellular membranes (Cavalcante et al., 2019), which may be fundamental in activating and speeding up ethanol production during alcoholic fermentation in the cell cytosol.

Other studies on the amount of ethanol released by seeds, such as by Barbosa et al. (2021) in red rice seeds, Ornellas et al. (2022) in canola seeds, Cavalcante et al. (2017) in ryegrass seeds, and Vergara et al. (2018) in quinoa seeds, used an ethylometer. However, these methods, similar to the one presented in this study using the ethylometer for quick and adequate analysis of exhaled air from the lungs and the presence and amount of alcohol in the body, provide results equivalent to the values found in alveolar air and are therefore inadequate for evaluating a similar metabolism in seeds. Each sensor has its characteristics, in this case, an electrochemical sensor was used, which is widely used and can replace the analog diaphragms commonly used in quick-response breathalyzers to measure the amount of alcohol present in human blood.

The compounds used in electrochemical sensors, or even hydrogel sensors, are well known for their reversible swelling process, making them interesting candidates for sensor materials. The mechanisms of expansion and contraction induced by ethanol were studied by Çaykara & Dogmus (2004) and Guenther & Gerlach (2009), demonstrating their effectiveness in using sensors of this nature during their use time.

Understanding the reading time is also necessary to program the choice of a specific period that encompasses all possible readings within the measurement range without compromising the reliability of the sensor since, in practice, seeds from the same lot can have different moisture and damage levels, and even with hydration, due to physiological quality, they can generate different amounts of ethanol during anaerobic respiration (Cavalcante et al., 2019). This highlights the importance of fully stabilizing the MQ-3 sensor.

As mentioned above, most breathalyzers are very close to precision and accuracy and can be used as screening or confirmatory tests for intoxication. However, the results of this equipment are expressed in mg L-1 of blood, which can cause inconsistent results when using it to check the amount of ethanol released by seeds in anaerobiosis because there is a need for sufficient volumes of air for reading.

The results also showed that all evaluated bean samples remained stable until the final evaluation time (618 seconds), as the model used has over 70% confidence represented by R^2, which is acceptable for statistical standards and demonstrates the reliability of the sensor in obtaining results, and can also be applied to other species. Anjum & Khainar (2016) found that after injecting 100 ppm of ethanol, the sensor resistance gradually increases and reaches a constant value that provides the sensor response time. This is similar to what was found and demonstrated in Figures 1, 2, and 3 of this work. The same authors found a response time of 180 s for ethanol, while the recovery time is 700 s. This work found a minimum response time of 13 s and a recovery time of 618 seconds, supporting their findings. The Nano-HAp sensor doped with CNT by Anjum & Khainar (2016) is a nanoparticle sensor and therefore behaves like an electrochemical sensor, which is not seen in sensors made of metallic alloys.

From this perspective, it is highlighted that the development of sensors of this type is significant for many applications due to their advantages, such as reduced size, high stability, sensitivity, and long lifespan. On the other side, current methods of ethanol detection are based on the indirect calculation of ethanol content by oscillating density meters or spectral determination using infrared spectroscopy (Mcginn et al., 2020). However, these systems require a laboratory environment and consume a lot of time and high costs, making them inaccessible for small businesses.

CONCLUSIONS

The average stabilization time of the MQ-3 sensor during ethanol measurements in bean seeds is approximately 24 seconds.

The MQ-3 sensor is efficient, practical, and quickly stabilized in measuring ethanol in bean seeds subjected to hypoxia, presenting promising results compared to conventional breathalyzers.

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