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Original Research

An Airborne Fungal Spore Mass Measurement System Based on Graphene Oxide Coated QCM

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Abstract

This study aims to develop a real-time QCM (quartz crystal microbalance)-based method for a fungal spore mass measurement system as the indoor air pollutants. *Penicillium camemberti* (B2), *Penicillium caseifulvum* (B3), and a mixture of both *P. camemberti* and *P. caseivulfum* (B4) were used as the bioaerosol samples in the form of fungal spores. These samples were collected and cultured inside an isolated chamber and mixed with fresh air (filtered by a filter paper) to generate bioaerosol with a diameter of less than 1 μm (fine particles). These bioaerosols were filtered to produce different particle diameters using a particulate cyclone (a filter paper and a suction pump). The developed system consisted of a GO (graphene oxide, a graphene derrivative)-coated QCM (Q1), a bare QCM (Q2), a crystal oscillator, and a frequency counter to process the output signal, QCM's frequency, and bioaerosol mass. The system shows a good performance with the sensitivity of $27x10^{-2}$ to $29x10^{-2}$ Hz/ng and $23x10^{-2}$ to $29x10^{-2}$ Hz/ng for coated and uncoated sensors, respectively. The best performance is obtained from the coated QCM sensor Q1. The system works well in measuring bioaerosol concentrations with an accuracy of 82% for the coated QCM and 66% for the uncoated QCM. The coated QCM has the potentials of being developed as a fungal spore sensor.

Keywords: fungal spores, graphene oxide, indoor air pollutant, measurement system, quartz crystal microbalance

Introduction

Bioaerosols are the biological constituent of PM (particulate matter). Bioaerosols are produced during many activities in landfills, agricultural sectors, food preservation, and many others in daily life [1, 2]. Bioaerosols can be generated from biomass burning activity, resulting in bioaerosols with a diameter <2.5 μm (fine bioaerosols) [2]. Bioaerosols commonly have many forms like fungal spores, pollen, bacteria, and even viruses. As confirmed before, bioaerosols consist of *Aspergillus*, *Alternaria*, and *Cladosporium* species [4, 5]. Another previous study investigated bioaerosols from bacteria species in landfill areas, such as *Staphylococcus aureus*, *Staphylococcus gordonii*, *Alloiococcus otitis*, *Kocuria rosea*, *Pediococcus pentosaceus*, and many others [1].

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Fungal spores are one common kind of bioaerosol that may adversely affect human health and the environment. Some fungal spores can cause allergic asthma [6, 7]. Another study shows the individual effects of the daily exposure to *Alternaria* and *Cladosporium* species as the biological constituents of PMs to the lung function in schoolchildren [7]. Especially for *Penicillium sp*, this bioaerosol can be found indoors as mold, with different species such as *Penicillium crustosum*, *Penicillium chrysogenum*, and *Penicillium brevicompactum* [8].

Fungal spores concentration can be measured using many techniques. They can be measured using an indirect measurement, such as a real-time polymerase chain reaction [9]. Particle number concentrations of bioaerosols can also be measured using a scanning mobility particle sizer (SMPS) or an optical particle sizer (OPS) [10]. Another study used UV-APS (ultraviolet aerodynamic particle sizer) to measure bacterial aerosols, such as *Staphylococcus epidermis* and *Escherichia coli* [11]. These measurement systems are not portable, are high cost, generally use indirect measurement, and need extra maintenances or specialized training.

A quartz crystal microbalance (QCM) sensor can measure a nanogram-scale change in mass among various measurement methods by recording its frequency shift. This sensor has been widely used due to its real-time performance, high sensitivity, ease of installation, and low cost [12, 13]. Thus far, this sensor has a resonator surface that can be modified as a sensitive layer related to its function. For sensitivity purposes, a QCM sensor needs specific coating material on the surface of its electrode. For example, a previous study fabricated QCM sensors using polystyrene particles coated with transferrin to predict nanoparticle in vivo behavior [14].

Recently, graphene has been studied as a coating material due to its superior electronic conductivity and high thermal stability [15]. The graphene oxide (GO) nanosheet (thin film for coating material) has good hydrophilicity, specific surface areas, and dispersion stability, as GO is the graphene derivatives [12]. GO inherently represents a good mechanical modulus which may cause a small probability of the swelling effect (false crystal frequency response due to over mass) that influences the QCM response [16]. GO has been used as a specific coating material in QCM and utilized for many sensors. GO-coated QCM has a good response in sensing formaldehyde concentration [17]. As a composite, GO/ polydopamine has been developed as a good humidity sensing device due to its ultra-sensitive behavior in water contents [12]. As a gas sensor, GO/TiO_2 composites can be deposited on the QCM's surface [15]. Bioaerosol hazard measurement research is still at an early stage. Since fungal and other bioaerosols have mycotoxins, accurate and sensitive analytical methods are urgently required. These kinds of particulate matter are also easily spread and suspended in ambient air, making them easier to breathe and may increase respiratory health problems. Our study aims to develop a bioaerosol measurement system based on a QCM sensor and to identify the performance of a graphene oxide-coated QCM for a fungal spore sensor.

Materials and Methods

Measurement System

This study used a microcontroller (Atmega 328), self-developed crystal oscillators (maximum crystal input = 16 MHz of frequency f), QCM sensors (uncoated and GO-coated AT-cut QCMs, fundamental frequency = 5.0 MHz), and suction pumps (1.0 m/s of the flowrate) as the main parts of the developed system. According to the preliminary study, the best suction pump flow rate was lower than 2.0 m/s [18]. These parts were integrated inside a polycarbonate prototype box (15 cm (length) x 15 cm (width) x 8 cm (height)). Both QCMs were placed inside a sensor box and connected to the oscillators to drive the signal. The signals from the output pins were connected to the microcontroller to process the output signals (Fig. 1). The microcontroller worked as the frequency counter that counted the given frequency and changed them into bioaerosol mass. The input and output suction pumps were controlled to suck the bioaerosol sample into the sensor box.

Bioaerosol Concentrations

This study used filtered fresh air (B1) and three fungal spores as bioaerosol samples. They were *Penicillium camemberti* (B2), *Penicillium caseifulvum* (B3), and a mixture of both *P. camemberti* and *P. caseivulfum* (B4), which were purchased from a local distributor. The fungal samples were cultivated inside a chamber (20 cm (width) x 30 cm (length) x 20 cm (height)) and cultured on potato dextrose agar (PDA, Merck, 1.10130.0500) using glass Petri dishes (dimension: 9 cm x 1.5 cm) for a week at room temperature. *Penicillium sp.* were chosen for safety purposes, with low mycotoxin productions and the cheese strain, and their existence as indoor air pollution substances [19, 20]. After incubation, the fungal spores were injected into an exposure chamber and mixed with fresh air (filtered air using a HEPA filter, 2.1 m/s of the air flowrate using a suction pump) for 60 seconds) to identify the sensing capability of the developed system [21].

Sensor Preparation

This study used GO-coated (Q1) and uncoated QCM (Q2) sensors (fundamental frequency f_0 = 5 MHz, AT-cut, purchased from PT. Great Microtama Electronics Indonesia). These sensors had a diameter of

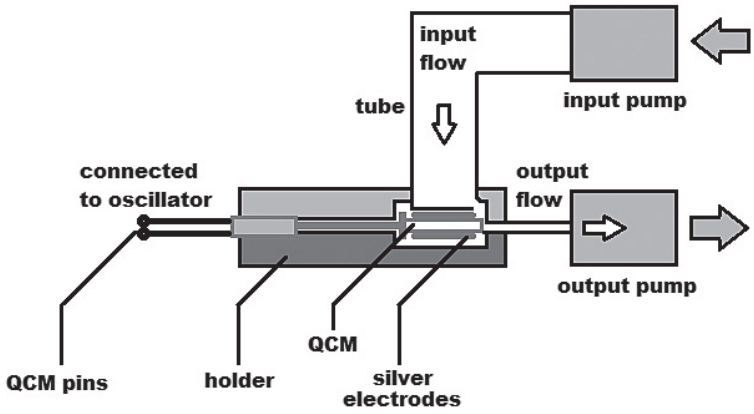

Fig. 1. Schematic diagram of the measurement system.

8.7 mm (silver electrode). All QCM samples were tested before being used and placed in a dust-free box. Each QCM was mounted on a holder made with polylactide and polycarbonate and then connected to a self-made oscillator module [18]. The output signal was then connected to the frequency counter circuit and laptop. The QCM's mountings or holders were also made for the input and output flows and connected to suction pumps (Fig. 1) (modified from Kleo et al. [22]).

Measurement Set-Up

All measurements and treatments were conducted inside an experimental chamber. The fungal spores were introduced to the chamber using a suction pump for 60 seconds (modified from Wardoyo et al. [23]) with a flow rate of 2.0 m/s. A filter paper (Whatman™ filter paper Grade 5) was installed on the pump's outlet to allow bioaerosol diameter ≤1.0 µm to pass through the filter. In line with this, each bioaerosol sample, including B1, was exposed to the chamber. The bioaerosol concentrations were measured by evaluating the developed system's frequency shift (Δf) until the value of Δf was stagnant [18]. The bioaerosol concentrations were also measured using a Kanomax Digital Dust Monitor Model 3443.

System Performances

Sensitivity (S) was evaluated by measuring the frequency shift and varying the bioaerosol samples from B2 to B4 [24]. Since low concentration was not easy to evaluate, the injection time was determined as 110 seconds for a minimum bioaerosol concentration. These tests were conducted at a fixed room temperature at ±25-26ºC. The following equation was used to calculate S:

$$S = \Delta f / m \tag{1}$$

System accuracy was investigated by comparing the results with the Digital Dust Monitor.

Statistical Analysis

The collected data were presented as the mean value and standard error of the means (SEM). Differences between groups were evaluated using the Analysis of Variance (ANOVA) test. All tests were conducted at $p<0.05$ [25].

Results

Bioaerosol Mass Measurement

Fig. 2 shows the bioaerosol concentration measurement results from Q1 as the coated QCM (Fig. 2a) and Q2 (Fig. 2b), the uncoated QCM. According to the results of the Q1 group, B1, B2, and B3 have bioaerosol concentrations of 221±17, 248±15 µg/m^3, and 257±30 µg/m^3, respectively. Meanwhile, no significantly different results can be seen in B4 with the concentration of 266±9 µg/m^3. In Q2, the measurement results show similar concentrations for B1, B2, B3, and B4, resulting in 229±16, 252±16, 257±25, and 263±5 µg/m^3, respectively. Fig. 2 shows that the filtered air has the lowest concentration. These results are obtained consistently for all repetitions.

The bioaerosol masses (m, Table 1) were calculated (Eq. (2)) by multiplying the total flow rate (ΣQ), sampling duration time t, and total concentration per bioaerosol sample (ΣC_t). According to the experiment results, the measurement of the particulate concentrations needed 110 seconds of the total sampling time t to reach the ambient concentration C_0. Besides, the total flow rate is obtained from the measurement device and the suction pump flow rates (44.93 cm^3/ second). As expected, the most exposed bioaerosol mass is referred to as B2-B4. The results show no significant difference between Q1 and Q2 sensor groups for all samples (p = 0.36). These values are then used to compare the calibrated measurement device and the developed system to get the accuracy level.

$$m = \Sigma Q \,.\, \Sigma C_t \,.\, t \tag{2}$$

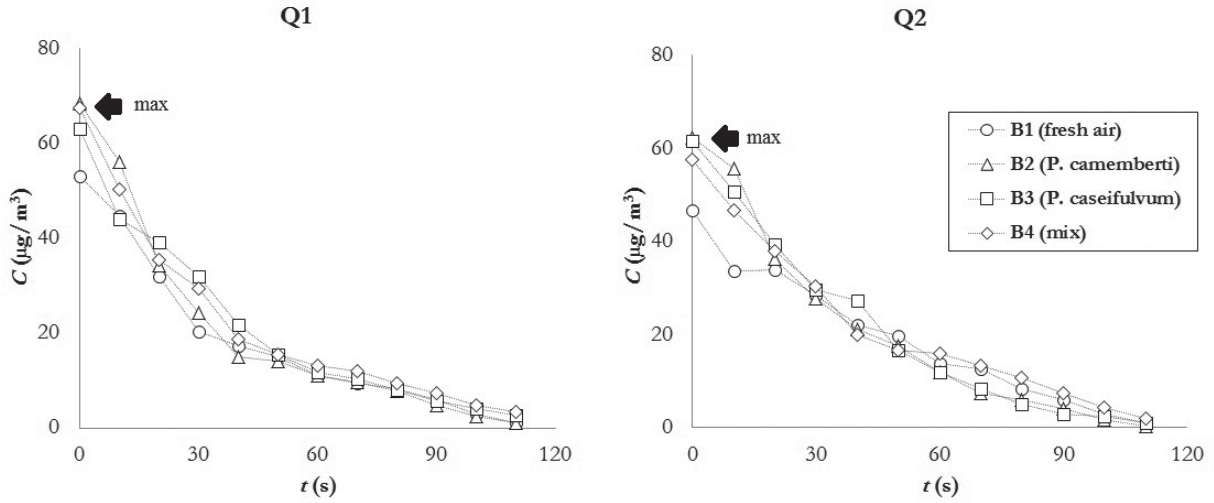

Fig. 2. Measurement results of bioaerosol concentrations (*C*) of all samples (B1-B4) with a diameter ≤ 1 µm (mean): Q1 (GO-coated QCM) and Q2 (uncoated QCM).

Table 1. Exposed sample masses were obtained from Q1 (GO-coated QCM) and Q2 (uncoated QCM).

QCMs	*Mass (x10^{-4} ng)*			
	B1 (fresh air)	B2 (*P. camemberti*)	B3 (*P. caseifulvum*)	B4 (mix)
Q1	11±1	12±1	13±2	13±0
Q2	11±1	12±1	13±1	13±0

System Outputs

Fig. 3 shows that Q1 has better adsorption-desorption reproducibilities than Q2. The frequency recovers to the initial baseline (f_0). The frequency shifts Δf increase with the increasing bioaerosols concentration. According to Fig. 3, the highest Δf is referred to as Q1. The most Δf is detected on B2, B3, and B4 for Q1. The average peak Δf of Q1-B2, Q1-B3, and Q1-B4 are 423, 430, and 447 Hz, respectively. Q1-B1, which was exposed to the filtered fresh air, has a smaller Δf (337 Hz) than other Q1 groups exposed to bioaerosol samples. Similar treatments were also applied in Q2. Q2-B1 has only 187 Hz of Δf, which is smaller than Q1-B1. This result indicates that the coated QCM sensor has a better response than the uncoated one.

System Performances

According to the equation below, Δf is influenced by the deposited bioaerosol sample mass (m, g) on the sensor's surface. A is the electrode surface area (0.196 cm^2), while μ_Q and ρ_Q are the shear modulus (2.947x10^{11} g/cm s^2) and the density of the sensor (2.684 g/cm^3), respectively.

$$\Delta m = [\, A \,.\, \Delta f \,.\, (\rho_q \,.\, \mu_q)^{1/2} \,] \,/\, [\, 2 f_0^2 \,] \qquad (3)$$

The correlation between Δm and Δf is valid only if $\Delta f << f_0$. The calculated masses on Q1 for B1, B2, B3, and B4 are (12±1)x10^{-4}; (15±1)x10^{-4}; (15±0)x10^{-4}; and (16±0)x10^{-4} ng, respectively. Q2-B1 and Q2-B2 generate (7±3)x10^{-4} and (9±0)x10^{-4} ng. No significant results are found at Q2 using B3 and B4, generating (9±1)x10^{-4}; and (8±0)x10^{-4} ng of the deposited masses. There are more deposited masses on the surface of the coated QCM. Although the deposited masses are varied, there is no significant difference in the sensitivity between Q1 and Q2 (p = 0.43). According to Eq. (1), the sensitivities are varied from 23x10^{-2} to 29x10^{-2} Hz/ng.

Furthermore, this study also approaches the accuracy of the developed system by comparing the results with the calibrated measurement device. Compared to the Digital Dust Monitor, the developed system has 82% and 66% accuracy for the coated and uncoated sensors, respectively. These values interpret that the developed system has an accuracy of 82% to measure bioaerosol concentrations when using Q1, with the mean sensitivity of (28±1)x10^{-2} Hz/ng. On the other hand, Q2 gives a lower accuracy, 66%, than Q1. Q2 also has a lower sensitivity ((27±3)x10^{-2} Hz/ng) than Q1.

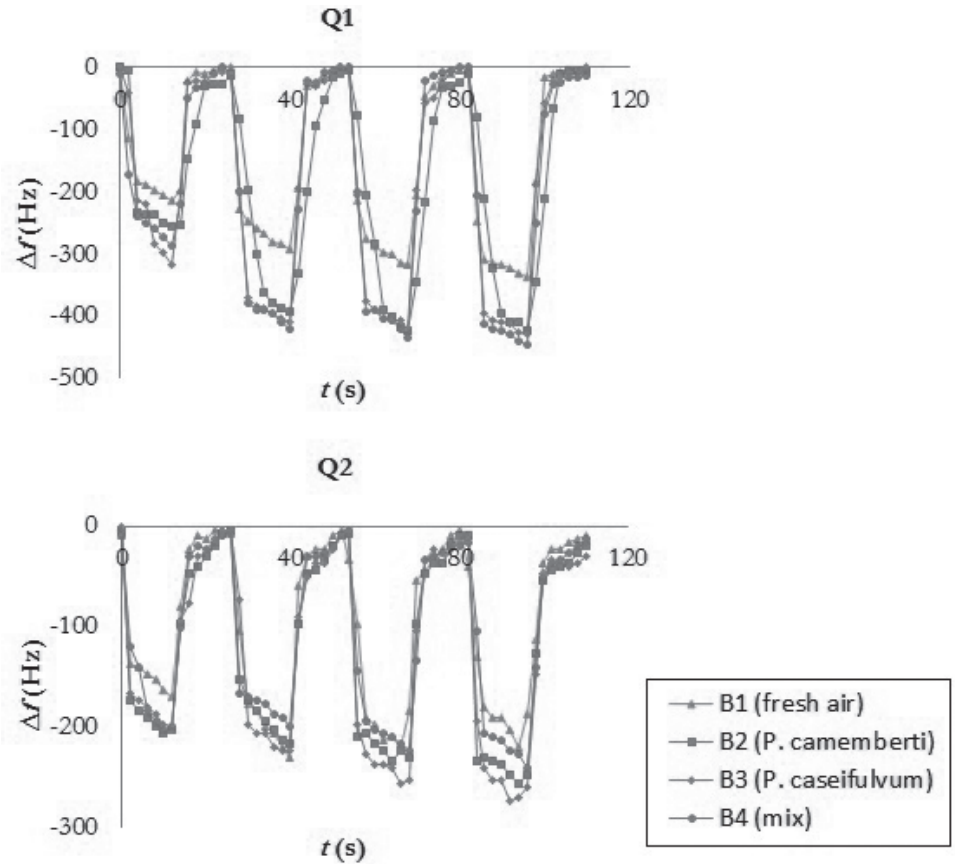

Fig. 3. Output response curves (Δf) of: Q1 (GO-coated QCM) and Q2 (uncoated QCM).

Discussion

A QCM can be used as a well-established biosensor, as developed by a previous study to detect vaccinia virus DNA [22]. In this section, the use of nanoparticles or another surface modification method in material sciences proved to be a good sensing ability technique, whether using electrochemical, magnetic, or piezoelectric sensors. The system's selectivity, sensitivity, feasibility, and response time are influenced by many factors, such as the coating material, layer, deposition technique, and many others [12, 25-27]. This study uses the system to measure the bioaerosol concentrations with various biosamples. The use of different QCMs is to identify the performance of the coated-QCM compared to the uncoated one. The results are obtained from the estimation of change in the resonance frequency of QCMs under different exposures to bioaerosol samples (B2-B4). The decrease in f (represented by Δf) is considered proportional to the deposited substances (bioaerosols, represented by Δm) on QCM's surface [12, 26, 27]. Thus, there will be a different result between coated and uncoated-QCMs.

As an alternative method in sensing particulate matters, whether bio or non-bio aerosols, the uses of GO as the coating material for the QCMs are investigated in this study. The specific GO coating was chosen because its large surface area might cause more volatile or aromatic substances of the samples to be adsorbed on these active sites [15]. GO has rich hydrophilic functional groups (carboxyl, hydroxyl, and epoxy), which may increase the active and specific surface area [12]. For this reason, the swelling effect and the accumulation of the samples on the QCM's surface become important points. Fortunately, GO inherently represents a good mechanical modulus and a high hydrophilic surface area, which may cause a small probability of the swelling effect [16].

Moreover, GO has an antibacterial substance that may influence the GO-coated QCM. As well explained in a previous study, the interaction between chitosan chloride-GO composites and gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus* was investigated [29]. In this study, the used materials generated antibacterial properties that were inactive. GO can potentially be an anti-fungi and anti-bacteria when combined with phosphoramide as a nanocomposite [30]. The interaction of this mechanism may exist in aromatic substances and epoxy groups of GO and cause plasma membrane damage and cell death [30]. GO may cause extreme damage to fungal cell walls and destroy cellular organelles [31]. As expected, these previous studies support the results of this study, resulting in a better performance of a GO-coated QCM than the uncoated one. As seen in the results, the highest frequency shift

is obtained at Q1. The value of Δf in Q1 is higher than the value in Q2 for all bioaerosol samples.

In addition to bioaerosols detection, GO has also been developed for other applications. As a composite, GO (with polydopamine) has been developed as a good humidity sensing device due to its ultra-sensitive behavior in water contents [12]. Another study also uses GO for the QCM coating materials and develops it as a humidity sensor [16]. As found in a previous study, GO/TiO_2 composites are deposited on the QCM's surface as a gas sensor [15]. In this study, GO alone gives a good sensitivity for gases and vapors detection compared to TiO_2.

As reviewed before, QCMs are proven application-oriented sensors that can detect a wide range of bioaerosols [13]. Similarly, GO-coated QCM has a better performance than the uncoated QCM in sample detection. The GO-coated QCM has a better mass change, resulting in more Δf than the uncoated one. Our results are supported by many previous studies that the uncoated QCM cannot bind hydrophilic molecules or other substances due to its hydrophobicity [32]. For this reason, the silver surface must be modified with a hydrophilic structure to optimize the crystal. As expected, the GO-coated QCM has better accuracy (82%) than the uncoated one (66%) for fungal spore sensing.

Apart from these two parameters, data on the system performance and dissipation energy factor can become additional attractions of QCM. Our system results can be used as a preliminary study to develop a better GO treatment. There is a limitation like the coating material used in our developed system. The sensors need to be optimized under many further treatments. There are also limitations like sensitivity and selectivity limits with other substances, compatibility in differing each bioaerosol sample, and response time.

Conclusions

This study develops a fungal spore mass measurement system based on graphene oxide coated QCM. The developed system shows a good performance with $27x10^{-2}$ to $29x10^{-2}$ Hz/ng sensitivity and $23x10^{-2}$ to $29x10^{-2}$ Hz/ng for coated and uncoated sensors, respectively. The best performance is obtained from the GO-coated QCM sensor. The system works well in measuring fungal spore mass with an accuracy of 82% (GO coated QCM) and 66% (uncoated QCM). The coated QCM has the prospects of being developed as a fungal spore sensor.

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Conflict of Interest

The authors declare no conflict of interest.

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