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Small Optical Measurement System for Stable Detection of Mold Growth

Varuni Rathnayake [1,2], Thomas Schweizer[1,2], Sander van den Driesche [1,2,3], and Michael J. Vellekoop[1,2,3]
[1] Institute for Microsensors, -actuators and -systems, University of Bremen, Germany
[2]Microsystems Center Bremen (MCB), Germany
[3]MAPEX Center for Materials and Processes, University of Bremen, Germany
vrathnayake@imsas.uni-bremen.de

Summary: A small, stable sensor system is proposed to detect mold growth. The system measures the pH change in culture medium using the corresponding colour shift of pH indicator dyes in response to mold metabolism. The system employs a spectral sensor and a LED that measures the transmittance across 150 μl of culture media. The configuration of the optical system assures the accuracy of the measurements with a fixed optical path with no mold colony hindrance. A calibration method was introduced to calibrate small volumes of dyed culture medium.The system allows the detection of mold growth and identification of growth phases of mold measuring the pH value of the culture medium.

Keywords: mold, Bromocresol purple(BCP), agar, pH value, colour sensor

Background, Motivation and Objective

Mold contamination is a significant issue in agriculture, biogas facilities, industrial plants, and indoor environments. It can break down organic matter, can lead to structural damage in buildings and can result in crop losses. Additionally, mold spores have been shown to impair air quality, causing respiratory diseases such as asthma and allergies in both humans and animals. Conventional methods for detecting mold involve using Andersen impactors with agar-filled petri dishes and counting colony-forming units (CFUs). This is laborious process that requires several steps. Other methods, such as PCR, qPCR, and microscopic analysis, require experienced professionals. Colourimetric measurement methods have been previously investigated and shown to be effective for mold detection [1]. However, mold growth on the surface of agar plates disturb the optical pathway which leads to inaccurate measurement results.

Mold Detection System and Characterization

In this work a stable optical measurement setup was realized to detect the growth of mold. An optical path was chosen such that it does not cross the surface of agar where mold colonies are growing, which would otherwise affect the transmittance measurements [1]. Mold spores start germinating in environments, which are humid and have sufficient organic matter. Agar mixed with pH indicator dye is used as growth medium in the micro reactors to determine mold growth. When spores are in contact with Agar, they start germinating and feed on the organic matter. As a function of metabolism, when mold grows, they release acidic and basic waste [2]. These waste products changes the ionic composition of the culture medium and thus changing the pH of agar, which can be detected by pH indicator dyes. Spores can be introduced into the system by air or liquid samples. To prevent the agar from drying out caps are fitted onto the microreactors.

The system consists of a cylindrical microreactor that has a volume of 400 μl (diameter: 8mm, height: 8mm). It is filled with agar mixed with Bromocresol purple(BCP) dye with a concentration of 0.025% w/v. The measured dye colours for different pH values are shown in Fig. 1.

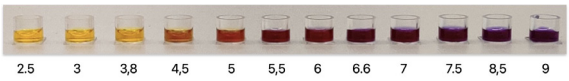

Fig. 1: Colour in BCP dyed Agar for different pH values

The optical setup to measure the colour intensity through a small optical path of 8 mm consist of a 3000K LED and a spectral colour sensor (AS7341) that were configured in such a way that light passes through the microreactor filled with agar. The LED was powered with 20 mA current to illuminate the microreactor. The colour sensor has 8 channels sensitive to different wavelengths in visible range and provides intensity data for each wavelength.

To measure the pH of the agar medium accurately, a calibration curve for BCP dyed agar is necessary. One significant challenge when measuring the pH of (semisolid) textured agar with a standard pH probe is that the sensitive membrane gets obstructed by agar particles resulting in inaccurate readings. Also, changing pH and measuring pH of small volumes of Agar is not straight forward. To address the above challenges, a liquid fraction with BCP dye is used. As the first step, a calibration curve of BCP dyed

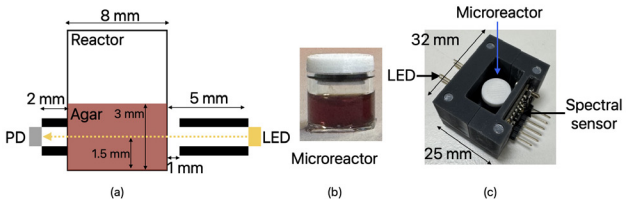

Fig. 2: (a) Optical setup (b) Microreactor (c) Resin printed holder for LED and color sensor

water for a pH range 2.5-9 was established as the first reference. By adding a liquid fraction with multiple samples covering a pH range of 0-14 on top of agar gel, the pH value of the liquid and the agar fractions reach equilibrium. Then by comparing the colour of the liquid fraction on top of the agar, the pH value of the two fractions can be read out using the first reference curve. A second reference curve can then be created for the agar fraction itself. This will be used further as the calibration curve to measure the pH of the medium when mold is growing. To minimize the possible drifts due to temperature fluctuations and varying LED properties, each wavelength was normalized using 680nm wavelength because it is not affected by the change in colour of the dye.

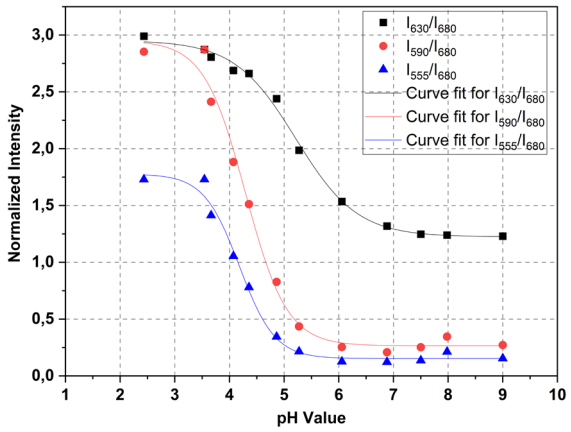

Fig. 3: Calibration curve for 0.025% w/v BCP dyed Agar, including sigmoidal Boltzmann curvefit

Fig. 3 outlines the correlation between pH values and the intensity ratios at three distinct wavelengths: 555 nm, 590 nm, and 630 nm, all normalized to the intensities at 680 nm. The I_{630}/I_{680} (black curve) is suitable for pH values ranging from 4 to 7. In contrast, the I_{590}/I_{680} (red curve) is effective for detecting pH values between 3 and 5.5. The normalized intensity at 555 nm (blue curve) does not offer any additional information compared to the other wavelengths. Therefore, the system can effectively operate along a pH range of 3 to 7 using only 3 wavelengths: 590 nm, 630 nm and 680 nm(reference).

Results

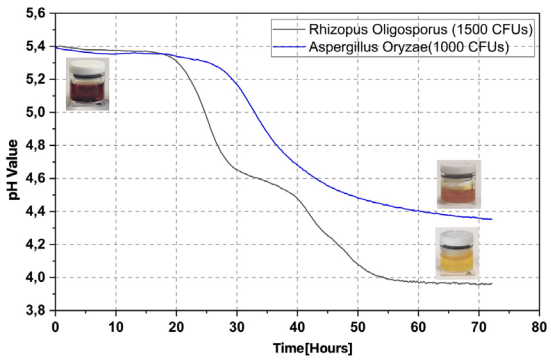

Fig. 4: pH change in the culture medium for 2 different species of mold

Fig. 4 shows the change in pH of the culture medium for two types of mold: *Rhizopus Oligosporus* and *Aspergillus Oryzae*. For the measurements, liquid samples with different spore concentrations were used for each species. The intensity measurements were recorded with a 10 minute sampling time. Initial pH of agar was determined as 5.4±0.2. During the lag phase, the pH value of the medium remains unchanged as the spores adjust to the moist medium. Following this phase, mold spores start growing and release waste into the agar, altering its pH. A pH change of -0.5 is shown within 25 hours for *R. Oligosporus*. For *A. Oryzae* spores, it takes a bit longer and is observed after 35 hours. Both species demonstrate a gradual decrease in pH over time, indicating that the medium becomes more acidic.

Conclusion

The growth of two mold species has been successfully detected using stable optical readings with 150 μl volume of culture medium. Intensity ratio measurements from 2 wavelengths and a reference wavelength enable the system to detect pH values in the range of 3 to 7. The results are very promising for the realization of a mold spore quantification platform.

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