CORPUS PUBLISHERS

Journal of Mineral and Material Science (JMMS)

ISSN: 2833-3616

Volume 4 Issue 4, 2023

Article Information

Received date : September 30, 2023

Published date: October 16, 2023

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DOI: 10.54026/JMMS/1069

Key Words

Micro-Oxymax Instrument; Calibration; Exponential Growth; Biochemical Reaction; Concentration

Short Communication

Effect of Nutrient on Oxygen Concentration and Consumption Rate by Soil Microbials Measured Using ER-10 Electrochemical Oxygen Sensor

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Summary of Tests Results

To stimulate microbial growth in the soil to illustrate their effects on the oxygen O_2 concentration and consumption rate, a test measurement was conducted in a sealed test bottle (350 ml) containing 100 g of sandy soil material from the large-scale column (Mesocosm) [1,2], to which 2 g of sugar (nutrient) and 20 ml of water were added. This test was designed specifically because O_2 and CO_2 gas analyses and their associated stable isotope analyses clearly showed that the pore-gas chemistries of the sand material from the Mesocosm were controlled by the oxidation of organic carbon present in the Mesocosm. The O_2 concentration and consumption rate were measured using the ER-10 respirometer. The ER-10 is a computerized apparatus for measuring very low levels of gaseous O_2 uptake (Figure 1). An IBM-PC-compatible computer maintains and displays the operation of the Micro-Oxymax instrument.

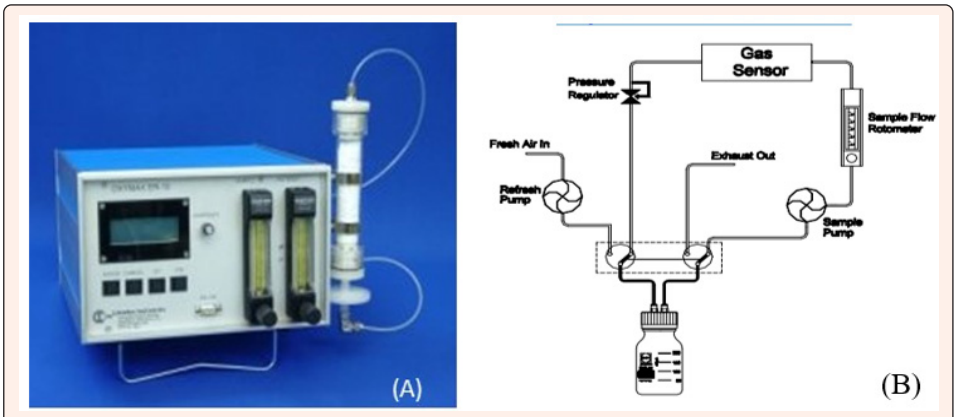

Figure 1: (A) Photo of ER10 and (B) simplified block diagram of the gas through ER-10 gas respirometer. (Micro Oxymax Instruction Manual)

The sample reactor consists of a bottle, lid and seal assembly. The standard lid assembly contains fittings that connect the sample tubing to the system sample pump through "Quick Connect" type fitting. The fitting itself contains a small O'ring that rests against the tubing as it is inserted into the fitting to ensure a proper seal. The air going to the chamber is supplied at the tubing fitting on the rear of the cabinet. After the volume is measured the system leakage can be measured as a double check that everything is connected properly. An Excessive leakage is a leak that is larger than +/- 0.3ml/min. Calibration of O_2 gas analyzer is performed automatically at specific time interval with air and consisted of drawing air into the sensor and adjusting the oxygen sensor to 20.93 %. The maximum value of concentration of oxygen the sensor could measure was 21.47% (factory setting). The ER-10 Respirometer was set to automatically calibrate the gas sensor at the beginning of each experiment. Before starting the measurement, the system needs only the time interval between samples to be specified and the chamber volume, which is computed automatically during system calibration. When the experiment starts, the software assumes control of information and storage of results acquisition and/or presentation to the printer. Measurements are performed in a closed-loop fashion whereby the sampled gas is returned to the chamber. The measurement principle involves air sampling from the chamber's headspace, circulating it through the gas analyzer, and returning it to the chamber without any contact with the sample. Samples are continuously aerated with adjustable airflow (100 ml/min. to 1,500 ml/min.), except for the short time interval when the gas analyzer measures a particular sample.

The continuous measurements of headspace temperature, O_2 consumption rate, and O_2 concentration for a test period extending to 4000 min (56 h), are presented in Figures 2 and 3. Measured data show that the temperature (plot a) in the measuring bottle rose then dropped sharply periodically by 2 °C from 22 to 24 °C at equally spaced time differences. The periodic change in temperature was due to the temperature fluctuation in the room during the three days' test period that started at 12:45:07 (time 0 of day 1) on June 13, 2001. The first temperature rise occurred at 17:45 h on day 1. The second rise occurred after 48 h of the test period (around 17:45 h) on day 2, and finally, the third rise in temperature occurred after 72 h of the test period (around 17:45 h) on day 3 of the test period. The pressure remained constant throughout the test period (results not shown here). Data in Plot (b), and Plot (c) of Figures 2 and 3, respectively, can be viewed as three stages of measurements: (1) the Lag Phase extending from time 0 up to 1800 min (30 h) of measurements (Plot (b)), a period when cells may be metabolizing but are not yet growing after addition of nutrient (2) the Exponential Growth stage extending from 1800 to 2260 min (8 h) where the cell number doubles within this period and (3) the Stationary Phase, the period immediately

following Exponential Growth when the growth rate of the population falls to zero (from 2260 to 4000 min). Data show that during the Lag Phase, the temperature change affected the O_2 concentration to a greater degree than the consumption rate (Figures 2 (Plot b) and 3 (Plot c)). For example, the sharp drop in temperature by 2 °C caused a sharp drop of 0.06% in O_2 concentration. Moreover, it is well established that the O_2 concentration increases or decreases with increasing or decreasing temperature.

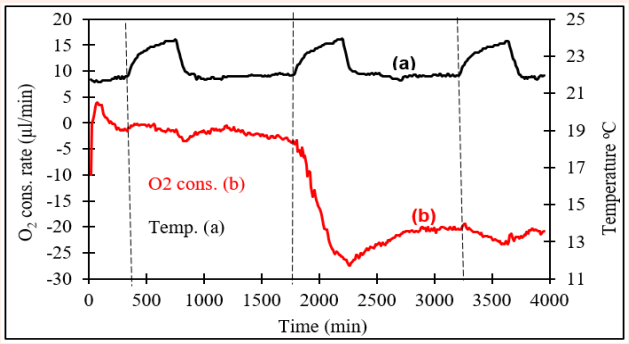

Figure 2: Measured (a) temperature and (b) O_2 consumption rate measured using the ER-10 respirometer from a measuring bottle containing soil mixed with water and sugar.

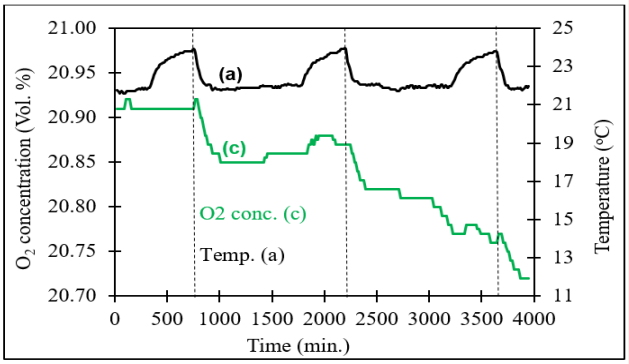

Figure 3: Measured (a) temperature and (c) O_2 concentration using the ER-10 respirometer from a measuring bottle containing soil mixed with water and sugar..

The average consumption rate during the Lag Phase was -2.10 µl min^{-1} (std. dev. = 0.026 µl min^{-1}) (Plot b), most of it represents the quantity of O_2 consumed by the sensor in making O_2 measurement. The typical factory setting value is 3.9 µl/min. and it is an indication that the apparatus was operating correctly. These early measurements indicate that it took more than 1800 min (30 h) for the microbial cells to begin growth in number during this study. Measurements data in Plot (b) of Figure 2 show a sharp increase in O_2 consumption rate from -2.10 µl min^{-1} to about -27 µl min^{-1}. This change occurred just past 1800 min and lasted over 500 min. (8.3 h) of measurements period. Results show that the consumption rate changed by a factor of 12.9. At the same time, the O_2 concentration in the headspace started decreasing gradually at a relatively faster rate than that of the Lag Phase (Figure 3 Plot (c)). The rapid O_2 consumption and concentration changes signalled the onset of the Exponential Growth stage. However, the time required for a complete growth cycle in bacteria is highly variable and depends on several nutritional and genetic factors. Under the best nutritional conditions, the bacterium *Escherichia coli* can complete the cycle in about 20 min [3]; a few bacteria can grow even faster than this, but many grow much slower. Other major environmental factors influencing microbial growth are temperature, pH, water potential, and O_2. In this batch experiment, the exponential growth could not occur indefinitely. What generally happens is that either an essential nutrient (i.e., sugar) is used up or some waste product of the organism builds up to an inhibitory level, and exponential growth ceases. The microbial population has reached the Stationary Phase. As seen in this study, there was no net increase or decrease in cell numbers in this Phase. However, although no growth occurred in this Stationary Phase, the cell functions continued, including energy metabolism and some biosynthetic processes. The subsequent effects of the periodic change in temperature during the Exponential and Stationary Phases can be seen on both the O_2 concentration and consumption rate plots. The small temperature variations also reflect the small variations in the O_2 consumption rate.

In summary, these measurements show that this biochemical reaction is time-dependent to a larger degree and temperature-dependent to a lesser degree [4]. When a microbial population is inoculated into a fresh medium, growth does not begin immediately but only after a period of time called the Lag Phase, which may be brief or extended depending on the history of the culture and growth conditions. The rate of O_2 consumption (exponential growth) is influenced by environmental conditions (temperature, composition of the culture medium) as well as by genetic characteristics of the organism itself. The population has reached the Station Phase generally when either an essential nutrient of the culture medium is used up in the medium to an inhibitory level and exponential growth ceases. These experiments illustrate the capability of continuous measurements, the accuracy, and the resolution of the ER-10 Respirometer.

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