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Original article

Monitoring of microbial activity in soil using biological oxygen demand measurement and indirect impedancemetry

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Abstract

This paper describes a technique that allows one to monitor simultaneously oxygen consumption and carbon dioxide production in order to better characterize microbial activity in soil. The experimental methodology is based on biodegradation tests in biometric flasks filled with soil and equipped with a CO_2 trap (KOH solution) and OxiTop® measuring heads (used for Biological Oxygen Demand [BOD] determination). CO_2 was measured using both indirect impedancemetry and acid/base titration. First, results showed that the concentration of the KOH solution concentration is a key design parameter for the sensitivity of the method. Second, respiratory quotient was calculated during the biodegradation tests in microcosms. Biomass evolution was also monitored to study the possible correlation between the respiratory quotient and biodegradation phases. As a conclusion, it is stated that on-line monitoring of the respiratory quotient can provide relevant information concerning *in situ* soil microbial activity.

Keywords: Microbial activity; Oxygen consumption; Carbon dioxide production; Indirect impedancemetry; Respiratory quotient

1. Introduction

Bioremediation is a well-recognized method for the treatment of contaminated soil [1]. However, bioprocesses are often operated under sub-optimal conditions due to the difficulty of identifying on-line the limiting parameters to biodegradation. In particular, oxygen and carbon dioxide concentrations in the gas phase are the two only on-line measurements that provide qualitative information about the microbial activity, and indirectly about the contaminant biodegradation activity. Respiratory quotient, which is the molar ratio of carbon dioxide production to oxygen consumption, can display variations depending on composition of the examined microbial community as well as their available growth substrates [2]. Therefore, respiratory quotient could provide a valuable tool for a qualitative evaluation of microbial activity during bioremediation processes. For an on-line determination of the respiratory quotient, simultaneous and accurate measurements of oxygen and carbon dioxide evolutions are needed. Oxygen consumption in microcosms is usually determined by monitoring the air pressure after trapping the produced carbon dioxide into a strong base solution. This method is known as the Biological Oxygen Demand (BOD) test [3]. It is more difficult to monitor on-line carbon dioxide in the same microcosm and only few methods are reported in the literature. Because carbon dioxide is trapped into a base solution, direct measurement in the gas phase (as infra-red

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analysis and gas chromatography) cannot be used. A study suggested the measurement of pH change within the base solution resulting from CO_2 absorption [4]. This method may be accurate but pH sensors are prone to drift and require several calibrations during a single biodegradation test. Finally, carbon dioxide trapped in the base solution can be monitored by measuring the variation of the electrical conductivity. The chemical reactions corresponding to CO_2 absorption by the base solution can be represented by the following equations:

$$CO_2(g) + H_2O(l) \rightarrow H_2CO_3^-(aq)$$

$$H_2CO_3^-(aq) + 2OH^-(aq) \rightarrow CO_3^{2-}(aq) + 2H_2O(l)$$

As the conductivities of hydroxide ions (OH^-) and carbonates (CO_3^{2-}) are significantly different, the amount of CO_2 absorbed by the base solution can be deduced from the conductance measured in the solution and the stoichiometric coefficients of the above equations. This method, also called indirect impedancemetry, was tested by Govind et al. [4] using a barium hydroxide solution as the CO_2 trap and was applied to aqueous and soil slurry media. Strotmann et al. [5] evaluated this technique, for the determination of the biodegradability of substances, by following CO_2 evolution during the degradation of aniline in activated sludge. Indirect impedancemetry was also used to monitor low production of CO_2 in agro-food industries [6].

The aim of this study is to investigate the possibility of a correlation between respiratory quotient and microbial activity during biodegradation in soil, and to evaluate the reliability of indirect impedancemetry as a tool for quantifying CO_2 production, and, combined with O_2 consumption for characterizing *in situ* soil microbial activity. Diuron was used as contaminant in this study. This is a phenylurea herbicide considered as a Priority Hazardous Substance [7]. Due to its high persistence, diuron can be found in many environments such as soil, sediments and water. Microcosms equipped with BOD sensors and base solutions were contaminated with diuron.

The paper is organized as follows. Biodegradation tests in microcosms and analysis techniques are first described. Then, in Section 3, the choice of KOH solution is discussed, followed by an evaluation of indirect impedancemetry as a measuring technique for CO_2 evolution in comparison to acid/base titration, a well-recognized approach to determine CO_2 production [8]. Finally, respiratory quotient was calculated and its evolution compared to biomass analysis. The importance of this study is outlined in the conclusion.

2. Materials and methods

2.1. Microcosms preparation

Compost soil (Soldor, France) was used in this study. It was characterized by a dry weight of 46% (w/w), water-holding capacity of 720 ml L^{-1}, and a pH of 6.5. The biodegradation test was performed in 500 ml bottles containing 25 g of non-contaminated homogenized soil. The soil was artificially contaminated with diuron $C_9H_{10}Cl_2N_2O$ (Sigma) dissolved in acetone at 500 mg kg^{-1} of soil. The bottles were manually shaken and acetone allowed to evaporate. This contamination procedure was adapted from Widehem et al. [9] and the removal of acetone from soil was verified by weight loss test in control microcosms. A tube filled with 10 ml KOH (0.1 N) was placed in each microcosm. Bottles were closed with BOD measuring heads (OxyTop®-C controlled by the OxiTop® OC110 system, WTW, Weilheim, Germany). Non-contaminated and abiotic microcosms were used as controls. Abiotic microcosms were prepared by adding 1% of sodium azide (NaN_3). All microcosms were incubated at 20 °C for the entire experiment. The water content of soil was constant in all microcosms for the duration of the measurements. KOH was removed and replaced regularly in all bottles.

2.2. O_2 and CO_2 analysis

Oxygen consumption was measured with BOD heads based on pressure drop in the bottles following CO_2 trapping by KOH solution.

Removed KOH solution was analyzed by two methods: indirect impedancemetry and acid/base titration, to monitor CO_2 production. A volume of 5 ml of KOH was used to measure its conductivity with a WTW inoLab measuring instrument. The other 5 ml, was mixed with 3 ml of 1 N barium chloride solution ($BaCl_2 \cdot 2H_2O$) to precipitate carbonates, and titrated with standardized 0.1 N hydrochloric acid (Chem-Lab).

2.3. Biomass analysis

Biomass evolution was monitored by sacrificing regularly a contaminated microcosm. CFU (colony forming units) counts were used to monitor diuron degraders and total population. Microorganisms were extracted from soil matrix by adding 28.5 ml of sterile physiological water (0.85% NaCl) to 3 g of soil. The mixture was shaken vigorously on a vortex for 10 min followed by a centrifugation at $1400 \times g$ for 1 min to precipitate soil particles. Different dilutions were prepared from