Development of a microbial indicator to monitor soil bioremediation: the respiratory quotient

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Introduction

Bioremediation is a well-recognized method for the treatment of contaminated soil [Aspray et al.]. However, bioprocesses are often operated under sub-optimal conditions due to the difficulty of identifying on-line the limiting parameters to biodegradation. 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 [Dilly et al.]. 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. CO2 production is determined by acid/base titration of this solution. 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 impedancemetry as a tool for quantifying CO2 production, and, combined with O2 consumption for characterizing in situ soil microbial activity. RQ evolution is monitored to investigate (1) the effect of the water content; and (2) the effect of nutrient amendments. Hexadecane (C16H34) was used as a characterized aliphatic hydrocarbon contaminant.

Material and Methods

Soil samples were collected from a natural field of Hanoi suburb.

The first, gas activities and residual hexadecane concentrations were monitored for soil microcosms’ experiments at five gravimetric water contents: 15, 20, 25, 30 and 35% (w/w). Each microcosm was constituted by a bottle of 500 mL containing 50 g of soil. Hexadecane (5.8 mg/g-dry soil) was added to each bottle. The C:N:P ratio of 100:10:1 was adjusted by adding external N and P sources in the form of (NH4)2SO4 and KH2PO4.

The second, gas activities and residual hexadecane concentrations were monitored for soil microcosms’ experiments at three conditions of nutrient: (1) hexadecane (called sample “H”); (2) hexadecane + (NH4)2SO4 (called sample “HN”) and; (3) hexadecane + (NH4)2SO4 and KH2PO4 (called sample “HNP”).

A tube filled with 10 mL of 0.5 M KOH solution, placed into each bottle, was used as alkaline trap to fix CO2. KOH solution was removed from the tubes and renewed daily in all microcosms. CO2 production was determined by acid/base titration of this solution. O2 uptake was determined daily by manometric measurement using OxiTop system. Identical microcosms were sacrificed at day 0, 2, 5, 8, 10 and 14 to monitor residual hexadecane concentration. All microcosms were incubated at 20 °C for all experiments.

Results and Conclusions

Hydrocarbon degradation in all microcosm experiments followed a 3-step pattern (Fig.1 and Fig.2). The first phase (from 0 to 2 days) may correspond to the lag phase when the indigenous microbial population adapts to the source of hexadecane. The hexadecane depletion in this phase was minimal. During the second “exponential” phase (from 2nd to 5th day), a higher hexadecane depletion was observed. It corresponds to the maximum growth of the biomass. During the last phase (after 5th day), the quantity of hexadecane remained stable. Hexadecane degradation was correlated to microbial activity, expressed as CO2 production, for all microcosms tested (Fig.1 and Fig.2). Figure 1 showed that the soil water content seems to strongly affect hexadecane degradation. The highest hexadecane depletion was obtained at 20% of water content and the lowest at 35%. Figure 2 showed the important role of simultaneous presence of N and P on hexadecane biodegradation in soil.

  
**Figure 1:** Hexadecane degradation and gas activities at five water contents after 14 days: (a) residual hexadecane concentration; and (b) cumulative carbon dioxide production



**Figure 2:** Hexadecane degradation and gas activities at three conditions of nutrient after 14 days: (a) residual hexadecane concentration; and (b) cumulative carbon dioxide production

 



**Figure 3:** Evolution of the RQ values during hexadecane degradation at all five water contents (a) and at three conditions of nutrient: H (b), HN (c), and HNP (d)

The respiratory quotient (RQ), defined as the ratio of molar CO2 production to molar O2 uptake, is an integrative parameter that characterizes the respiration activity. Its temporal evolution is presented at Fig. 3 for all water contents and all conditions of nutrient tested. For all the contaminated microcosms, a similar RQ profile was observed (Fig. 3). Initials RQ values ranged from 0.86 to 1.96. They increased from 1.2 to 2.4 during the first day of incubation. A decrease of RQ values was then observed between day 2 and day 4. Finally, RQ values increased until the day 5, before decreasing slowly until the end of the experiment.

A clear relationship was observed between RQ evolution, microbial activity and contaminant depletion. The RQ may be an indicator for easily biodegradable carbon sources in the soil and presents a relevant, quick and easy determinable indicator for the efficiency of bioremediation. In addition, RQ is highly influenced by environmental factors, such as soil water content, nutrient amended. Thus, pre-conditioning and standardization of the soil before measuring RQ is necessary to minimize the effect of these variables.

References

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