



## The $^{13}\text{C}/^{12}\text{C}$ fractionation by microbial cells immobilized on a solid-phase carrier during the growth on glucose

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**Problem.** In microbiological ecology, the level of basal  $^{12}\text{CO}_2$  respiration and the potential of microbial activity defined as substrate-induced respiration (SIR) are used as criteria of the metabolic state of soil microbiota. The peculiar feature of glucose metabolism in soil is its utilization by microbial cells immobilized on soil particles as a solid-phase carrier. The efficiency of substrate utilization and  $^{12}\text{CO}_2$  production in such cases depend on the rate of microorganisms' growth and colonization of the solid-phase carrier surface, where the substrate is located. The products of microbial metabolism are supposed to inherit the substrate isotope composition correct to the isotopic effects accompanying substrate utilization and metabolic transformations. However, all experiments in carbon isotope fractionation during microbial utilization of glucose as a substrate have been carried out with microorganisms growing in liquid media.

**Objective:** Study of the kinetics of glucose utilization as a test substrate during the growth of soil microorganisms immobilized on a solid-phase carrier and ascertainment of peculiarities of the formation of carbon isotope composition of produced metabolic  $^{12}\text{CO}_2$ .

The objects of research were *Pseudomonas aureofaciens* BS1393(pBS216) (culture A) and *Rhodococcus* sp. 3-30 (culture B) as representatives of pseudomonades and rhodococci, which occur in the soils of different genesis and are of defining value in development and implementation of biotechnological schemes for degradation of toxic organic pollutants in the environment.

**Results and discussion.** The cultures under study had different rates of growth on glucose. Specific rates of  $^{12}\text{CO}_2$  production during the growth of cultures A and B on glucose were  $0.34 (\pm 0.05)$  and  $0.078 (\pm 0.01)$   $\mu\text{g} \cdot 2 \text{ h}^{-1}$ , respectively. The lag periods of culture (A and B) growth were about 4.3 and 26 h, respectively. Comparison of the lag periods of these representatives of pseudomonades and rhodococci shows that the conventional application of a fixed time interval (4-8 h) in the case of SIR registration reveals only the microorganisms that metabolize glucose during a short lag period. The considerable part of soil microorganisms playing an important role in the cycle of organic matter in soil cannot be taken into account in this case. The value of respiration quotient (RQ) as a ratio of mole concentrations of the formed  $^{12}\text{CO}_2$  and consumed  $\text{O}_2$  varied from 0.5 – 0.6 at the stage of slow growth (lag period) to more than 2.0 at the maximum rate of  $^{12}\text{CO}_2$  production. Then, as the rate of  $^{12}\text{CO}_2$  production decreased, the RQ value approached 1.0 as a theoretically expected value during glucose oxidation. Carbon isotope characteristics of metabolic  $^{12}\text{CO}_2$  in the experiments with cultures A and B during the lag period also varied and were characterized by the  $\delta^{13}\text{C}$  values demonstrating noticeable depletion of  $^{13}\text{C}$  in the  $^{13}\text{C}$  isotope as compared with glucose. As the rate of  $^{12}\text{CO}_2$  production increased and reached its maximum value, the  $\delta^{13}\text{C}$  values of carbon dioxide increased as well, reaching the characteristic of glucose (substrate) isotope composition. Later on, when glucose was exhausted and rate of  $^{12}\text{CO}_2$  production decreased, the isotope characteristic  $\delta^{13}\text{C}-2$  indicated the tendency to reduction of the amount of  $^{13}\text{C}$  isotope in produced carbon dioxide.