Measurement of Respiratory CO₂ Production of Roots in an Aquatic Medium


Institute of Botany, Czechoslovak Academy of Sciences, Dukelská 145, CS-379 82 Třeboň, Czechoslovakia*, and Department of Biology, Humboldt-University, Invalidenstrasse 43, 1040 Berlin, GDR**

Abstract. Changes in pH, total alkalinity and O₂ concentration were followed in an aquatic medium with excised wheat roots (Triticum aestivum L.). Concentrations of total inorganic carbon and free CO₂ were calculated from total alkalinity and pH according to carbonate equilibria. The total inorganic carbon was estimated by flow-injection infra-red gas analysis. Total alkalinity increased in the root medium during incubation. Respiratory CO₂ production was estimated best from the increase in total inorganic carbon measured with an infra-red gas analyser.

Gasometric studies of root respiration are limited by the difficulties involved in measuring oxygen and carbon dioxide simultaneously (cf. Wiedenroth 1984). While oxygen can easily be followed with a Clark-type sensor in aquatic systems, there is no simple tool for simultaneous CO₂ measurements. Infra-red gas analysis, commonly used for continuous CO₂ measurements in gaseous systems, is not so suitable for aquatic systems, because the solution must be vigorously bubbled to equilibrate its CO₂ concentration with that of the carrier gas. The study of CO₂ exchange in aquatic systems is further complicated by the behaviour of CO₂ itself: equilibrating with dissolved HCO₃⁻ and CO₃²⁻ according to the pH of the solution (Golterman 1969, Loewenthal and Marais 1976).

In photosynthetic studies of submerged aquatic plants, the difficulties encountered in the direct measurement of CO₂ have often been avoided by using an indirect method: CO₂ concentration is calculated from pH and total alkalinity, assuming an equilibrium between free CO₂, HCO₃⁻, and CO₃²⁻ (e.g., Van et al. 1976, Allen and Spence 1981, Titus 1982, Pokorný et al. 1985). This approach makes it possible to follow changes in the concentrations of free CO₂ and the other carbon species simultaneously with changes in O₂ concentration.

The aim of the present work is to evaluate the application of the method of CO₂ estimation from pH and total alkalinity to studies of root respiration. It follows from

Received June 10, 1988; accepted August 23, 1988
the definition of total alkalinity in the carbonate system that total alkalinity does not change if CO₂ is the only substance whose concentration is changing. Thus, total alkalinity has often been assumed to be constant in gas-exchange studies in aquatic plants (Allen and Spence 1981). However, such an assumption would not be valid if the roots themselves were able to change the pH of the medium through other processes operating simultaneously with CO₂ production (Smith and Raven 1976). Care was therefore taken to check changes in total alkalinity during the experimental runs. As an additional safeguard, the concentrations of total inorganic carbon derived from the pH and total alkalinity model were compared with those measured directly by infra-red gas analysis.

MATERIAL AND METHODS

Ten-day-old wheat plants (Triticum aestivum L. cv. Hatri, yielded 1985) were used for the experiments. Root respiration was measured as the exchange of both O₂ and CO₂ of excised roots incubated in closed glass vessels containing an aquatic medium. The experiments were made in two series, differing in the composition of the incubation medium and the preceding cultivation treatment.

In the first series (experiments 1 to 4 in further text), the seedlings were cultivated in sand, either moistened two times per day (variant S, control of this series) or flooded continuously (variant F) with Knop solution. In the second series (experiments 5 to 8) the seedlings were cultivated in pure Knop solution, either aerated (variant A, control of the second series) or continuously bubbled with nitrogen (variant N). For further details of cultivation see Erdmann et al. (1986).

In the first series, the incubation medium consisted of 4.2 mM Ca(NO₃)₂, 1.0 mM MgSO₄, 1.6 mM KCl, and 0.37 mM KH₂PO₄. The initial alkalinity of the medium was adjusted with KHCO₃. In the second series, a pure NaHCO₃ solution was used as the incubation medium. Within each experimental series the experiments differed in: initial alkalinity (0.3 to 0.6 mM), incubation time (1 or 2 h), and vessel volume (30 or 70 ml). Each experiment was replicated three times.

Prior to the experiment, the incubation medium was bubbled with CO₂-free air before measuring initial values of pH and total alkalinity. The roots were gently washed with tap water and then with the incubation medium. Excised roots were enclosed in the vessels with the medium and incubated at 25 °C, with continuous shaking to ensure mixing. At the end of incubation, final O₂ concentration, pH and total alkalinity were measured. The roots were then dried to constant dry mass at 80 °C.

Oxygen concentration was measured with a Clark-type sensor, manufactured by Chemoprojekt Satalice, Czechoslovakia (Čap et al. 1987). pH of the solution was measured with a combined glass electrode (Chemoprojekt Satalice, Czechos-