USE OF A CO_2–LASER–BASED OPTOACOUSTIC SPECTROMETER IN STUDYING GAS EXCHANGE OF VEGETATION

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In this paper we present a description of a modified optoacoustic CO_2–laser–based spectrometer. This instrument can be used for studying gas exchange of vegetation (especially of CO_2) under varying temperature and pressure in a real time scale. We also discuss the measurement technique and some preliminary results of such investigations.

Analysis of the plants gas exchange with the environment makes it possible to obtain important information about changes in the rate of processes of ontogenesis, and the plants response to the environment influence.[1] Some results of the study of the gas–exchange dynamics can be used to estimate the resistance of a given type of plants to external stress influences, namely, drought, frosts, soil salinity,[2] pollution of the atmosphere by the gases or industrial aerosols.[3] It is shown that the plants gas–resistance inversely depends on gas–exchange intensity and the rate of absorption of toxic gases as well as directly depends on the level of critical dose of toxicants in leafs.[4] When studying the plants gas exchange the commercial IR gas analyzers with non–laser sources are usually used.[2] Generally these analyzers are produced for industrial needs and enable one to measure CO_2 and H_2O concentrations. Gasometric setups created on this basis do not allow simultaneous recording of the emission of other physiologically active gases. It is known, that in addition to the main gaseous products (CO_2, O_2, and H_2O), the plants, in the process of their vital activity, are capable of emitting the ethylene, C_2H_4, which takes part in the hormone balance, the ammonia, NH_3, characterizing protein metabolism, and a number of other volatile metabolites, that are typically measured *in vitro* with the biological and biochemical methods.[5]

In recent years a few papers were published abroad concerning the use of the optoacoustic spectroscopy for studying photosynthesis[6] and recording the ethylene emitted by plants.[7] The improvement of the equipment and techniques for investigation of the plants gas exchange *in vivo* as well as the expansion of the set of simultaneously recorded components of gas–exchange cycle are the key problems of further development of this research branch.

This paper describes a modified optoacoustic CO_2–laser–based spectrometer for measurements of the dynamics of such components of gas–exchange cycle as CO_2, C_2H_4, NH_3, and H_2O, in real time scale under temperature and pressure variations and modeling the effect of various polluting gases on the plants. We also consider the methodological aspects of measurements and some results of the initial stage of the investigations.

EXPERIMENTAL SETUP

The setup has been designed around an optoacoustic (OA) spectrometer based on a frequency tunable CO_2 laser (Fig. 1). The choice of the radiation source is determined by the fact that some products of vital activity of the plants (CO_2, C_2H_4, and NH_3) have strong absorption lines near a CO_2 laser wavelength of 10.6 μm. For this work we used a commercial ILGN–705 CO_2 laser. For wavelength tuning

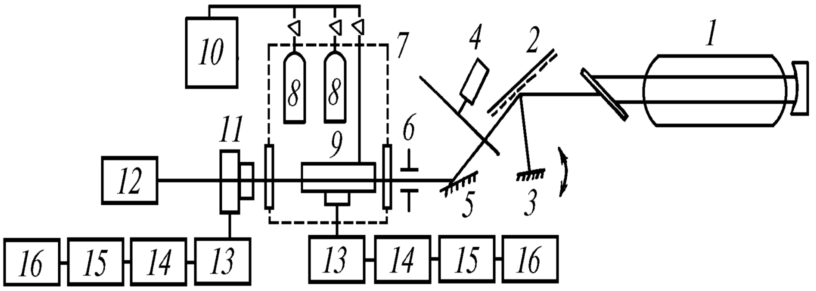

FIG. 1. Block–diagram of setup: gas–discharge tube (1), diffraction grating (2), mirror of resonator (3), modulator (4), take–off mirror (5), diaphragm (6), thermostat (7), exposure chambers (8), optoacoustic cell (9), vacuum system (10), meter of radiant flux (11), spectrum analyzer (12), preamplifier (13), lock–in amplifier (14), voltage converter (15), and recorder (16).

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the laser output mirror was replaced by the combination of the diffraction grating (100 grooves/mm) and plane 100% mirror.[8] The mirror was adjusted so that the first–order reflected radiation should fall back onto the grating and the rear spherical mirror (with 100% reflectivity). This spherical mirror was tightly welded to an active element of the laser. Radiation was emitted through the zeroth order of the grating diffraction. The radiation wavelength was tuned via the plane mirror swiveling.

The resonator construction enables one to obtain the generation at the IP(10)–IP(32) lines. Identification of the laser generation lines was carried out by panoramic spectrum analyzer, whose scale is in absolute wavelength values. Amplitude modulated radiation was directed with the plane mirror through the diaphragm of 3 mm diameter into the optoacoustic cell 100 mm long and 10 mm in diameter, whose windows were made of BaF_2 . Pressure in the OA–cell was measured with the plane condenser microphone especially designed for this investigation.

Electric signal from the microphone was preamplified and then recorded by the recording system (lock–in microvoltmeter, voltage converter, and recorder), simultaneously the reference signal from the modulator arrived at the input of the voltage converter. Similar channel was used to record a signal from the nonselective OA–detector mounted behind the measuring OA–cell and intended for measurements of the radiant flux passed through the cell.

Reference and experimental plants were placed in the exposure chambers connected to the vacuum system and OA–cell.

To investigate the influence of temperature on the gas–exchange processes, a temperature chamber was designed. A set of exposure chambers, OA–cell, heating element, and thermocouple can be placed inside it. Temperature regime of the chamber was specified by the device of KSVU–4 type. This device makes it possible to monitor temperature within 293–373 K with an accuracy of ± 5°C.

EXPERIMENTAL PROCEDURE AND MEASUREMENT RESULTS

During the experiment the gas samples from the exposure chambers were successively thrust in evacuated measuring OA–cell. When absorbing radiation at a given wavelength the amplitude of electric signal from OA–detector U is directly proportional to the absorption coefficient of the gas mixture K. The ratio characterizing the absorptance of the gas under study A was determined during the experiment as follows:

$$A = I/W = \mathrm{a}K ,$$

where W is the laser radiant flux and α is the sensitivity of the OA–detector; α is the function of the total gas pressure P in the cell.[9] For the used OA detector $\alpha = \alpha_{\max}$ for $P \simeq 60$ Torr, thus all measurements were carried out at this pressure.

To analyze the dynamics of respiration processes and to identify the gases being envolved in gas exchange we observed temporal variations of A for two CO_2 laser wavelengths $\lambda_1 = 10.591$ μm (P(20)) and $\lambda_2 = 10.532$ μm (P(14)). These wavelengths were chosen because of carbon dioxide (at λ_1) and ethilene (at λ_2) provide the main contributions to absorption.[7] Extraction of CO_2 from gas samples was carried out with ascarite, the chemical absorber of CO_2 .

Let us present some results of analysis of the air composition in the exposure chambers as an example characterizing the potentialities of this setup for studying the plants gas exchange. Figure 2 shows the temporal dependence of A for peas (*Pisum sativum L*) under the natural aeration conditions (curve *1*) and under hipobaria (curve *2*).

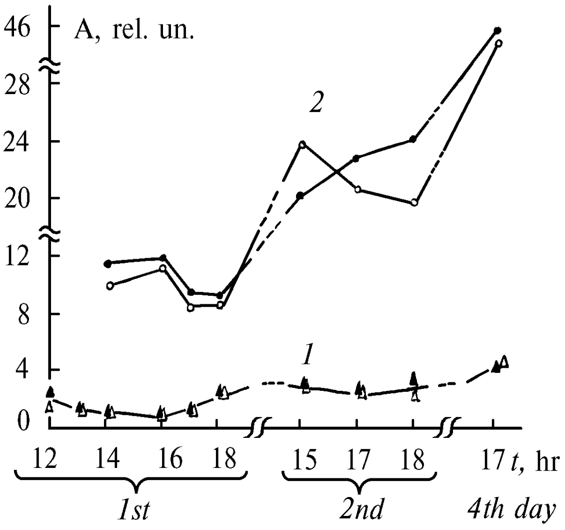

FIG. 2. Temporal dependence of A for peas at $P = 760$ Torr, P(20) (Δ)*, and* P(14) (Δ) *(curve 1) and at* $P = 100$ Torr, P(20) *(▲), and* P(14) *(o) (curve 2).*

To exclude photosynthesis in the process of which the partial absorption of CO_2 occurs, the exposure chambers were located in the light–tight covers. Measurements have shown that the quantity of absorption substantially exceeds the reference one under conditions of a low density atmosphere. This is indicative of the different rate of release of gaseous exchange products from the plants surfaces and the increase of emission of intracellular gases to the environment.

After gas passing through the ascarite, the signal value was reduced to the background ones, that is, carbon dioxide can be considered as a principal absorbing component of analyzed gas. The ratio between signal values for two generation wavelengths remained approximately similar during the experiment $A_{\mathrm{P}(20)} : A_{\mathrm{P}(14)} \approx 1.2$. Thus, plants mostly emit CO_2 to the environment in the dark, shielding the low concentrations of accompanying gases (or preventing its emission under conditions of unaerobic medium caused by hipobaria[5,10]). The revealed effect is not characteristic of a specie, but common to the plants from other taxonomy classes. Figure 3 shows the measurement results on the pine sprouts (*Pinus sylvestris L*) gas exchange justifying these assumption. Relative increase of the radiation absorption as the duration of action of experimental factor (hipobaria) increases takes place for conifers as well as for peas.

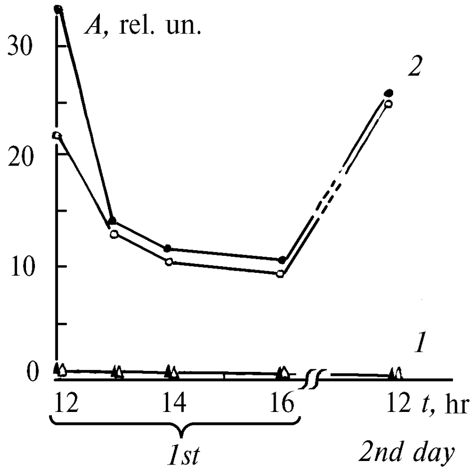

FIG. 3. Temporal dependence of A for pine sprouts at P = 760 Torr, P(20) *(*Δ*), and* P(14) *(*Δ*) (curve 1) and at P =* 100 Torr, P(20) *(* ▲ *), and* P(14) *(*o*) (curve 2).*

Thus, the obtained results are indicative of the potentialities of the developed spectrometer for recording the temporal variations in the plants gas exchange.

Further improvement of the equipment as well as the recording and processing techniques will allow one to use this approach for the ecological and physiological investigations.

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