Laser based spectrophotometer for real-time monitoring of carbon monoxide production and utilization by plants

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Abstract. A carbon monoxide analyzer based on tunable diode lasers was developed and used to study CO in the microcosm of some plants. Designed analyzer has high sensitivity (at the level of 5 ppb) and close to 100% selectivity to water vapors and CO2 and allows real-time on-line monitoring of CO in plants microcosm. In our in-vitro experiment the CO formation and release into the surrounding atmosphere were observed in the early stages of plant development (stage of germination and growth of seedlings wheat, cucumbers and colza). An intense CO absorption from the surrounding air was observed when studying the CO content in the microcosm of the formed plants.

1. Introduction
The unique analytical capabilities provided by the use of laser based spectral methods, in particular, diode laser spectroscopy (DLS) methods, allow highly sensitive measurements of gas composition in studies of plant physiology. The relevance of such studies is determined, in particular, by the needs to study the characteristics of the vital functions of living organisms in closed artificial ecological systems, for example, in future space or underwater inhabited stations.

One of the relevant objects for such studies of gaseous molecules is carbon monoxide (CO) [1-5]. It is widely known that CO is detrimental to humans and animals, as it forms a stable compound with hemoglobin in the blood and impedes the delivery of oxygen to organs and tissues. Being one of the combustion products of all hydrocarbon fuels, it is constantly released into the atmosphere of the earth in huge quantities due to human industrial and economic activities. In spite of this, the background level of CO in the atmosphere is maintained at a constant level of about 0.2-0.3 ppm. And a large role in the disposal of CO belongs to the plant world. Thus, in 1974 Bidwell R.G.S. and Bebee G.P. found that the leaves of 35 plant species studied by them are capable of absorbing carbon monoxide in the light [6-8]. Among the plants they studied were wheat, oats, carrots, buckwheat, beans, alfalfa and others. However, later Seiler W. [9,10] discovered the opposite phenomenon - plants do not absorb in the light, but emit carbon monoxide, and it is especially active at noon. The resulting contradiction in the results of various scientific groups remained unresolved for several decades [11,12].

The results of our research carried out using tunable diode lasers could resolve this paradox by allowing the experiment in real time to observe the processes of gas exchange in the atmosphere around the plants placed in a closed environment.
2. Laser carbon monoxide analyzer

To study plant gas exchange at the microconcentration level, in particular, carbon monoxide (CO) metabolism, we used laser based methods of spectral analysis [3-5]. A highly sensitive CO analyzer is shown in Figure 1. It is based on the use of a PbSSe solid-state diffusion tunable diode laser (TDL). Precise selection of the chemical composition of the laser provides the generation of infrared radiation in the range of 2100-2180 cm\(^{-1}\), where the most intense vibration-rotational absorption band of CO is located. To pump the laser crystal, short current pulses with an amplitude of ~1-2 A, duration of up to 100 μs and a repetition rate of ~ 100 Hz are used. Tuning of the laser generation frequency occurs with velocities of the order of 5*10\(^4\)-10\(^5\) cm/s. The radiation power in the mode is 0.3-0.5 mW. For TDL cooling, a liquid nitrogen optical cryostat with a flow rate of of about 1.5 liters per day is used. The selection of the appropriate mode of generation of TDL in this system is carried out only by changing the parameters of the pump current. The stability of the laser temperature is determined by the stability of the boiling point of liquid nitrogen, the small slow variations of which, caused by an external conditions variations, are compensated by a special algorithm for processing the recorded spectra.

The TDL radiation emerging from the cryostat using a CaF\(_2\) double-lens optics, which allows to reduce losses due to spherical aberrations, is introduced into a multi-pass cell (MPC), adjusted according to the White scheme. The volume of the cuvette is ~1.2 liters with the base length of the cell ~ 30 cm. When using mirrors with aluminum coating, the optimal optical path in the MPC is ~20 meters. After passing through the cell, the laser radiation focuses on a cooled InSb photodiode with a speed of ~ 20 ns and a detection ability of ~1*10\(^{11}\) cm*Hz\(^{1/2}\)sr\(^{1/2}\)W\(^{-1}\).

![Figure 1. Optical design of a tunable diode laser spectrophotometer.](image1)

1 - TDL in the cryostat; 2 - IR photodetectors in a cryostat; 3 - Reflecting mirrors; 4 - Two-lens IR optics; 5 - Calibration cell; 6 - Reference cell; 7 - Multi-pass analytical cell.

![Figure 2. The laser transmission spectrum of expired air (1), atmospheric air (2), a calibration mixture of CO: N\(_2\) (3) and an isotopic mixture with nitrogen (4) enriched in \(^{13}\)C\(^{16}\)O and \(^{12}\)C\(^{18}\)O at atmospheric pressure. The optical path length in the MPC is 18.6 m, in reference cells -5 cm.](image2)
To record the transmission spectra of CO, we used an electronic system based on a high-speed (50 ns) 8-bit ADC [13-15]. When recording the envelope of a TDL pulse with a duration of 40-60 μs, the CO absorption line scanned by a laser for a time of ~ 1.5-2 μs has about 30-40 points. The studied transmission spectrum, shown in Figure 2, is recorded with an accumulation of 128 times for ~5 s. The dynamic range of recording the amplitude of the signal is expanded to $4 \times 10^3$ due to hardware sweep of the signal [5,13].

To measure CO concentration, direct detection of absorption lines is used. During automatic processing of the recorded spectrum, the position of the center of the analytical line, the envelope of the absorption zero, optical zero, the absorption value at the resonance maximum, and the absorption line width at half maximum are determined. Based on the results of these procedures, as well as taking into account the length of the optical path in the MPC, temperature, atmospheric pressure, and spectral parameters of the analytical absorption line used, the concentration of CO in the gas mixture under study is calculated. The line parameters used for the calculation borrowed from the HITRAN spectral data bank [16], are set based on the identification of the absorption spectrum of CO using isotopomers $^{13}$C$^{16}$O and $^{12}$C$^{18}$O.

The main sources of errors in determining the concentration of CO in air using this analyzer are: the influence of atmospheric CO in the open part of the optical system, fluctuations in the boiling point of liquid nitrogen, the effect of the superluminescent stand in TDL radiation, the influence of optical interference, the influence of the discreteness of digitization of the spectral signal.

We especially note that for studies of gas exchange of plants at the micro level, the selectivity of the applied analytical method is essential. When using traditional spectral analysis methods and an 0-1 CO absorption band located near 4.7 μm, problems arise associated with interference in the CO, H$_2$O, and CO$_2$ absorption bands in this spectral range. The use of diode laser spectroscopy methods removes the interference problem and allows one to obtain high selectivity for gas analysis even at elevated concentrations of H$_2$O and CO$_2$ relative to ambient air that are usual. This is achieved due to the possibility of choosing an analytical CO absorption line, which is least overlapping with the absorption lines of H$_2$O and CO$_2$. In particular, one of such fairly well-isolated lines is the P(6) line of the P-branch of the band 0-1.

The main analytical parameters of the CO analyzer based on PDL:

- The sensitivity of the detection of the content of CO: ~ 5 ppb;
- Detection accuracy: ~ 3 %;
- Detection selectivity: ~ 100 %;
- CO concentration measurement time: ~ 5 s;
- MPC refilling time (at the level of 0.9): ~ 10 s;
- The time of continuous monitoring is not limited.

3. **Experimental results**

We used the laser based analyzer to study CO in the microcosm of some plant species. The objects of our research were plants of wheat, cucumbers and colza of different ages.

Studied plant of different species and age was placed in closed space. Air sampled from this space passed multipass cell of the TDL analyzer and then returned back with help of micropump. In Figures 3A and 3B two the most typical results are shown. In the case of juvenile wheat seedlings, Figure 3A, intensive CO generation was observed. This effect depends on the age of seedlings as well as on the intensity of illumination by day light. In the case of well developed wintercress on Figure 3B an opposite effect was observed. This plant assimilated CO from the environment atmosphere. Rate of this consumption is dependent on the CO content in the atmosphere and reached a value of 0.05 μl / h per gram for 0.5 ppm CO concentration. High sensitive studies of plant gas exchange and / or respiration of that kind can give some new ideas on global atmosphere chemistry.
4. Discussion
Thus, the obtained results showed that young seedlings really very actively release carbon monoxide into the atmosphere, and its formation rate in plants is thousands of times lower than carbon dioxide - the main product of plant respiration. In the first days of its development, seedlings continuously emit CO, which can lead to a multiple increase in its content in the air if the volume where the plants are grown is closed. As the seedlings grow and turn into adult plants, the process of carbon monoxide emission by them changes. First, carbon monoxide emission becomes light-dependent, i.e. goes in the light and ceases in the dark. Then, 3-5 weeks after the start of growth, herbaceous plants begin to absorb carbon monoxide from the surrounding air. Moreover, with age, this ability to absorb increases.

This pattern in the CO excretion and absorption by plants detected *in-vitro* by the used laser technique, may explain the global changes in background concentration of this gas in the atmosphere. In particular, the result obtained allows us to understand the results of our earlier field studies on monitoring of CO in an open atmosphere by S.N. Kotelnikov, in which there was an increase in the level of CO in the atmosphere of the Kara-Dag reserve during the periods of vegetative development of plants (spring and autumn) [17]. Using a laser analyzer similar to our one, but designed for research in an open atmosphere, he found that the level of carbon monoxide in the air rises sharply in the spring. The moment of this increase depends on whether early or late spring, i.e. from the exact time of awakening to life of herbaceous and woody plants. If warm autumn periods occur and conditions for repeated vegetation are created in Crimea, then in the fall carbon monoxide levels increase similar to the spring ones. In the summer, when the green is gaining strength, i.e. grass, trees and shrubs will completely form their leaves, there is a sharp decrease in the level of carbon monoxide - there is a cleansing of the earth's atmosphere. During the winter, while nature is sleeping, there is a gradual increase in the background level of carbon monoxide in the atmosphere. Thus, seasonal variations in the concentration of carbon monoxide in the atmosphere are observed.

![Figure 3. A – an increase of CO content in ambient atmosphere caused by CO production by juvenile wheat seedlings. B – CO decrease in ambient atmosphere caused by well developed wintercress CO absorption.](image-url)
The ability of plants to absorb carbon monoxide can be used for practical environmental purposes. In particular, these properties could be useful for creating artificial ecosystems and systems of long-term life support in confined spaces. As known, in such systems the problems associated with the utilization of the vital products of plants, animals and humans, including gaseous ones, and maintaining the ecological balance are especially acute. For long space flights or manned stations on the Moon or Mars, plants would simultaneously provide a person with food, oxygen and dispose of carbon dioxide, carbon monoxide and other gases. In particular, the accumulation of carbon monoxide, which, as it turned out, is intensively secreted by animals and humans [3-5,15], can be avoided by selecting the appropriate plants in the ecosystem. And the volumes of the enclosed space where the plants will be germinated can now be recommended to isolate for some time from the living compartments until the initial stage of vegetation has passed. If you do not take into account such subtle effects and patterns when creating artificial ecosystems, then this can lead to the disruption of even grandiose scientific projects, as happened in the past with the American project “Biosphere”.

Conclusion
A carbon monoxide analyzer based on tunable diode lasers was developed and used to study CO in the microcosm of some plants. Designed analyzer has high sensitivity (at the level of 5 ppb) and close to 100% selectivity to water vapors and CO$_2$ and allows real-time on-line monitoring of CO in plants microcosm. In our in-vitro experiment the CO formation and release into the surrounding atmosphere were observed in the early stages of plant development (stage of germination and growth of seedlings wheat, cucumbers and colza). An intense CO absorption from the surrounding air was observed when studying the CO content in the microcosm of the formed plants.

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