Cockroaches and tomatoes investigated by laser photoacoustics

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Abstract Technical details of two sensitive gas photoacoustic experimental systems based on the use of tunable CO (cryogenic) and waveguide CO\textsubscript{2} lasers as radiation sources are given and their application to specimens of biological origin (cockroaches and tomatoes) described. The results obtained present feasibility and flexibility of this technique in studies requiring high stability and fast response over extended measuring intervals.

1. INTRODUCTION

In the past years laser photoacoustic (PA) spectroscopy capable of detecting gas concentrations at the trace level, has also been recognized as an important tool for studies of biological processes. Very high sensitivity and fast time response (10 seconds) in on-stream analysis have already been achieved with powerful and line tunable CO and CO\textsubscript{2} lasers \cite{1,2}. Such short response times are of interest in studies that include metabolic changes in plants exposed to external stress factors. For example, small concentrations of ethylene, the only gaseous plant hormone, were found to influence wilting, supergrowth or germinating effects in plant material. Due to its high sensitivity laser PA spectroscopy enables practically on-line measurements, i.e. monitoring the response of plant to a variety of ambient conditions without a need to accumulate (concentrate) gases in a space adjacent to plant tissue; accumulation would change and falsify the plant response. The existence of some degree of spectral coincidence between a specific (CO or CO\textsubscript{2} lasers having a line to line spacing of several cm\textsuperscript{-1}) laserline and absorption line of a given gaseous specie under investigation is an impetus for PA detection. At infrared wavelength and atmospheric pressure (typical for a PA measurement) the total width of a gas absorption line is about 2-3 GHz \cite{3}. For small (diatomic) molecules, probability for such spectral overlap is low. On the other hand big molecules (e.g. ethanol) are characterized by broad spectral features missing a clear and an easy to recognize fingerprint. Interference effects due to the absorption caused by other gases present in the cell, impedes performance of PA measurements to a considerable extent \cite{4,5}. For biological studies, use of passive approaches (such as for example scrubbing and freezing) provide a remedy in combating this restriction. Hydrocarbon compounds can be removed effectively from the streaming gaseous mixture by using
Granulated potassium hydroxide (KOH) helps in eliminating the effect of CO$_2$ residual gas. A cryogenic trap operating at 120 K serves to collect substantial quantities of ethanol produced in plant material under anaerobic conditions.

In this study described here we report on what is believed to be the first PA studies of C$_2$H$_4$ emission from a tomato and on the real time monitoring of CH$_4$ gas (due to the activity of methanogenic bacteria found in anaerobic intestines) exhaled by a cockroach. Strong water vapor absorption across a large part of the CO (Av=1) laser emission spectrum is often annoying. However, in many biological applications fast and sensitive determination of water vapor concentration is desired. In this paper we present rapid changes of water vapor concentration due to respiration of a single cockroach.

2. EXPERIMENTAL

Characteristic parameters of CO$_2$ [8] and CO laser (continuous wave) PA systems used in this study (with PA cell placed in intracavity configuration) are displayed in Table 1.

The CO$_2$ laser emission covers the spectral region from 900 to 1100 cm$^{-1}$ and provides some 90 laser transitions (for most abundant CO$_2$-isotope). The liquid nitrogen cooled CO laser [6] emits between 1250 and 2050 cm$^{-1}$, with about 300 transitions for $^{12}$C$^{16}$O. The CO (Av=2) overtone version of the same laser has a comparable number of usable transitions in the range from 2400 to 3800 cm$^{-1}$ [7].

The operation of both systems is fully automated; measuring periods of 100 hours are typical. In order to reduce the effect of parasitic signals caused by periodic heating of Brewster windows, the PA cell is equipped with properly dimensioned buffer volumes [2] and additional $\lambda/4$ tubes at the location of cell windows as shown in Fig. 1. The cell is designed to allow rapid removal of a gas from the central portion (resonator). The sampling gas is admitted through the port located in the middle of the resonator (flow 1 l/h), while larger buffer volumes (1.5 liter each) are purged by additional dry air to improve the response time of the cell. Without this additional flow the gas will have a long residence time in the buffer volumes; due to diffusion it will re-enter the resonator. The main objective in designing the PA cell is to obtain highest possible sensitivity with a minimum loss in
### Table 1: Technical specifications of the intracavity CO laser and CO\(_2\) laser sources used in photoacoustic experiments described here.

<table>
<thead>
<tr>
<th>Laser configuration</th>
<th>Cryogenic cw CO-laser liquid N(_2)-cooled</th>
<th>cw Waveguide CO(_2)-laser water cooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of laser cavity</td>
<td>2 m</td>
<td>1.2 m</td>
</tr>
<tr>
<td>Diameter of discharge tube</td>
<td>11.4 mm</td>
<td>2.8 mm</td>
</tr>
<tr>
<td>Intracavity laser power</td>
<td>1 W (1304.97 cm(^{-1}))</td>
<td>100 W (949.48 cm(^{-1}))</td>
</tr>
<tr>
<td>Photoacoustic cell configuration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of resonator</td>
<td>150 mm</td>
<td>100 mm</td>
</tr>
<tr>
<td>Diameter of resonator</td>
<td>15 mm</td>
<td>6 mm</td>
</tr>
<tr>
<td>Waist of the laser beam</td>
<td>4 mm</td>
<td>1.2 mm</td>
</tr>
<tr>
<td>Q-factor</td>
<td>40</td>
<td>31.8</td>
</tr>
<tr>
<td>number of microphones</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>type of microphone</td>
<td>B&amp;K 4179</td>
<td>Knowles EK 3024</td>
</tr>
<tr>
<td>Refilling cell volume</td>
<td>26 ml</td>
<td>22 ml</td>
</tr>
<tr>
<td>Chopping frequency</td>
<td>1 kHz</td>
<td>1.6 kHz</td>
</tr>
<tr>
<td>Minimum response time (flow 1 l/h)</td>
<td>70 s</td>
<td>80 s</td>
</tr>
<tr>
<td>Sensitivity of photoacoustic cell (1 Watt laser power)</td>
<td>(3 \times 10^{-9}) cm(^{-1})</td>
<td>(1.4 \times 10^{-8}) cm(^{-1})</td>
</tr>
<tr>
<td>Gas: (see text)</td>
<td>CH(_4)</td>
<td>C(_2)H(_4)</td>
</tr>
<tr>
<td>Laser wavelength</td>
<td>1304.97 cm(^{-1})</td>
<td>949.48 cm(^{-1})</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>3 atm(^{-1})cm(^{-1})</td>
<td>23.7 atm(^{-1})cm(^{-1})</td>
</tr>
<tr>
<td>limiting sensitivity</td>
<td>1 ppbv.</td>
<td>6 pptv.</td>
</tr>
</tbody>
</table>

speed of response. Values quoted in Table 1 should not be considered as ultimate limits but rather as practical figures instead.

Detection limits achieved for several gases when using the CO\(_2\) laser PA spectrometer have been reported [5]. Table 2 contains detection limits attainable by the CO laser (\(\Delta v=1\)) PA system shown in Fig. 1. Note that less favorable conditions (such as interference of water vapor) may result in performance inferior to that reported in Table 2.

### 3. RESULTS AND DISCUSSION

#### 3.1 Cockroaches

Millipedes, termites, scarab beetles and cockroaches are insects accommodating methanogenic bacteria in a symbiotic fashion. The bacteria reside in the anaerobic environment of the intestinal tract and render digestible poisonous or otherwise unusable parts of the insect's food. Some insects have built-in structures in their intestines to accommodate great numbers of these microbes. Chemically, the insects obtain energy from anaerobic fermentation, with H\(_2\) as one of the fermentation products. Too high concentrations of H\(_2\) would lead to detrimental levels of propionic acid and butyric acid; in addition, the excess energy of the reaction CO\(_2\)+4H\(_2\) \(\rightarrow\) CH\(_4\)+2H\(_2\)O would remain unused.
Historically, the archae bacteria (methanogenic bacteria) or their predecessors prospered in the originally anaerobic atmosphere of the earth. After the "invention" of photosynthesis and respiration methano-bacteria withdrew into anaerobic niches, like the intestines of animals. The world-wide rate of methane production from insects amounts to some \( (30 \text{ to } 300) \times 10^9 \text{ kg/year} \) (about 20\% of the total earth production).

In collaboration with dr. J. Hackstein (Dept. of Microbiology, University of Nijmegen, The Netherlands) cockroaches were chosen to study the time dependence microbiologically produced \( \text{CH}_4 \) emission. To this end a single insect was placed in a measuring cuvette, a dark cell vented by air for some days. After passing through the cuvette the airflow was led along a cryo-trapping surface to remove \( \text{H}_2\text{O} \) from the gas mixture before the gas was analyzed in the photoacoustic cell, utilizing the about 1 Watt intracavity laser power around 1300 \( \text{cm}^{-1} \).

For low methane signals, the CO-laser wavelength was switched between two neighboring transitions \((1308.01 \text{ cm}^{-1} v=33\rightarrow 32 J=9\rightarrow 10 \text{ and } 1304.97 \text{ cm}^{-1} v=33\rightarrow 32 J=8\rightarrow 9)\), one absorbing weakly, the other strongly. The difference signal was proportional to the \( \text{CH}_4 \) concentration in the sampled air. To improve the response time of the system strong methane signals were recorded only at the CO-laser line corresponding to strongest absorption.

The \( \text{Periplaneta americana} \) (a frequently investigated cockroach, about 30 mm long) yielded a minimum production of less than 10 nmole/h (to be compared with the detection limit of 10 picomole/h). However, quite unexpectedly, every 15 minutes or so a peak in emission occurred, reaching values exceeding 300 nmole/h (see Fig. 2). For an air flow of 1 l/h these peak emissions correspond to easily detectable concentrations of 6 ppm.

In order to clarify the origin of these periodic methane bursts the \( \text{CO}_2 \) emission was monitored simultaneously by a commercial infrared gas analyzer (URAS, Hartmann and Braun). Figure 2 displays the synchronous character of carbon dioxide and methane emissions. The conclusion, therefore, is that the methane produced by methano-bacteria in the hindgut of the cockroach near to the rectum is practically entirely emitted together with the respiratory product \( \text{CO}_2 \), through the respiratory pathways. These pathways (spiracles, trachea) are normally closed (probably in order to reduce water loss) and open only periodically to release \( \text{CO}_2 \) and take up \( \text{O}_2 \) \([9,10]\).
Figure 2: The respiration of the cockroach *Periplaneta americana* as evidenced by the periodical CO$_2$ emission. It is shown that the methane produced by methano-bacteria in the hindgut near to the rectum is practically entirely emitted through the respiratory pathway.

Figure 3: The reduced methane production of the *Periplaneta americana* after exposure to a temporarily increased CO$_2$ level which narcotizes the methano-bacteria.
Figure 4: The respiration of the cockroach Gonfedrina with a period of 1.3 hour. Variations of the water vapor concentration are within 1 minute, reflecting pumping and venting motions of the spiracles.

Figure 3 shows the "narcotizing" effect of a temporarily increased CO$_2$ level on the archae bacteria, yielding a substantial reduction in methane production while the respiration of the cockroach remains practically unchanged.

Especially interesting are results of Fig. 4 where the time variation of water vapor concentration can be compared to the simultaneously measured CO$_2$ levels. These measurements were performed on a larger cockroach (Gonfedrina, 50 mm long, sturdier than Periplaneta americana), possessing a respiratory period of about 1.3 hour. Variations of the water vapor concentration can be seen to occur within 1 minute, reflecting pumping and venting motions of the spiracles. Note that these sensitive and fast H$_2$O measurements were performed with the photoacoustic detector of Fig. 1, with the CO laser tuned to 1729.76 cm$^{-1}$ ($v=16 \rightarrow 15, J=6 \rightarrow 7$). The measurements demonstrate the influence of motion of the closure parts of the spiracles without leading to an effective opening. Already enlarging the intact water film covering the outlet of a spiracle, yields an increase of the water vapor concentration.

3.2 Tomatoes

Figure 5 features design of a double cuvette that allowed us to measure the C$_2$H$_4$ emission at different positions from a cherry tomato. In particular, emission from the calix area (where fruit is attached to the plant) can be compared to the remaining emissions integrated over the complete skin-surface. The gas is sampled alternately from the outlet at the top of the central part of the cuvette and from the outlet on the right side (Fig. 5). The C$_2$H$_4$ emission is calculated from the air flow (typically 2 l/h) and the concentration determined from the signal detected by the photoacoustic cell. This concentration follows from the difference of signal, recorded at CO$_2$-laser transitions at 949.48 cm$^{-1}$ (10P14) and 951.19 cm$^{-1}$ (10P12). Details of photoacoustic C$_2$H$_4$ measurements are given elsewhere [8]. In addition, these local emission rates have been determined from sensitive photo deflection measurements, results which will appear in a forthcoming publication.

Tomato fruit develops from superior ovaries, i.e. what remains of sepals and petals forms the green coronet where the "berry" is attached to its plant. From the suspension area, open connections extend to the center part of the fruit; through these gas exchange with the surroundings can take place.
When detached from the plant tomatoes react by increasing their respiration. However, their ripening process and C$_2$H$_4$ production are not influenced [11]. If after disconnection the coronet part is removed and the appearing fresh scar sealed (e.g. using a piece of parafilm or by high vacuum grease) ripening processes are suspended and rotting occurs while the fruit remains green. In its normal ripening phase, the tomato is characterized by enhanced respiration accompanied by autocatalytic C$_2$H$_4$ production typical for climacteric fruit [12]. It is expected that gas exchange via the coronet area plays an important role under such conditions.

In Fig. 6 it is shown that 95% of emitted ethylene originates from the coronet zone, with small relative variation during the development from green/orange to red. Little is known about the overripe stage. In Fig. 7 maximum productivity amounts to about 60 nl/h, shortly after the climacteric rise in ethylene production. The different maturity at time "zero" causes horizontal shifts of two production curves. The development was followed for 9 days. From the synchronous character of superimposed modulation it is clear that external factors - of presently unknown and therefore uncontrolled nature - influence the measurements.
The effect of sealing the coronet area (4 hours before zero) was investigated under anoxic and aerobic conditions (Fig. 8). Opening the coronet area under anoxic conditions (at 5 hours) results in a small peak due to accumulated ethylene. Switching to aerobic conditions at 6.5 hours yields renewed ethylene production. Note that oxygen is needed for the production of ethylene. In the ethylene synthesis, starting from methionine via SAM to ACC and ending up in \( \text{C}_2\text{H}_4 \), oxygen is required for the conversion of ACC to \( \text{C}_2\text{H}_4 \) [13]. The shape of the curve reflects initially the ethylene production due to accumulated ACC and thereafter the restart of the ACC production. The tomato measurements form part of a collaboration with dr. E. Woltering and dr. H. van der Valk (ATO, Wageningen, The Netherlands) and demonstrate the usefulness of photoacoustic measurements in research of fruit storage. Simultaneously, other gases (like CO\(_2\) and C\(_2\)H\(_5\)OH) were measured. Likewise specimens of other fruits, such as apple, pear, banana and fig are investigated, they exhibit quite a different behavior during ripening as will be discussed in a forthcoming publication.

![Graph](image-url)

**Figure 7**: Climacteric rise in \( \text{C}_2\text{H}_4 \) production of two cherry tomatoes (■ and □) in different stages of maturation at \( t=0 \) hour.

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Figure 8: Emission rates of C$_2$H$_4$ under changing conditions (anoxic at the beginning and aerobic after 6.5 hours); the coronet area was sealed for first 5 hours.

References


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