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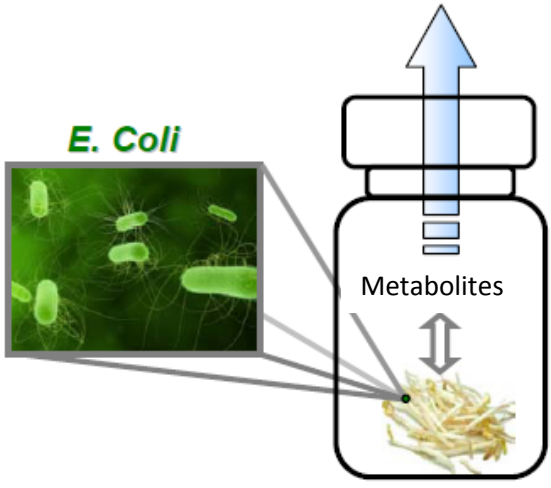
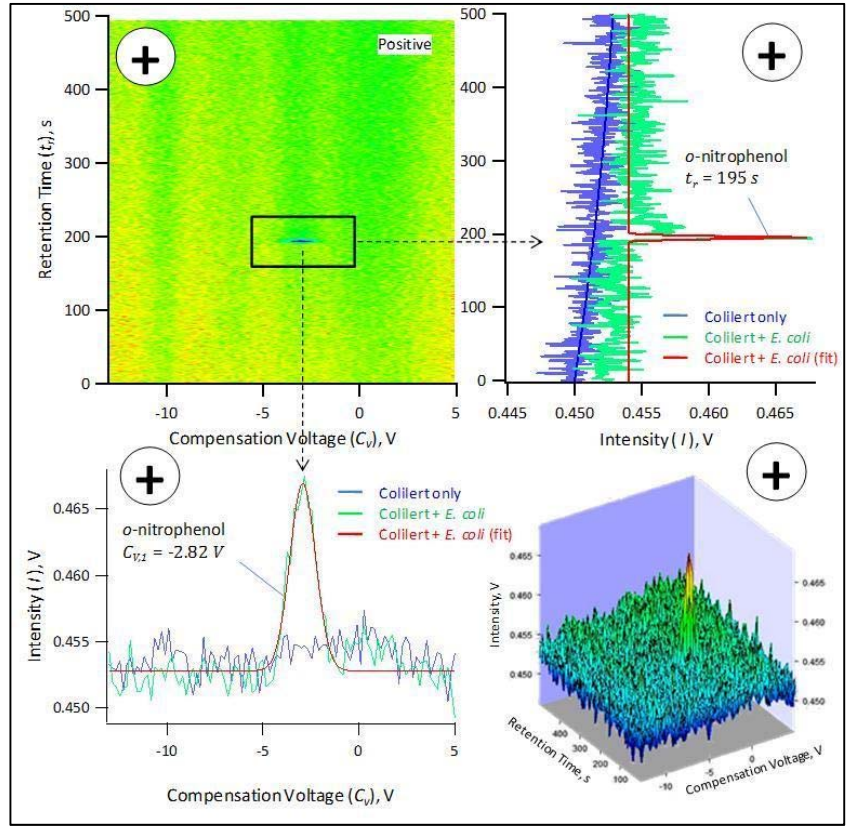
(GC-DMS)

microAnalyzer™





Highlights:

- New GC-DMS based method for detection of *Coliform* is presented
- *o*-Nitrophenol released by *Coliform* in Colilert®-18 was taken as a specific biomarker
- *o*-Nitrophenol can be detected by GC-DMS after 3-18 h of incubation

Gas Chromatography – Differential Mobility Spectrometry and Gas Chromatography – Mass Spectrometry for the detection of coliform bacteria

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Abstract

The use of miniaturized Gas Chromatography – Differential Mobility Spectrometry (GC-DMS) is shown for the detection and identification of coliform bacteria (including *Escherichia coli*) grown in five different media: Colilert®-18, glucose broth, M9-medium, tryptophan broth, and tryptic soy broth. After incubation in the different media, headspace containing the volatile compounds were analyzed by the GC-DMS and the results were validated by Gas Chromatography – Mass Spectrometry (GC-MS). Results showed that the GC-DMS and GC-MS were able to detect *o*-nitrophenol released by coliform bacteria incubated in Colilert®-18. In addition to that, GC-MS was able to detect indole compound released by coliform bacteria grown in all media. Neither GC-DMS nor GC-MS could detect 4-methylumbelliferone from the headspace of *E. coli* grown in media containing 4-methylumbelliferyl-β-D-glucoronide (MUG) substrate, which was available in Colilert®-18. With the miniaturized GC-DMS being portable and can be operated using ambient pressure, this method offers a potential on-site detection of coliform bacteria.

Keywords: Coliform bacteria, growth media, biomarker, Gas Chromatography – Differential Mobility Spectrometry, Gas Chromatography – Mass Spectrometry

1. Introduction

Coliform and *E. coli* bacteria are traditionally used as indicator organisms for fecal contamination in water. Due to persistence outbreak of *E. coli*, there is an urgent need to develop a method that could detect the bacteria in timely and accurate manners [1; 2; 3]. The main limitation of standard methods is the time to obtain the result (18 – 48 *h*) [4; 5; 6].

Commercial methods that are widely used today, such as Colilert (IDEXX Laboratories, Portland, ME, USA), Colisure (Millipore corporation, Bedford, MA, USA), and Coli-Quick (Hach, Loveland, CO, USA), are developed based on enzymatic methods. Colilert-18®, which utilizes the defined substrate technique with o-*n*itrophenyl-β-D-

galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucoronide (MUG) is the most widely used method among others and is included in the Standard Method for the Examination of Water and Wastewater [39]. However, the method still needs 18 h to 22 h to complete.

Alternative methods such as spectrometric methods have been gaining interest in past decade. Most spectrometric methods were based on the detection and identification of the bacteria through the presence/ absence of biomarker compounds [7; 8] or through comparison of chromatograms as fingerprints [9; 10]. It is well known that as bacteria grow and reproduce, they release various volatile compounds that can be profiled and used for their identification and speciation [11]. However, there are concerns over reliability of such methods in both approaches (biomarker and fingerprint analysis) [9]. The concerns are usually associated with variations, particularly in temperature of growth [9; 12], cell age [13], and food sources [9; 11; 14].

In the absence of control of these variations, fingerprint methods are disqualified since libraries are rendered unreliable from changes in the chemical components of bacteria [9]. Bos et al. (2013) [40] gave a review on volatile metabolites produced by six different bacterial species (Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae and Enterococcus faecalis) determined by chromatographic techniques in vitro and in vivo and found that there are only very few VOCs specific for one bacterial strain but the most are produced by several ones. It was concluded that reliable identification of bacteria can rarely be done according to single VOCs, but must be carried out according to specific VOC patterns, as very few metabolites were found which are specific for one single microorganism. Beside of this, these methods show technical complexities, such as the size of the analytical instruments, the need for vacuum, the need for oven and the high energy requirement which make them laboratory based and not suitable for on-site monitoring.

In order to develop a specific and fast method for the detection of coliform bacteria (including E. coli) the reaction of coliform bacteria with ONPG to β-D-Galactose and *o*-nitrophenol was combined with GC-Differential Mobility Spectrometry.

GC-DMS is an advanced gas detector that requires low power consumption, has a built-in GC column, compact and portable, and therefore suitable for on-site purpose [15; 16; 17]. DMS is an ion-separation technique that characterizes chemical substances using differences in the gas phase mobility of ions in alternating strong and weak electric fields that are generated using a high frequency asymmetric waveform [18]. The basic concept of separating ions based on ion mobility using a high frequency asymmetric field was first introduced by Buryakov and co-workers in 1993 [18; 19]. Unlike mass spectrometry which is a vacuum-based technique, DMS is operated at ambient pressure, where the separation of ions is achieved by exploiting the difference in the ion mobilities between alternating high and low electric fields within the DMS drift cell [8; 9; 10; 20; 21]. During the last decade DMS has been primarily employed for detecting volatile organic compounds (VOCs). DMS has been successfully coupled with GC for the analysis of human breath, bacterial odors, jet fuel analysis, and for the detection of biological and chemical agents among others [8; 9; 10; 20; 21; 22; 23; 24; 25].

As reported in earlier work [2], in which headspace over aqueous solutions of twelve standard compound which are suspected to be possible volatile metabolites [4; 26; 27; 28] were analyzed by miniaturized GC-DMS. The sensitivity of the analytical system was not sufficient for the analysis of indole, undecanone, and 2-tridecanone. In this work bacteria were grown in five different media in order to increase the concentration of the expected metabolites. The kind of cultivation medium has an immense influence on the growth of coliform bacteria and the release of characteristic metabolites in sufficient quantities. In order to determine a suitable cultivation medium as a requirement for a subsequent fast analytical determination, five media commonly used to grow *E. coli*, i.e. Colilert®-18, glucose broth, M9-medium, tryptophan broth, and Tryptic Soy Broth (TSB) were investigated and the result is presented in this study.

2. Material and Methods

2.1 Reagents and Samples Preparation

2.1.1 Bacteria

Escherichia coli DSM 30083 was obtained from the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) GmbH, Braunschweig, Germany. Prior to use, the bacteria was grown on NB agar and incubated overnight at 37 °C.

2.1.2 Chemicals

o-Nitrophenol (98%), 4-methylumbelliferone (≥ 98%), and indole (≥ 99%) were purchased from Sigma-Aldrich (Steinheim, Germany) and used as received.

2.1.3 Growth Media

The growth media were used and were prepared freshly. Except for Colilert®-18 (which was prepared according to its manufacturer instruction), all other media were prepared according to recipes from *Handbook of Microbiological Media* by Atlas [29]. The growth media were:

- **Colilert®-18**: 4 packages of Colilert®-18 (IDEXX, cat. nr. WPO2OI-18) were used in this experiment. Each package was dissolved in 100 mL of sterile deionized water as per manufacturer instruction.
- **Glucose broth**: 1 g of meat peptone (Oxoid, LP0034), 0.3 g of meat extract (Oxoid, LP0029), and 0.5 g of NaCl (Prolabo, 27810.295) were dissolved in 100 mL deionized water. The pH was adjusted to 7.2, the volume was brought to 142.5 mL, and then autoclaved. Shortly before use, 7.5 mL of sterile M9-glucose-solution was added to it.
- **M9-medium**: the following compounds were firstly dissolved in 100 mL of deionized water: 0.7 g of $Na_2HPO_4{\cdot}2H_2O$ (Riedel-de Haën, 30412), 0.3 g of KH_2PO_4 (Fluka, 60218), 0.1 g of NH_4Cl (Riedel-de Haën, 31107), and 0.05 g of NaCl (Prolabo, 27810.295). The solution was then brought into 132 mL using deionized water and was autoclaved. The following solutions were then prepared and autoclaved separately: 10 g of D(+)-Glucose (Oxoid, LP0071) in 50 mL of deionized water, 2.5 g of $MgSO_4{\cdot}7H_2O$ (Fluka, 63142) in 10 mL of deionized water, and 0.2 g of $CaCl_2{\cdot}2H_2O$ (Fluka, 21101) in 10 mL of deionized water. Shortly before use, 15 mL of the $D(+)-$Glucose solution, 1.5 mL of the $MgSO_4{\cdot}7H_2O$ solution, and 1.5 mL of the $CaCl_2{\cdot}2H_2O$ solution were added into the first solution.
- **Tryptophan broth**: 2.5 g of DEV-tryptophan (Merck, 1.10694.0500) was dissolved in 150 mL of deionized water, adjusted to pH 7.2, and was autoclaved.
- **Tryptic Soy Broth (TSB)**: 4.5 g of a premixed powder of TSB (Merck, 1.05459.0500) was dissolved in 150 mL deionized water and autoclaved for 15 min at 15 psi pressure and at 121 °C.

Each growth medium was distributed into headspace vials; one vial for one type of medium only. The volume of the vial was 20 mL. The volume of the medium in each vial was 10 mL. Some of these growth media were later inoculated by

E. coli, whereas some others were kept as blank samples (containing media only). For each analysis, the samples were prepared in triplicates.

2.1.4 Preparation of standard compounds

One liter of 10 mg/mL of *o*-nitrophenol, 4-methylumbelliferone, and indole solutions in water were prepared individually in ultra pure water (18.2 MΩ, Millipore). Each was then diluted and prepared as 1 mg/mL of standard solution. For each analysis, 10 mL of each standard solution was prepared as a working solution and was placed in a 20-mL vial which was closed with an air-tight septum.

2.1.5 Preparation of bacterial samples

Colonies of *E. coli* DSM 30083 which were previously grown overnight on NB agar were used to inoculate each growth medium (which was prepared as 10 mL medium in the 20-mL autoclaved headspace vials). The initial concentration of *E. coli* in each sample vial was 5×10^7 cells/mL. To achieve this exact concentration, the liquid cultures were prepared as follow (this is the example for inoculation in Colilert®-18 medium):

- Colonies that were grown on NB agar were transferred into an RG tube containing 10 mL deionized water until the water became a cloudy suspension. The cloudy suspension usually contained between 10^8 to 10^{10} cells/mL bacteria (the exact concentration is known after the cell counting).
- The tube was then placed on a vortex and shaken at 900 rpm for 10 s.
- Another three RG tubes were prepared, each contained 9 mL deionized water.
- A series of dilution was then prepared: the cloudy suspension from the first RG tube was diluted 3 times in the other three tubes, so that the cell concentration in each tube became 10^{-1}, 10^{-2}, and 10^{-3} of the first suspension, respectively.
- The cell concentration in the second and last series of tubes dilution (the one with 10^{-2} and 10^{-3} concentrations of the original suspension) was then counted using a Thoma counting chamber.
- It was found that the concentration of the first suspension was 1.08×10^{10} cells/mL. Therefore, 46 µL of aliquot was taken from this suspension and transferred into each 10 mL Colilert-18® solution so that the initial cell concentration in the each vial was 5×10^7 cells/mL.

All other liquid cultures in tryptophan broth, M9, glucose broth, and TSB broth were prepared analytically in the same way. All samples in the headspace vials were then capped with autoclaved septa and aluminum caps. These samples (containing *E. coli* and growth media) and the blank samples (containing only growth media) were incubated and shaken at 36 °C in a water bath GTL 1083 for 24 h, except those which were incubated in Colilert®-18 which were incubated for only 18 h according to the manufacturer's instruction. All samples were made in triplicate.

2.2 Instrumentations

2.2.1 GC-DMS

The experimental setup consisted of a miniaturized GC-DMS (microAnalyzer™ from the Sionex, USA) connected to a 0.5 bar nitrogen gas supply. The operational principle of the instrument has been described in the earlier work [2]. There are three stages in the operation of the miniaturized GC-DMS: sampling, loading, and detection. The instrumental setting for each stage is described below.

In the sampling stage, the suction pump was operated for 30 s, the flow rate of the suction pump was 80 mL/min, and the flow rate of the transport gas (the recirculation air) was 300 mL/min. Headspace sample (500 µL per analysis) was injected manually into the stream of nitrogen gas entering the sample inlet using a 500-µL gas-tight syringe.

In the loading stage, the temperature of the pre-concentration trap was programmed as follow: the initial temperature was set at 40 °C, after 1 s it was increased to 200 °C, after 4 s to 300 °C , and then was held at 300 °C for 100 s. The sample was then passed through the GC column. The GC column temperature was programmed as follows: the initial temperature was set at 60 °C and held for 60 s, increased 1 °C/s to 140 °C, and then held at 140 °C for 200 s.

In the analyzing stage (DMS detection), the operating parameters were set as follow: the RF voltage was set at 1100 V, the sensor temperature was set at 80 °C, the compensation voltage scanning range was set between −13 and +5 V, and the retention time was recorded until 500 s. Response was recorded in both positive and negative modes.

2.2.2 GC-MS

The experimental setup consisted of a GC-17A GC-MS system (Shimadzu) equipped with an Rtx-440 (30-m×0.25-m×0.25-µm) fused silica column at a flow rate of 1.4 mL/min of helium. The oven temperature was programmed as follow: the initial temperature was set at 40 °C and was held for 1 min, followed by a 10 °C/min increase to a final temperature of 250 °C which was held constant for 5 min. The injector and interface temperatures were held isothermally at 200 °C and 250 °C, respectively. The sample (500 µL) was injected into the column manually from a

500 µL gas-tight syringe (Hamilton, USA) and the detector was set with a scan interval of 0.15 s and m/z range of 45 – 350.

2.3 Procedure

2.3.1 Headspace analysis of bacterial samples using GC-DMS

For each analysis, after the incubation, from each sample of liquid cultures and blank media, 500 µL of headspace sample was collected using a 500-µL gas-tight syringe by injecting the syringe through the air-tight septum cap until the syringe reached the headspace gases area. The height of each headspace vial was 75 mm, approximately half of it was filled by sample, and the 500 µL headspace gases were taken 10 mm above the surface of the liquid sample. The headspace gases were then injected into the miniaturized GC-DMS, through the sample inlet in the modified sample introduction system whose schematic representation has been detailed in the earlier work [2]. The sample was then immediately stored in the dark at 4 °C to preserve the cell concentration. The cell concentration was then counted using Thoma cell counting chambers.

For each analysis with GC-DMS, the GC-DMS retention time was limited to 500 s. Between each analysis, post cooling, cleaning, and preheating were done until all operating conditions were equal to the determined operating conditions. All analysis was made in triplicate.

2.3.2 Headspace analysis of bacterial samples using GC-MS

For each analysis, after the 24 h of incubation, from each sample of liquid cultures and blank media, 500 µL of headspace sample was collected using a 500-µL gas-tight syringe and injected into the GC sample inlet manually. The sample was then immediately stored in the dark at 4 °C. The cell concentration was then counted using a Thoma cell counting chamber. For each analysis with the GC-MS, the span of retention time was recorded until 22 min. All analysis was made in triplicate.

2.3.3 Cell counting

The bacterial cells concentration were counted using a Thoma cell counting chamber (with 0.1–mm depth) after the incubation and after each run of analysis. For each cell counting process, 20 areas were selected and the total sum of the bacteria was averaged. The procedure was duplicated and the final concentration was averaged from both counting.

2.3.4 Headspace analysis of standard compounds using GC-DMS

Prior to the headspace analysis of the standard solutions of *o*-nitrophenol, 4-methylumbelliferone, and indole using GC-DMS, each sample was preheated for 15 min at 36 °C and mixed using a magnetic stirrer. Headspace gases (500 µL per analysis) were then collected using a 500-µL gas-tight syringe and injected into the miniaturized GC-DMS sample inlet manually. Before the headspace gases of the standard compounds were injected, at least 100 blank spectra were collected and each analyte was measured in triplicate.

2.4 Data Analysis

2.4.1 GC-DMS data

For each analysis, the GC-DMS spectra for positive and negative ions were recorded using Expert™ and the chromatographic data was automatically stored as Microsoft Office Excel workbook. IGOR Pro 6 was then used to generate the GC-DMS spectra, to process the data, and to generate graphs.

In generating the retention time and compensation voltage graphs, IGOR Pro 6 was used. The data were processed using the Gaussian curve fitting functions.

2.4.2 GC-MS data

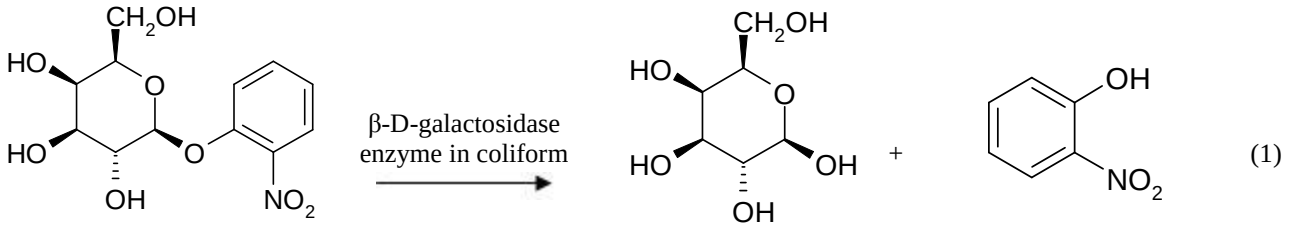
For each analysis, the GC-MS spectra were recorded using “LabSolutions” version 2.30 (Shimadzu Corporation) and the chromatographic data was automatically stored as “*.qgd” data file. The “PostRun” version of the software was then used to analyze the data and to generate graphs.

In identifying peaks, besides applying manual observation (by comparing the visually observed peaks from the blank samples and peaks from the spiked samples), auto-integration was also applied. Using “similarity search” function which is connected to the NIST library database, the peaks were then identified.

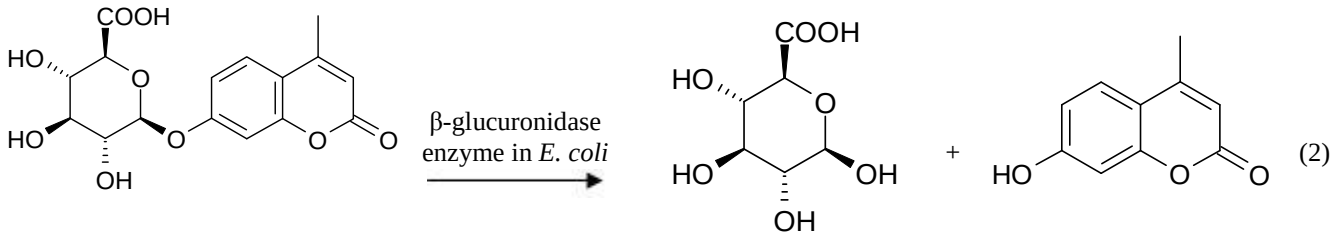
In presenting the spectra, each spectrum in the figure was also smoothed. The GC qualitative parameters were as follow: the smoothing was done using “Standard” filter, the number of smoothing times was 99, the smoothing width was 1 s; 5 highest peaks from each spectrum were auto-integrated and the identified peaks were listed in the fragment table. The GC-MS data were then presented in two forms: the total ion chromatograms (TIC) and multi-ions chromatograms (MIC).

3. Theory / Foundation for the Work

In a standard method using Colilert®-18, after 18 h of incubation, the presence/ absence of coliform (including *E. coli*) can be confirmed by the presence/ absence of yellow color of *o*-nitrophenol. Specifically for *E. coli*, the presence/ absence of *E. coli* can be confirmed by the presence/ absence of light blue fluorescence effect. These detections are based upon the following enzymatic approach [4; 30; 31]:



o-nitrophenyl-β-D-galactopyranoside (ONPG) | β-D-galactose | *o*-nitrophenol (yellow)



4-methylumbelliferyl-β-D-glucoronide (MUG) | β-D-glucuronate | 4-methylumbelliferone (light blue fluorescence)

Fig. 1: Enzymatic reaction of coliform and *E.coli* bacteria

Colilert®-18 contains *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucoronide (MUG) substrates. In the presence of ONPG substrate, the β-D-galactosidase enzyme in coliform bacteria would hydrolyze the ONPG, producing a yellow substance, *o*-nitrophenol. In the presence of MUG substrate, the β–glucuronidase in *E. coli* would hydrolyse MUG, producing a fluorogenic compound, 4-methylumbelliferone, which shows blue fluorescence effect under 365 nm, 6 Watt fluorescent UV lamp [31]. These changes could be distinguished after 18 h and used as indicators for the presence/ absence of coliform and *E. coli* in water sample. As such, *o*-nitrophenol and 4-methylumbelliferone could be used as biomarkers of coliform and *E. coli* grown in a specific controlled environment. This is also in accordance to the recently redesigned coliform taxonomy in which coliform bacteria are characterized by the presence of β-galactosidase enzyme and *E. coli* bacteria are characterized by the presence of β-glucuronidase enzyme [4; 5; 31; 32; 33; 34; 35]. These are more accurate than the classical definition of coliform and *E. coli* because some studies reported that not all coliform bacteria can ferment lactose [5; 36] and some *E. coli* strains neither ferment lactose nor produce indole [36], as defined classically. Upon this basis, in this work, *o*-nitrophenol will be used as the biomarker for the presence of coliform (including *E. coli*), whereas 4-methylumbelliferone will be used as the biomarker for the presence of *E. coli*.

4. Results and Discussion

Five different media were used to grow *E. coli*: Colilert®-18, glucose broth, M9-medium, tryptophan broth, and tryptic soy broth (TSB). Volatile metabolite compounds released by the bacteria were characterized using GC-DMS and the result was validated by GC-MS.

The detection of the volatile metabolites by GC-DMS in which *E. coli* were grown in Colilert®-18 is given in Figure 2, both for the positive and negative ion channels. As can be seen in the figure, signal peak of *o*-nitrophenol

appeared at a retention time t_r = 195 s. In the positive mode, this peak appeared at a compensation voltage C_v = –2.82 V, whereas in the negative mode, this peak appeared at C_v = –4.09 V.

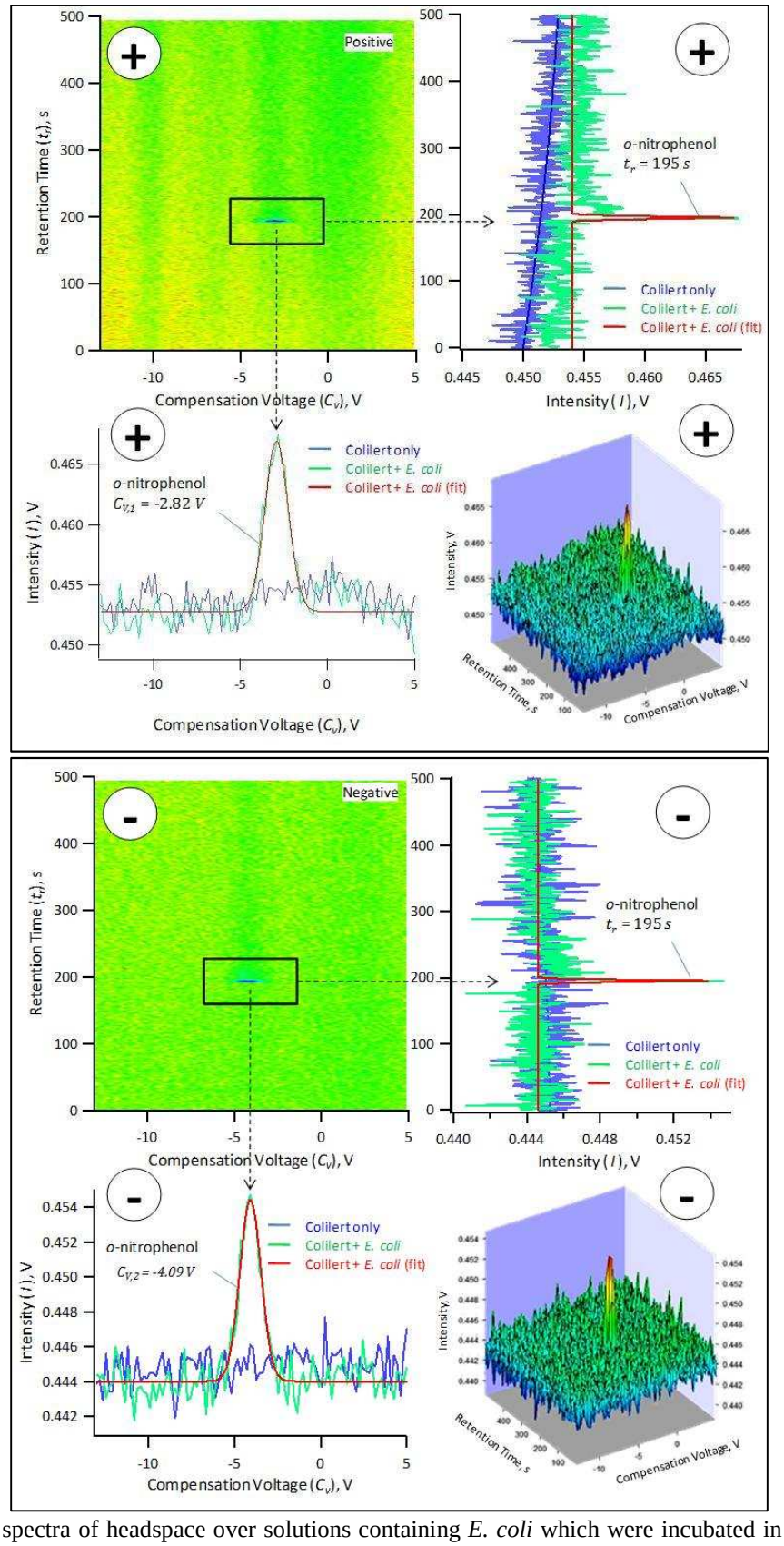


Figure 2. GC-DMS spectra of headspace over solutions containing *E. coli* which were incubated in Colilert®-18 for 18 h, both for the positive (top frame) and negative (bottom frame) ion channels, given as 2D spectra (top left), 3D spectra (bottom right) retention time profile, with and without *E. coli* (top right) and compensation voltage profile, with and without *E. coli* (bottom left)

After 3 h of incubation, the signal/noise ratio for *o*-nitrophenol was about 1:3. However, as can be seen in Figure 2, after 18 h of incubation a significant signal of *o*-nitrophenol was observed. Nevertheless, even after 24 h of incubation, 4-methylumbelliferone in the given concentration was not detectable. The detection of volatile metabolite compounds released by *E. coli* which were grown in other media is given in Figure 3. As can be seen in Figure 3, no biomarkers could be detected by the GC-DMS when the headspace gases were collected from sample vials containing *E. coli* which were grown in glucose broth, M9-medium, tryptophan broth, and TSB.

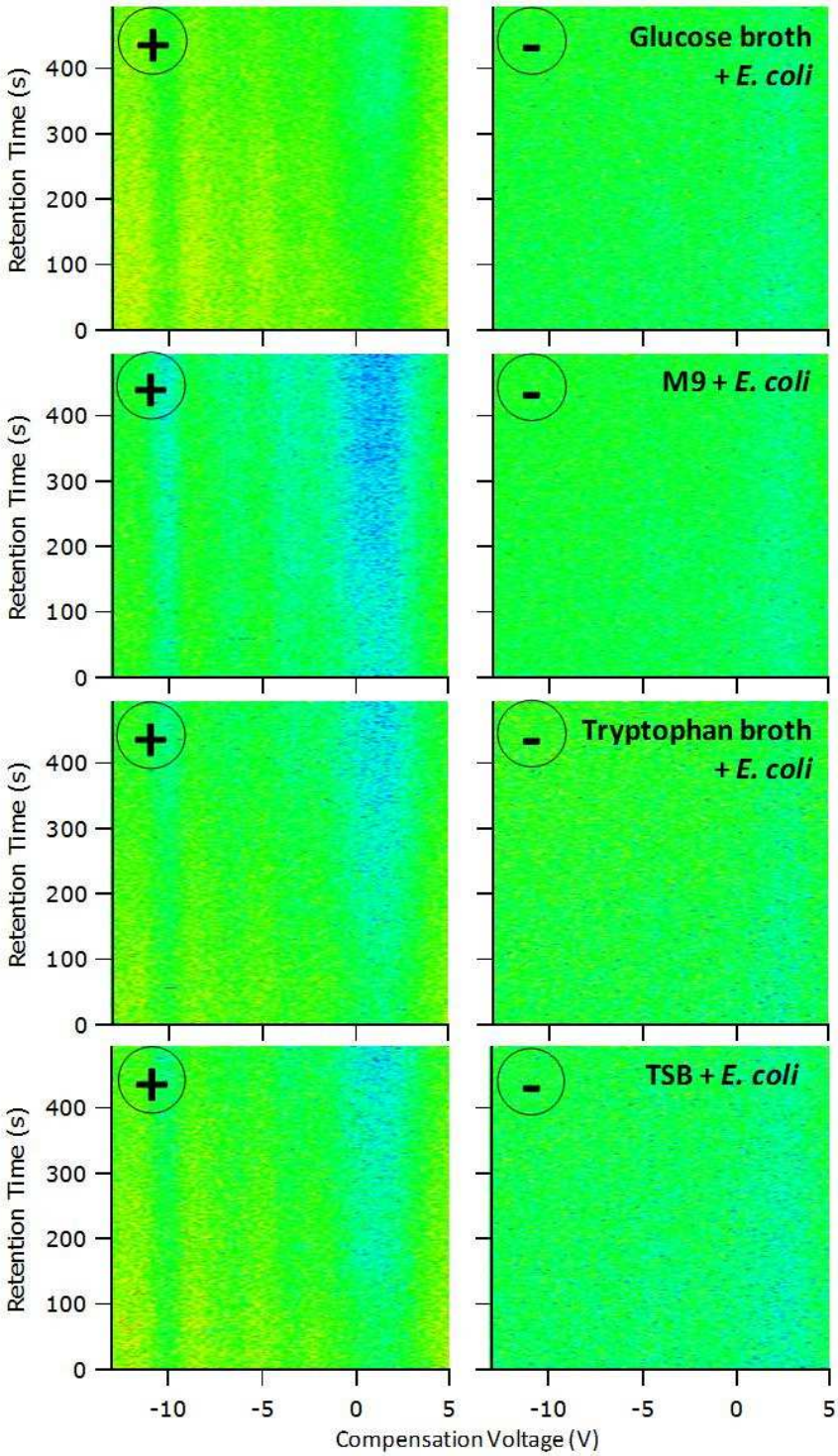


Figure 3. GC-DMS spectra of headspace collected from media (glucose broth, M9-medium, tryptophan broth, and TSB) spiked by *E. coli*, after 24 h incubation

The results from GC-DMS were validated by GC-MS. The results from GC-MS are given in Figure 4 and presented as total ion chromatogram (TIC) and multi-ions chromatogram (MIC).

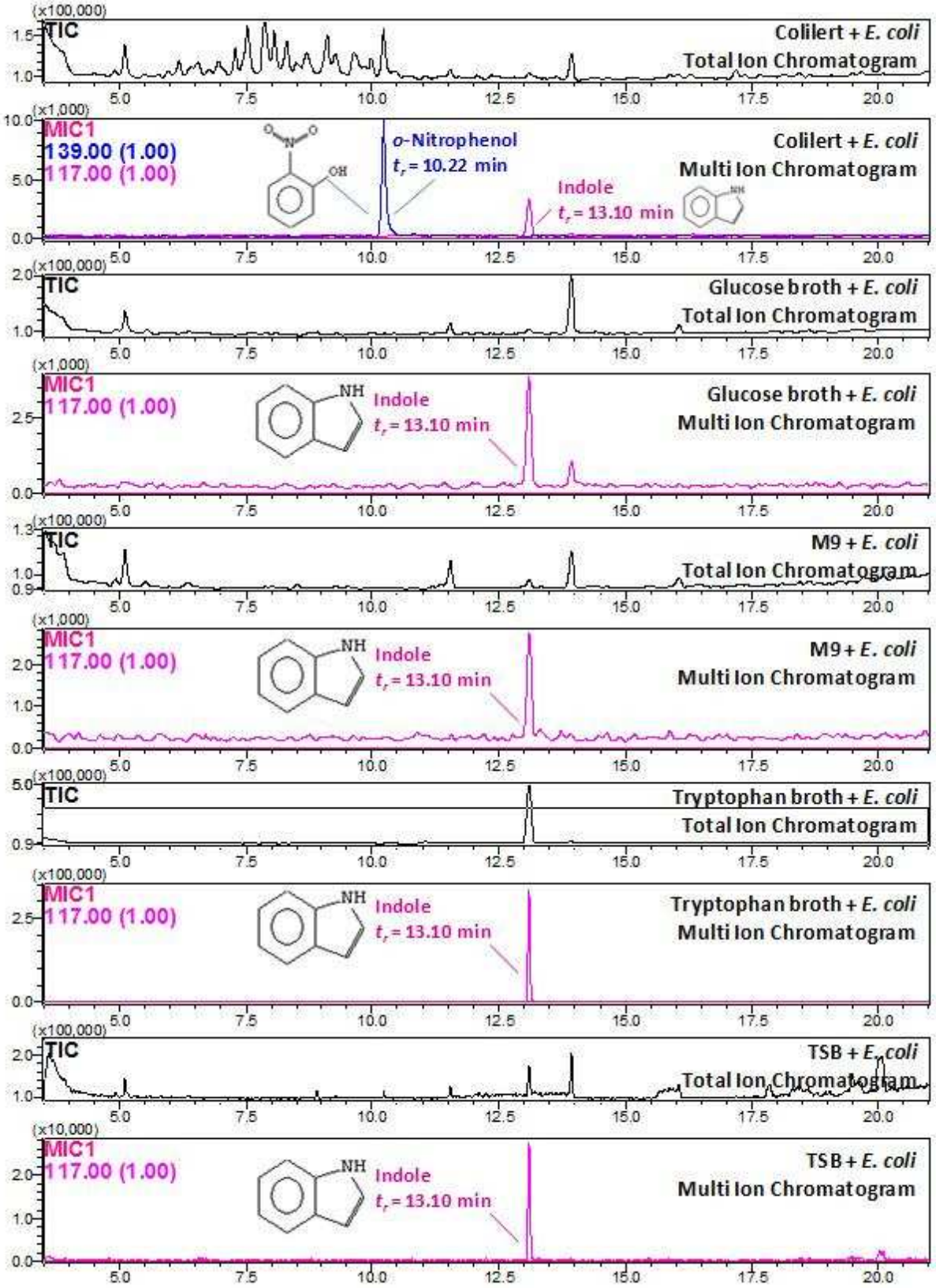


Figure 4. GC-MS spectra of volatile metabolites of *E. coli* incubated in five different media

As can be seen in Figure 4, in Colilert-18, *o*-nitrophenol was detected (at t_r = 10.22 min), whereas in all other media, *o*-nitrophenol was not detected. Indole peak was detected (at t_r = 13.10 min) in all media including Colilert-18. No peak of 4-methlyumbelliferone was detected in any of the media.

The results in Figure 3 and Figure 4 are summarized in Figure 5. In addition to that, the intensities of the signal peaks are given as normalized peak areas.

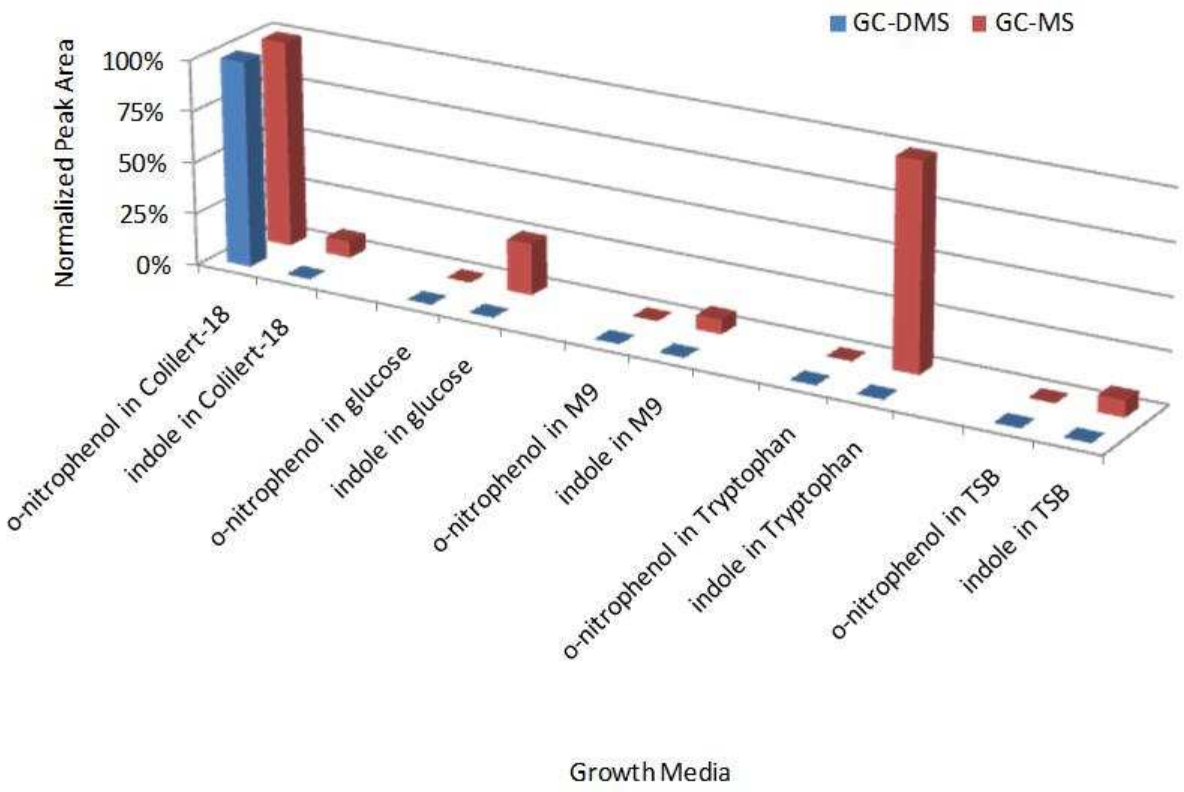
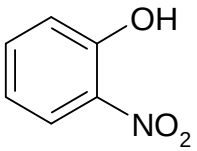
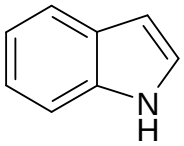
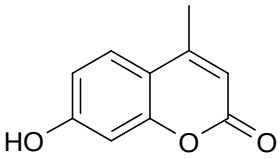


Figure 5. Normalized peak area of volatile metabolite compounds released by *E. coli* grown in different media andtheir detection by GC-DMS and GC-MS

The absence of *o*-nitrophenol and 4-methylumbelliferone in headspace over solutions containing *E. coli* in glucose broth, M9-medium, tryptophan broth, and TSB are attributed to the absence of ONPG and MUG in those media. The absence of 4-methylumbelliferone in headspace over Colilert-18 (despite the presence of MUG) containing *E. coli* is attributed to the physicochemical properties (low vapor pressure and Henry constant) of 4-methylumbelliferone and the sensitivity of the method. The presence of indole in all media when detected by the GC-MS and the absence of indole in all media when detected by the GC-DMS showed that GC-MS has a more sensitive detection towards certain compounds with low volatility such as indole than the GC-DMS. Several physicochemical properties of indole, 4-methylumbelliferone, and *o*-nitrophenol are given in Table 1. As also shown in earlier work [2], GC-DMS has better sensitivity towards compounds with lower molecular weights, high vapor pressure, and high inverse Henry's constants, which result in higher degree of volatilities and lower retention times. In compare to indole, the volatility of *o*-nitrophenol is much higher than the volatility of indole, as shown by their values of Henry's constants and vapor pressures.

Table 1. Properties of the standard compounds

Compounds	Structure	Molecular Weight, Mr ($\frac{gr}{mol}$)	Inverse Henry's constant, $K_{H,inv}$ ($\frac{atm.m^3}{mol}$ at $T = 298.15K$ and $\rho_{H_2O} = 997\ \frac{kg}{m^3}$)	Vapor Pressure, P ($mmHg$)	Reference
o-nitrophenol ($C_6H_5NO_3$)		139.11	1.63×10^{-5}	1 (at 49.3 °C)	[37]
Indole (C_8H_7N)		117.15	5.3×10^{-7}	0.0122 (at 25 °C)	[37]
4-methylum-belliferone ($C_{10}H_8O_3$)		176.17	n/a	n/a	[37]

In addition to that, in comparison to other studies, the result from this study produced fewer compounds. *E. coli* grown on BHI broth produced at least 12 different compounds including indole [26]; *E. coli* grown on LB agar produced several ketone compounds [11; 25]; *E. coli* grown on Minimal medium produced hexane, benzaldehyde, butan-1-ol, ethanol, acetone, and several ketone compounds (hexan-2-one, heptan-2-one, and nonan-2-one) [25]; and *E. coli* grown on TSB produced several alcohol compounds [25; 38].

The fewer types of compounds produced by the bacteria in this study is likely caused by the simplified approach used in the experiment, especially in the sample introduction method (in addition to the different type of medium used). As outlined in the material and methods, unlike in many other studies in which auto sampler and SPME fiber were used, only a simple gas-tight syringe was used in this study to inject the sample manually. The approach was simplified in accordance with the aim of the study to achieve a rapid, on-site detection of *E. coli*. In addition to that, the type of biomarker produced in this study (*o*-nitrophenol) is in agreement with the redefined coliform taxonomy because the presence of *o*-nitrophenol confirms the presence of β-galactosidase enzyme and hence coliform. To confirm the presence of 4-methylumbelliferone,, 6 Watt fluorescent UV lamp (365 nm) is still needed.

5. Conclusions

The presented results are significant enough to demonstrate the ability of the developed method to detect coliform bacteria (including *E.coli*) after 3-18 h of incubation. The achieved incubation time is already shorter than the time for standard methods (18-48 h). Nevertheless, further optimization of the method is required to reduce the overall analysis time. The developed method is based on the reaction of coliform bacteria with ONPG to β-D-Galactose and *o*-nitrophenol, which was detected by a miniaturized GC-Differential Mobility Spectrometer. The experiments described in this paper were performed under laboratory conditions. However, experiments under field conditions have not yet been carried out and will be the topic of our further research.

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Gas Chromatography – Differential Mobility Spectrometry and Gas Chromatography – Mass Spectrometry for the detection of coliform bacteria

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