Volatile organic compounds from halophilic Greenlandic bacteria as potential biomarkers of life on exoplanets

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INTRODUCTION

The direct observation of extraterrestrial life is hindered by technological limitations, necessitating the exploration of biomarkers as indicators of life beyond Earth's confines. Biogenic Volatile Organic Compounds (BVOCs) are one such potential biomarkers, because some are unequivocally biogenic and can contain chiral centers that can reveal enzymatic biases. Furthermore, in the presence of a well-established biosphere, BVOCs can measurably influence atmospheric chemistry and cloud formation on a global scale.

Peary Land (Northern Greenland) is characterized by low precipitation (25mm/yr) and low temperatures (annual average of -15°C), properties which makes this polar desert a terrestrial analogue of cold desert planets like Mars. Salinity can affect the freezing point of water, which is relevant in cold desert planets where liquid water may be scarce. Therefore, it is important to detail metabolic changes under such conditions. To investigate the potential of BVOCs as biomarkers, three psychrophilic, halophilic, gram-positive bacterial strains isolated from this harsh region (*Nesterenkonia aurantiaca*, *N. halotolerants* and *Oceanobacillus sp.*) were grown under different NaCl concentrations (5, 10 and 15%), and the volatiles produced were analyzed. The goal of this project is answering the following hypotheses:

1. Extremophilic bacteria isolated from North Greenland soils have distinct BVOC emissions.
2. Salinity affects the BVOC emissions from these bacteria.
3. The BVOC emissions can be used as biomarkers for the presence of extraterrestrial life.

Through this research, we aim to shed light on the potential utility of BVOCs as biomarkers in extraterrestrial settings.

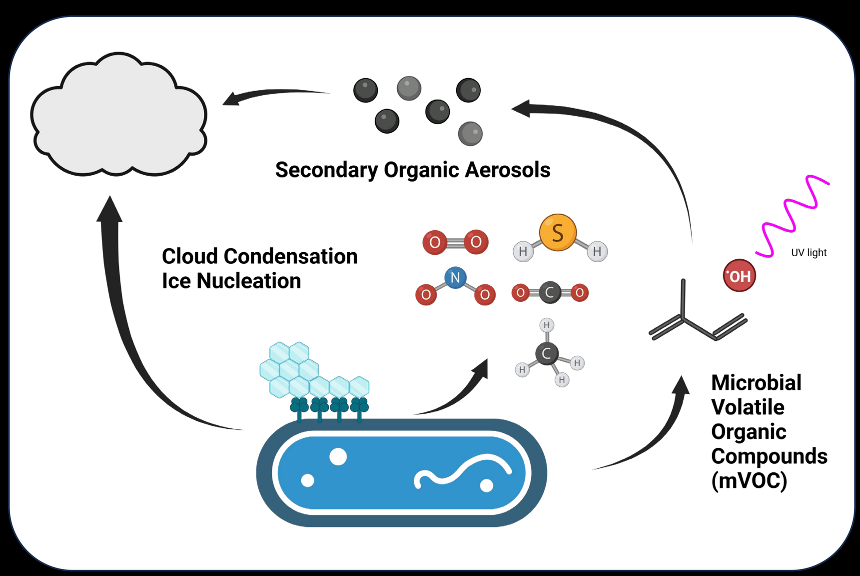

Figure 1. The biosphere and the atmosphere are inevitably linked. All life, including bacteria, release and consume a variety of gases, including BVOCs. These BVOCs affect redox balance of the atmosphere by reacting with hydroxyl radicals and other reactive species to produce NO_x , O_3 and secondary organic aerosols, which can have cloud nucleating properties. Furthermore, some bacteria produce Ice Nucleating Proteins, which nucleate ice in supercooled cloud droplets and induce precipitation.

MATERIALS AND METHODS

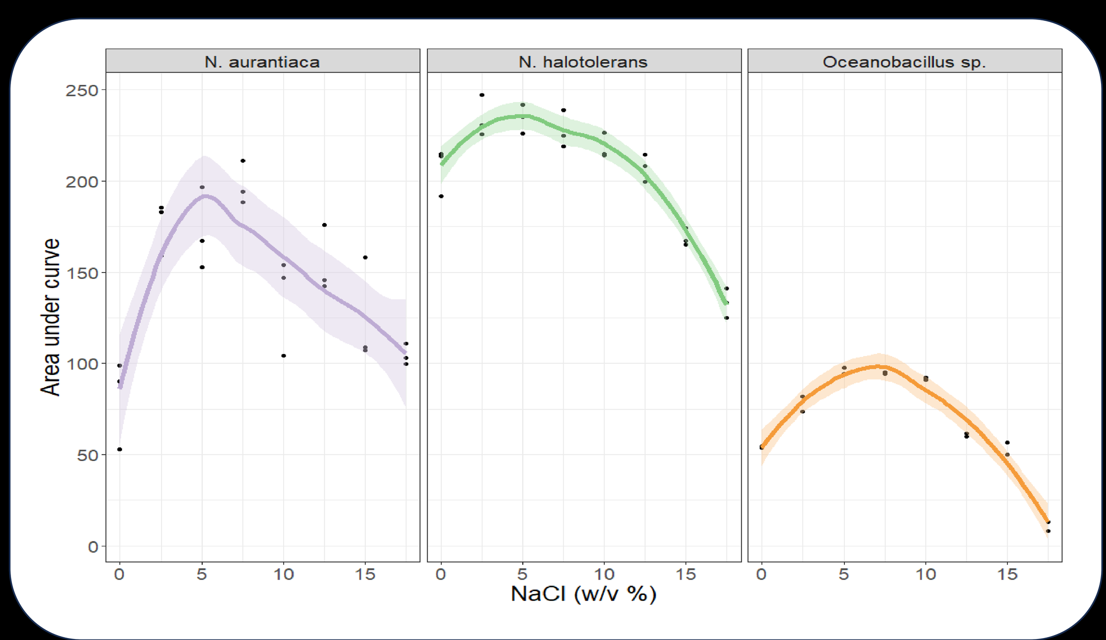

Figure 2. Growth of the selected strains in HM medium (1) under different salinity after 6 days. The response was calculated by fitting OD_{600} data measured at regular intervals to a Gompertz model, and then calculating the area under the curve.

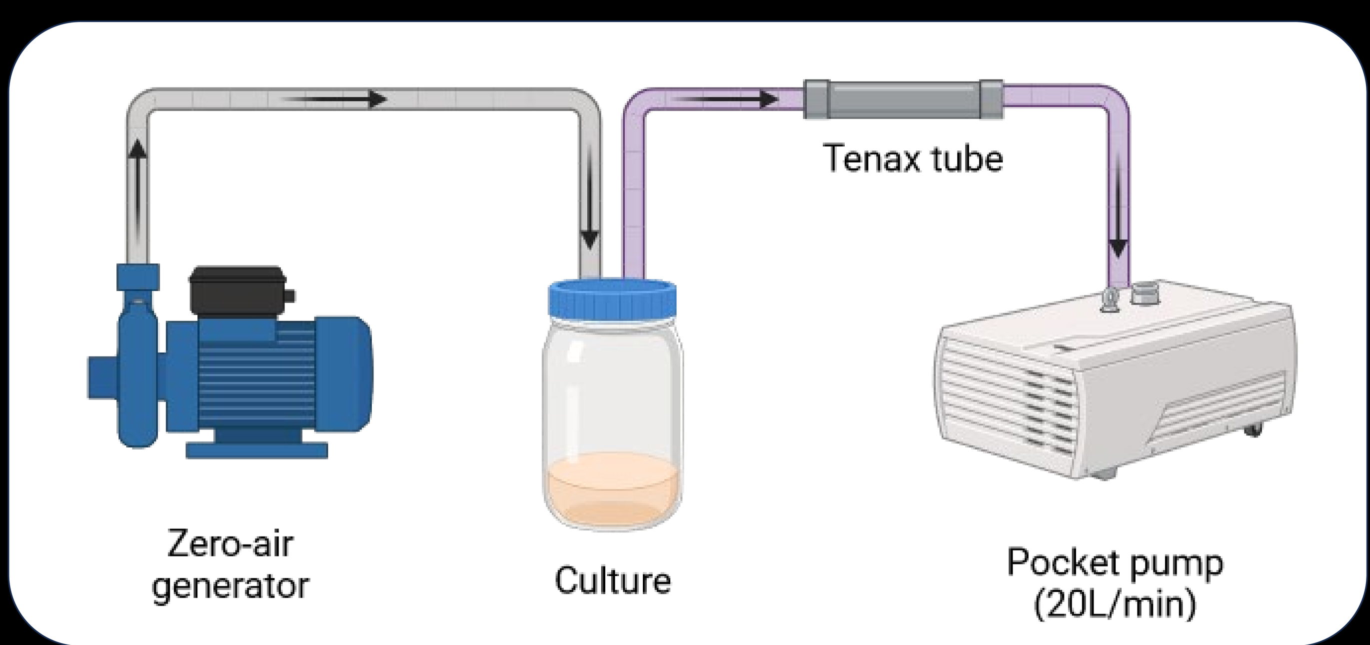

Figure 3. Dynamic headspace setup used to sample the volatiles produced by the strains. A zero-air generator was used to produce VOC-free air. Three conditions were tested: 5, 10 and 15% NaCl (w/v). After the cultures were grown aerobically in modified HM medium (1) at 25ºC until the late exponential phase (between 1 and 3 days, depending on the conditions and the strain), the headspace was sampled for 10 minutes at 35ºC with Tenax PDMS tubes. Negative controls were also incubated to subtract volatiles already in the medium during the data analysis.

The volatiles adsorbed to the tubes were later thermally desorbed and analysed via GC-MS. The GC-MS data was analysed with PARADISe (2). The compounds were identified based on MS data and the NIST14 database. They were then quantified using premixed standards ran before and after the samples.

RESULTS

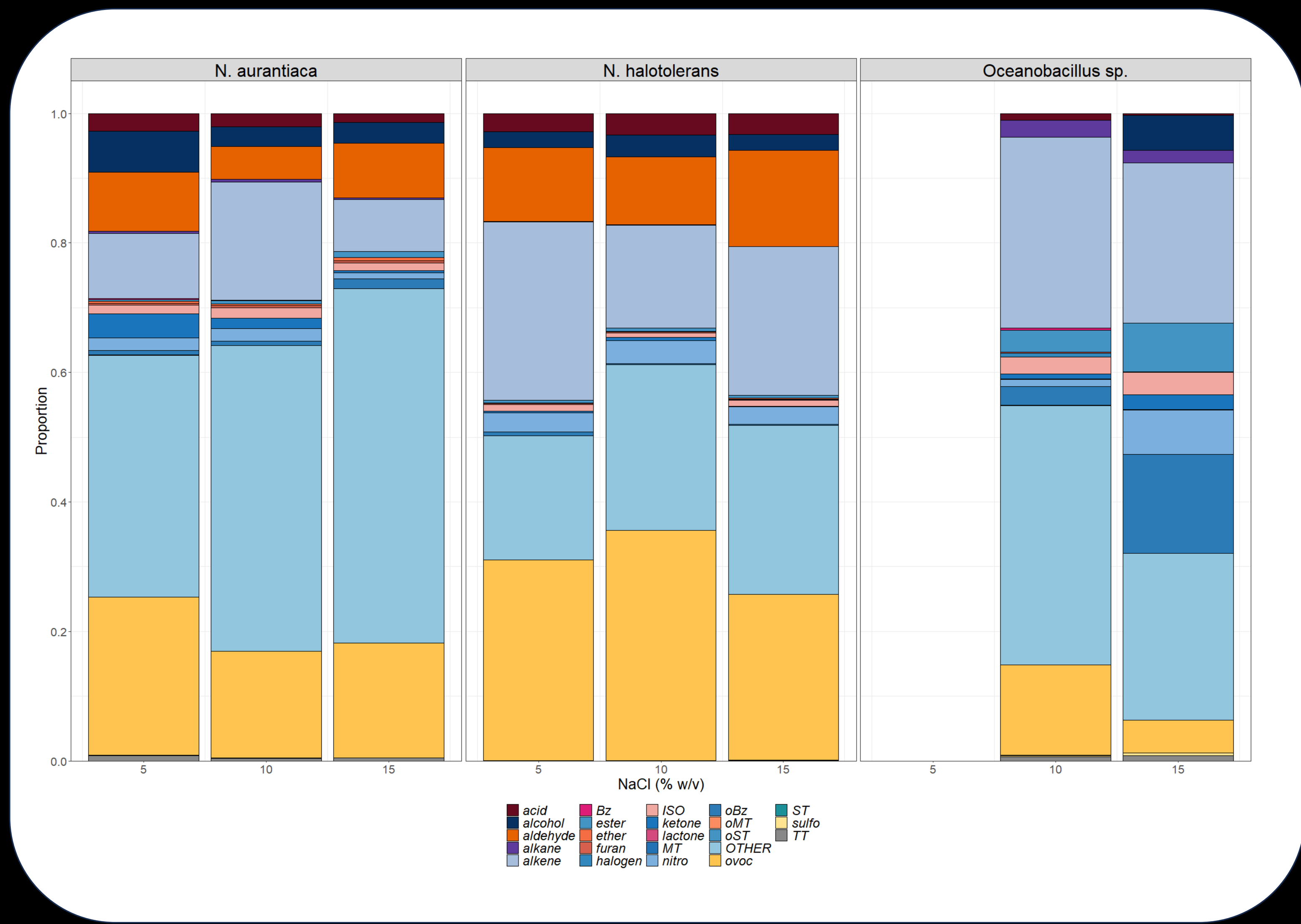

Figure 5. Average (n=3) blend of VOCs. Initials stand for benzenoid (Bz), isoprene (ISO), monoterpene (MT), nitrogenated compound (nitro), oxygenated benzenoid (oBz), oxygenated monoterpene (oMT), oxygenated sesquiterpene (oST), non-identified oxygenated VOC (ovoc), sesquiterpene (ST), sulfur-containing VOC (sulfo), triterpene (TT).

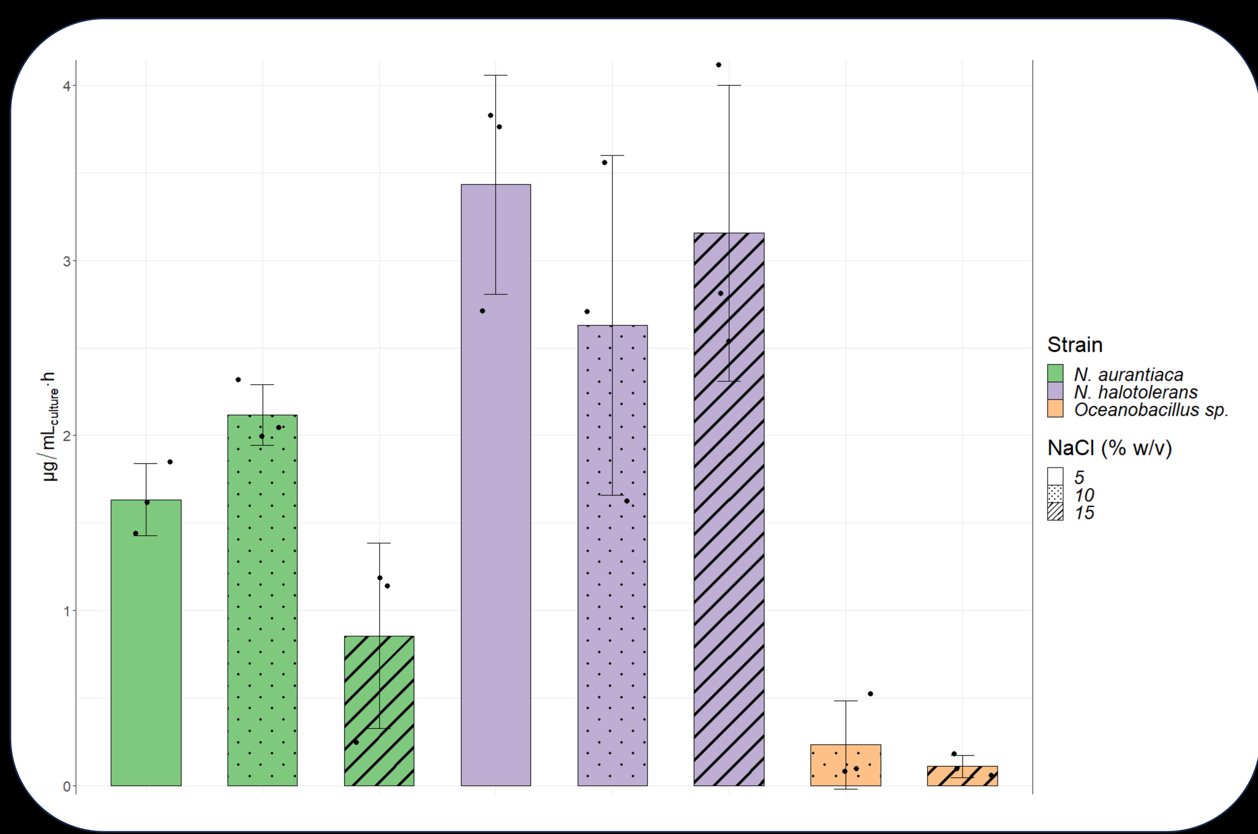

Figure 4. Total VOC emissions of the strains, in micrograms per millilitre of culture per hour.

Test	R value	P value
Emission rates		
Between strains	0,7292	0,0001
NaCl within *N. aurantiaca*	0,6543	0,0035
Between *Nesterenkonia* strains	0,6543	0,0001
VOC blend (%)		
Between strains	0,7059	0,0001
NaCl within *N. aurantiaca*	0,572	0,0069
Between *Nesterenkonia* strains	0,692	0,0001

Table 1. Similarity between samples (ANOSIM) (n= 3), for either the absolute emission rates, or the volatile blend. Only the statistically significant (p <0,01) results are shown. An extra test between both *Nesterenkonia* strains was also made to examine if the difference observed were only on the genus level.

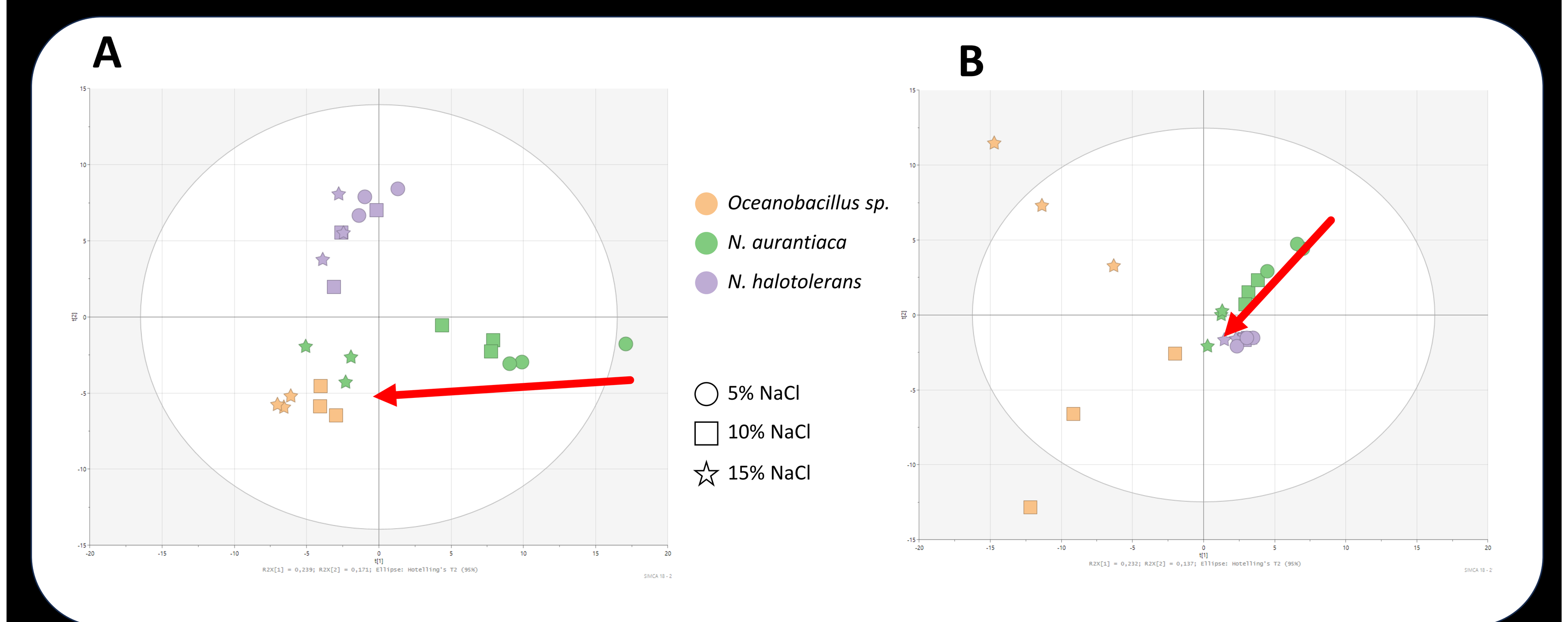

Figure 6. Principal Component Analysis of the absolute VOC emissions of the samples (A), and the VOC blend of each sample (B). Each strain forms a cluster, and there is a gradient of NaCl in *N. aurantiaca* (red arrows), corroborating the ANOSIM test results.

DISCUSSION, CONCLUSSIONS AND FUTURE DIRECTIONS

- All three strains exhibit distinct BVOC emissions (figure 6), with statistically significant effects of NaCl concentration observed in the case of *N. aurantiaca* (Table 1).
- The primary BVOCs produced by all three strains were 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propene, 2-methyl-butanal and 2-pentene.
- Future experiments should include better normalisation techniques to account for different growth rates and population densities, such as flow cytometry.
- We have demonstrated that at least some halophiles produce distinct BVOC blends. This discovery holds potential implications in areas like identification, taxonomy and biotechnology, including the production of biofuels by *Nesterenkonia* (3).
- Looking forward, the detection of these compounds on Earth-like exoplanets will require further technological advancement, but it is important to characterise the emission of these compounds for the time when such technology becomes available.

ACKNOWLEDGMENTS

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