REAL-TIME ANALYSIS OF VOC PRODUCTION FROM AN ALGAE BIOREACTOR USING SELECTED ION FLOW TUBE MASS SPECTROMETRY (SIFT-MS)

APPLICATION NOTE AS-251

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Abstract

Algae can be cultivated in large bioreactors, using a range of technologies, for purposes as diverse as biomass production, water treatment and CO_2 fixation. Algae biomass can be extracted or refined to produce compounds such as bioethanol or higher value products such as fatty acids and protein. Careful monitoring of the healthy of the bioreactor can significantly increase yields, leading to a commensurate increase in income from harvested products. The ability of SIFT-MS to measure gas phase volatile organic compounds (VOCs), in real-time, offers a unique approach to monitoring critical compounds produced by the bioreactor, potentially at low parts-per-billion by volume (ppbV) levels. This Application Note demonstrates this, on a pilot scale facility, over the course of several weeks and shows the utility of this approach in maximising outcomes.

INTRODUCTION

Algae bioreactors are used for cultivating algae for a range of purposes, such as biomass production, wastewater treatment and CO_2 fixation. Biomass production of algae can be used as foodstuff or by extracting and refining for compounds such as bioethanol or more high-value products such as fatty acids and proteins. A common approach is to use photobioreactors using LED lighting to provide energy and pumps to keep the aqueous system and dissolved gas and nutrients in constant circulation with the algae. Periodic harvesting of the biomass takes place over a production run of several weeks. In order to maximise the yields it is important monitor the health of the reactor and SIFT-MS offers a unique real-time approach to monitor a wide range of potentially critical volatile organic compounds (VOC) produced in the photobioreactor, potentially at low parts-per-billion by volume (ppbV) levels. Depending on the size of photobioreactor used and products being harvested, increasing yields can potentially lead to several hundred thousand pounds increase in production income per year.

In order to assess the capabilities, a Syft Technologies' Voice200*ultra* SIFT-MS was coupled to a pilot scale photobioreactor, consisting of several thousand litres of circulating aqueous media and allowed to passively monitor untargeted analysis of evolved VOCs over several weeks of runtime. The acquired data was periodically analysed to determine whether compounds of interest were being formed. In addition to the real-time analysis capability, when compared to GC-MS, the volatility of the expected analytes are also very amenable to SIFT-MS analysis

EXPERIMENTAL

Instrumentation

SIFT-MS: Syft Technologies Single Polarity Voice200*ultra* running in a flow-passed configuration.

METHOD

Approximately 8 metres of unheated ¼" Teflon tubing was run from the top side port of a large expansion tank attached to the flowing photobioreactor. In order to minimise residence

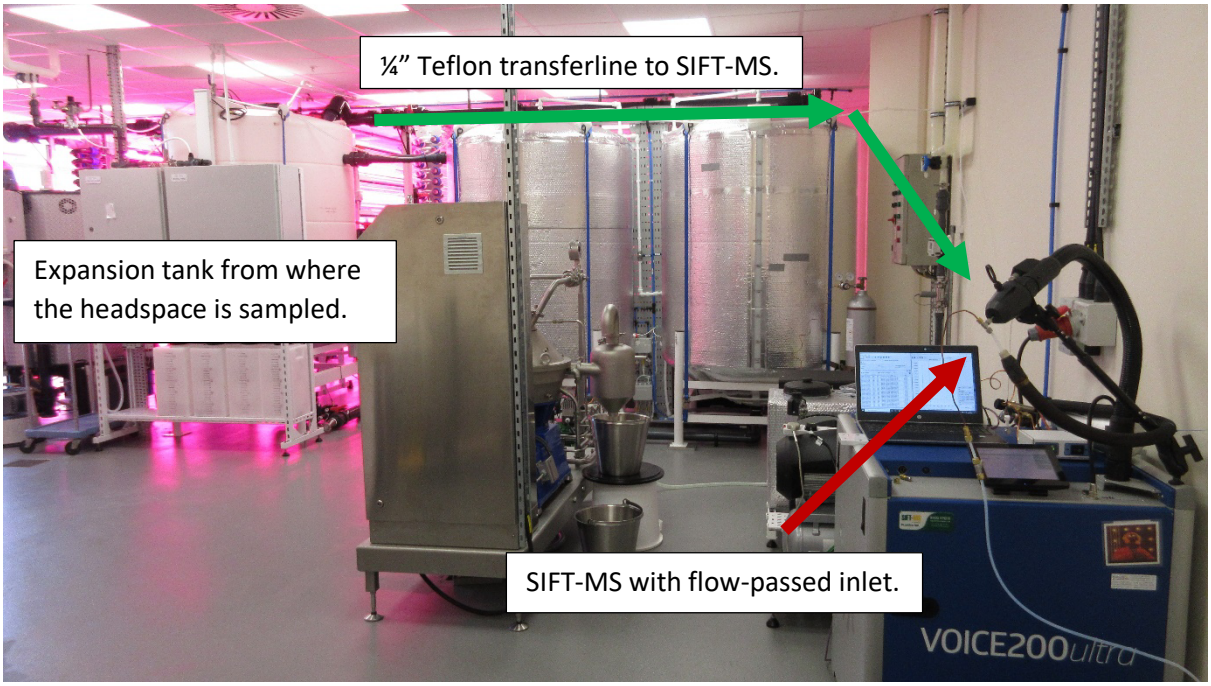

Figure 1: *Configuration for sampling*

time in the transferline, this was connected to the inlet of the instrument in a flow passed configuration, with a pump downstream from the inlet. Any volatile compounds produced during the production run were measured from the large headspace (several hundred litres) of the expansion tank (see figure 1).

Full scans for all three positive reagent ions – H_3O^+, NO^+ and O_2^+ – from 15 to 250 Da, were run at fixed intervals of about 16 minutes, with each run taking 1 minute, using the LabSyft batch scanner for over five weeks of continuous, passive sampling. It should be noted that there was minimal interaction between the instrument and the operators of the reactor. Examples of the scans obtained are shown in figures 2a and 2b (primary reagent ions have been removed from the spectra). Figure 2a is early in the production run, whilst figure 2b is from approximately 4 weeks later. It is clear from the increase in counts for the product ions in the spectra, that VOC generation has increased over the production run.

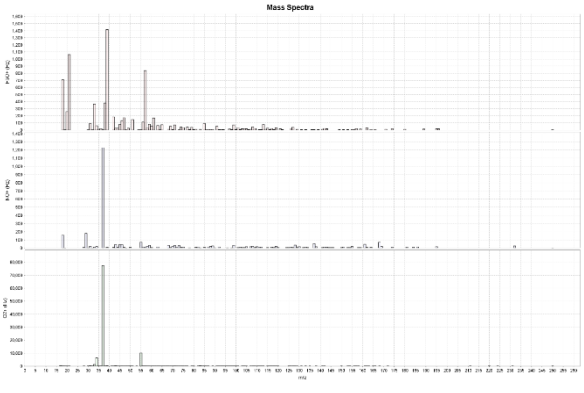

Figure 2a: *Full mass scan of headspace at the start of the production run.*

Literature searches to determine possible VOCs were carried out, but in addition, the full scans were analysed for possible compound matches, using the built-in LabSyft library and a list of possible VOCs present were selected and these are shown in Table 1.

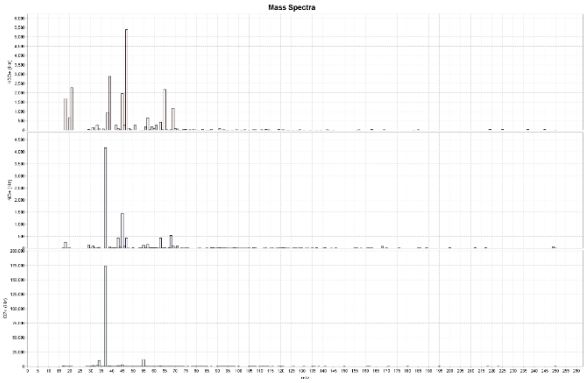

Figure 2b: *Full mass scan of headspace at mid-point of the production run.*

Table 1: *List of compounds extracted from full mass scan data.*

Compounds selected for analysis on full mass scans
Pentenal
Hexanal
Benzaldehyde
Pentenol
Methylbutanol
Diacetyl
Cyclocitral
Ethyl acetate
Methyl hexanoate
Methyl phenylacetate
Ethanol
Isopropyl alcohol
Isobornyl acetate
Heptadienal
Decadienal
Acetone
Acetaldehyde
Propanal
Isoprene
Ammonia
Methylamine
Dimethylamine
Trimethylamine
Dimethyl disulphide
Dimethyl sulphide
Dodecane

Using the data reprocessing function in the LabSyft method editor (see figure 3), it is possible to convert full scan data into VOC concentrations, by utilising the product ion and reaction rate data from the LabSyft library and hence, follow changes in VOC production over the entire production run. This was done for all analytes listed in Table 1.

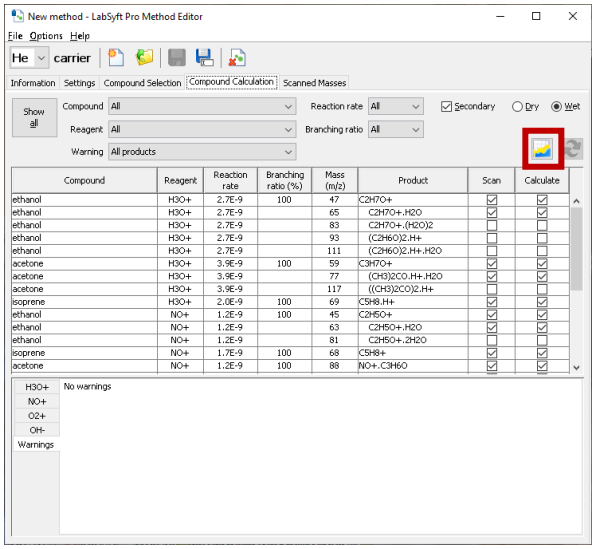

Figure 3: *Recalculate tool in LabSyft method editor.*

RESULTS

Analysis of the data showed that not all the potentially expected compounds were either present, or varied over the course of the production run, however, a number did show significant changes. Initial analysis seemed to suggest significant levels of ethanol being periodically produced, with significant spikes at days 12 and 31. Additionally, the small ethanol peaks appeared to occur regularly around midday. This can be seen in figures 4a and b. It was subsequently discovered that nutrients were added periodically into the expansion tank by removing the transferline tubing from the side port, which was cleaned with ethanol before reinsertion of the tubing, giving rise to periodic spikes of ethanol. Despite these significant excursions, the majority of other VOC measurements were not affected.

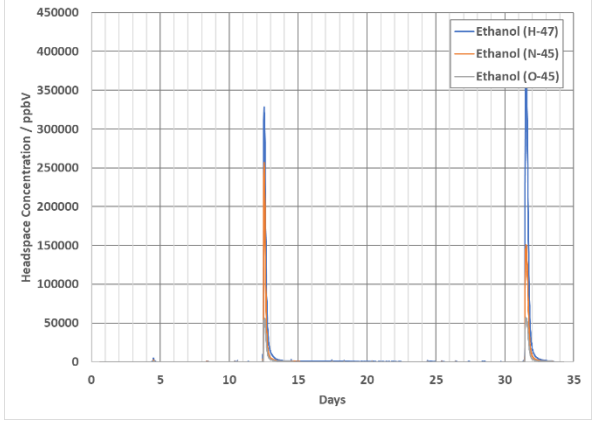

Figure 4a: *Significant changes in ethanol concentration can be seen at days 12 and 31.*

In the presence of water, both the H_3O^+ and NO^+ reagent ions will form water clusters, to generate $H_3O^+.H_2O$ at 37 Da and $NO^+.H_2O$ at 48 Da, that vary in intensity depending on the water vapour present. Therefore, from the full scan data, it is possible to follow changes in moisture, and figure 4c shows this for the $H_3O^+.H_2O$ water cluster.

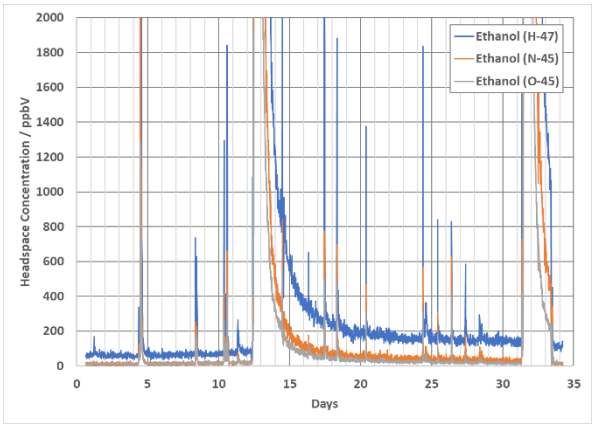

Figure 4b: *Enlarged plot of figure 4a showing small changes in ethanol concentration.*

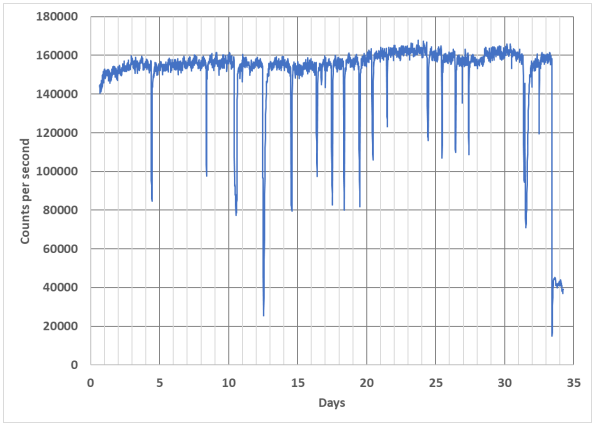

Figure 4c: *Detection of water vapour concentration using H_3O^+, which correlate with the ethanol spikes seen in figures 4a and 4b.*

It is clear, when compared to figure 4b, that there is a direct correlation between reduction in moisture levels and the spikes in ethanol due to sample port cleaning. The headspace inside the expansion tank will have a higher moisture content, due to the large amounts of water in the reactor, when compared to the ambient environment and thus, removal of the transferline tubing will lead to a reduction in measured water. Clearly, this allowsfor nutrient addition to be tracked in the volatile compound traces.

Figure 5a shows the concentration of isoprene measured using the four reagent ion-product ion pairs available and clearly shows, not just an overall rising concentration, but also variation within the traces over days or hours, but not at fixed intervals. By using all four product ions to measure the concentration changes, one can by confident that the variation is real and not underlying instrumental noise. Figure 5b shows the same plot with the moisture traces overlaid. Additionally, the red asterisks mark the points when the biomass was harvested. From this, the variations in isoprene concentrations can be related to the periodic addition of nutrients, in other words early growth after feeding followed by a gradual decline until the next feed point, suggesting that isoprene could be a key marker compound for tracking the growth of the algae, as isoprene is emitted by many species of algae.

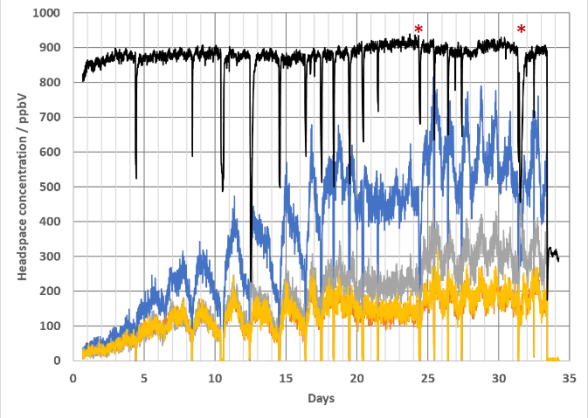

Figure 5b: *Figure 5a overlaid with moisture trace (black) and marked harvest points (red asterisk).*

In addition to compounds that may be showing beneficial growth of biomass, there are a number of VOCs that may be produced by unknown or unwanted constituents of the overall biome, for example bacteria. These are often nitrogen or sulphur-based compounds and may point to poor health in the photobioreactor. Figures 6a, 6b and figure 7 show the data obtained for ammonia, the methylamines and dimethyl sulphide. Rising concentrations of the small nitrogen containing compounds can clearly be seen. Dimethyl sulphide (DMS), shown in figure 7b, shows a number of sharp peaks, particularly at 12 and 31 days – these can be attributed to the significant spikes in concentrations of ethanol discussed above.

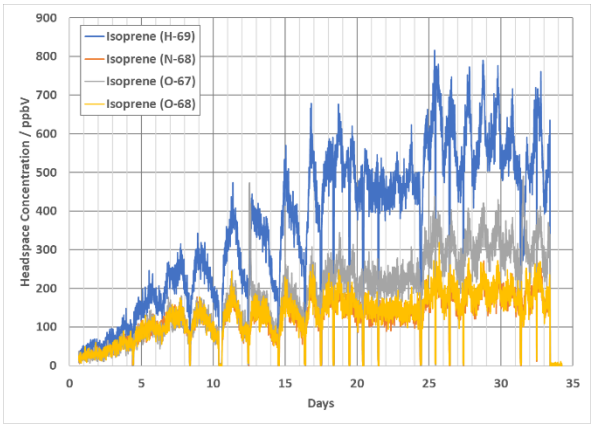

Figure 5a: *Concentration of isoprene, measured using all reagent ion-product ion pairs over 5 weeks of production run.*

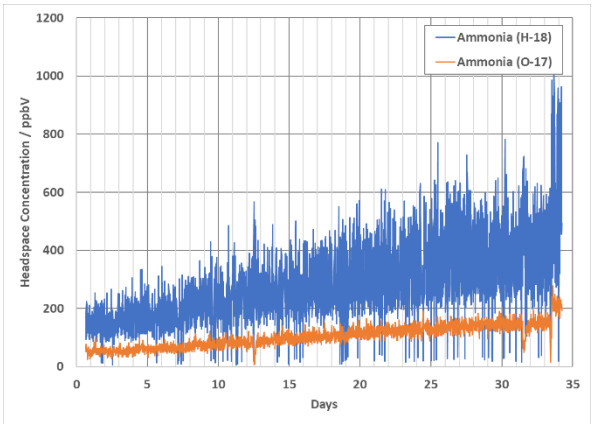

Figure 6a: *Concentration of ammonia over 5 weeks of production run.*

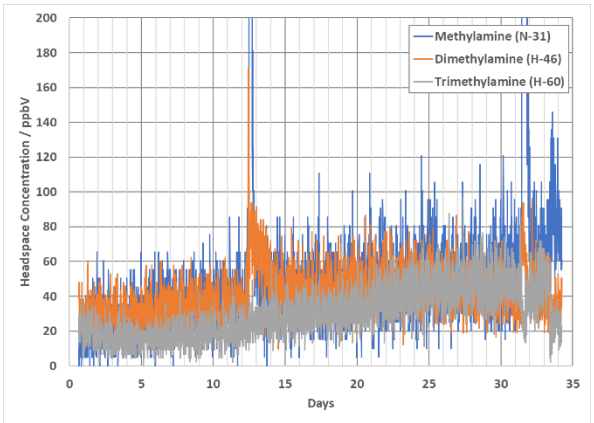

Figure 6b: *Concentration of mono, di and trimethylamine over 5 weeks of production run.*

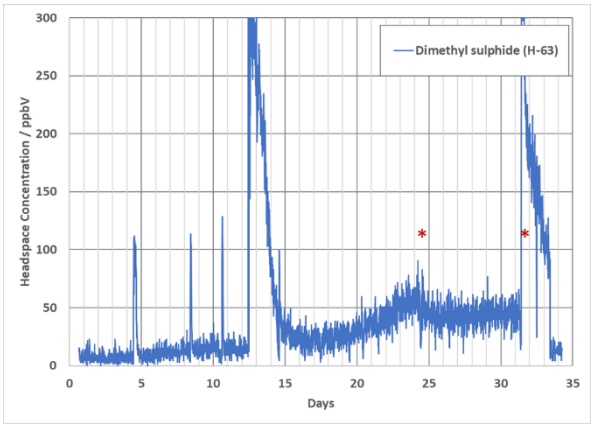

Figure 7: *Concentration of dimethyl sulphide over 5 weeks of production run (red asterisks show harvest points).*

Also shown in figure 7 are red asterisks, corresponding to biomass harvesting, and it is interesting to note that the rise in DMS concentration stops after the first harvest and only starts to recover immediately before the second harvest point.

A common concern raised with direct mass spectrometry techniques is the potential inability to separate isomers. One of the benefits of SIFT-MS having access to three positive reagent ions is that different reaction mechanism are available to each reagent ion, and consequently the ability to produce a range of different product ions and masses. Table 2 shows an example of this for propanal and acetone.

Table 2: *SIFT-MS reaction chemistry for acetone and propanal.*

Reagent Ion	Product Ion	m/z
	Propanal	
H_3O^+	Proton transfer	59
NO^+	Hydride extraction	57
O2+	Electron transfer	57, 58
	Acetone	
H_3O^+	Proton transfer	59
NO^+	Association	88
O2+	Electron transfer	43, 58

Figure 8a shows the concentration trace for either propanal or acetone as measured with H_3O^+ proton transfer reaction to give the 59 Da product ion. Clearly (as shown in Table 2), it is not possible to speciate with this single reagent ion and this signal could be either acetone, propanal or a combination of both. Figure 8b shows the same compounds measured using NO^+, which can separate the two analytes via the different reaction mechanisms available to it and it can be seen that the signal in figure 8a is entirely due to propanal.

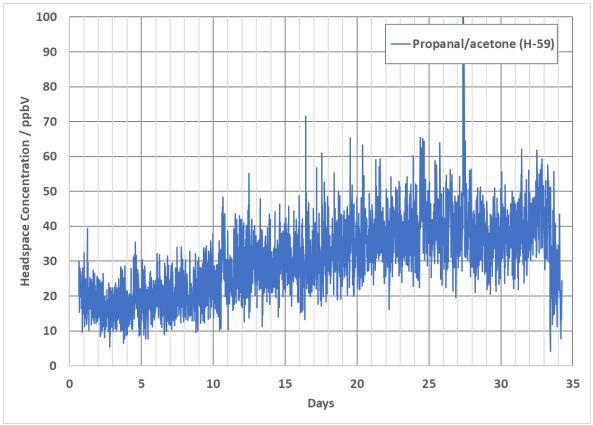

Figure 8a: *Concentration of either acetone or propanal measured using H_3O^+ - it is not possible to speciate using a single reagent ion.*

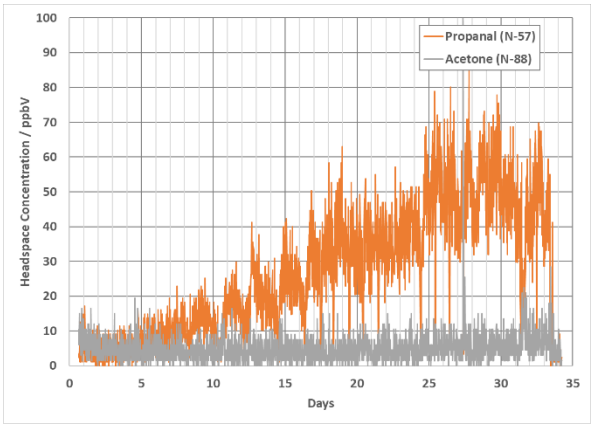

Figure 8b: *Separation of acetone and propanal, using the differential reaction chemistry of NO^+.*

Although only five weeks of data are presented in this Application Note, the entire production run lasted approximately nine weeks. Figures 9a and b show sampling carried out in the final day of the production run for isoprene and ammonia respectively. From the traces, the isoprene concentration has returned to baseline levels, whilst the ammonia has continued to climb towards 300 ppbV, suggesting the end of useful biomass growth can be detected through diminished isoprene generation.

CONCLUSION

The data presented in this Application Note shows the possibility of using a real-time VOC analyser, such as SIFT-MS to monitor long term VOC production from biological processes. The ability to monitor the entire mass range, followed by subsequent extraction of quantitative data allows for initial untargeted analysis to take place. Once compounds of interest have been identified, it is a straightforward process to convert the methods to target specific analytes. Often, the VOCs of interest are difficult to analyse by conventional means, either spot sampling or real-time – for example, ammonia and small sulphur compounds. Additionally, bioreactor environments have high moisture levels, increasing the difficulty further. SIFT-MS overcomes these problems with its ability to monitor small, polar molecules in wet environments with ease.

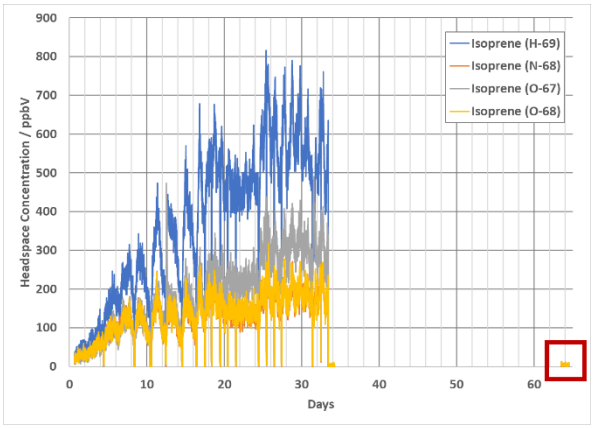

Figure 9a: *Concentration of isoprene, showing end-point concentrations at 9 weeks.*

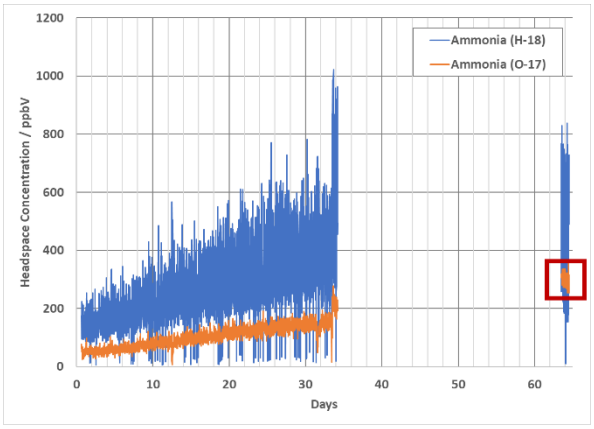

Figure 9b: *Concentration of ammonia, showing end-point concentrations at 9 weeks.*

The wide dynamic range, and fast sampling rate of SIFT-MS also meant that despite the significantly high, short term, concentrations spikes seen for ethanol during the periodic feed port cleaning there was minimal impact on the majority of the other product ions formed, allowing for meaningful measurements to continue. Also, the moisture detection inherent with this technique allowed for correlation of nutrient addition and relevant VOC productions. This should allow for more targeted feeding, increased biomass production, more frequent

harvesting and potentially enhanced yields of high value products.

In addition, the use of multiple reagents ions allows for the speciation of isomeric compounds, that would otherwise not be differentiated with other direct mass spectrometry techniques – as evidenced by the acetone/propanal separation.

Despite the relatively crude methodology used in this preliminary investigation, for example unheated transferlines and very large headspace volumes in the expansion tank, this Application Note demonstrates the ability of SIFT-MS to be of potentially significant help in bioreactor monitoring. By optimising the methodology, for example, the use of heated transferlines and minimising the sampling headspace to increase sensitivity, the capabilities should only improve.