Calculating Total Isoprene In Gas and Liquid Phase

Tuesday, October 16, 2012
2:37 PM

2012-10-24
Isoprene a...

Sean Weise 10-16-2012 The sample data in the attached sheet was from an experiment conducted for ZuvaChem on 10-02-2012 and is documented in lab notebook #166 pages 88-90.

The spreadsheet attached to this page is used to determine the amount of isoprene in moles or grams in a bacterial liquid culture from FIS data. The spreadsheet uses data from the FIS and uses the Henry's constant (explained in the OneNote page titled "Calculating Isoprene in the Liquid Phase") to determine the total isoprene amount in the flask both gas (headspace) and liquid phase. The spreadsheet integrates the photon counts in each peak by summing all the photon counts for the integration time (entered by the user) starting from the start time of each peak (entered by the user). The total photon counts from half the integration preceding and following the peak then are subtracted. For example if you had a peak that started at 120 seconds with an integration time of 30 seconds the total photon counts from 120 to 150 seconds are summed. The photon counts from 105 to 120 seconds and from 150 to 165 seconds are summed and subtracted from the photon counts from 120 to 150 seconds. This results in the photon counts of the peak with the baseline subtracted out.

As you are injecting samples into the FIS make sure the record the starting time of each peak in your lab notebook. **Record the starting time directly from the X-axis of the strip chart recorder display not from the time box in the left corner**. Record a starting time that is 2 - 3 seconds before you see the rise of the peak. If the sample isoprene concentration is too low to see record the time of the X-axis just after you finish injecting the sample. The lag time between injection and seeing a peak is around 5 seconds depending on the sample flow rate into the FIS.

A total flow of 1000 sccm and an oxygen flow of 800 sccm give good sensitivity when using the FIS in injection mode. Enter an integration time of 0.1 seconds in the FIS software to make sure enough point are recorded to adequately describe the peaks.

To calculate the total isoprene you need to know the following

- Total volume of flask or vial used to hold the liquid culture
- Volume of the liquid phase
- Temperature of flask or vial when headspace gas sample was taken
- Volume of headspace sample taken and injected onto FIS
- Concentration (mole fraction as ppm) of isoprene standard (This spreadsheet assumes that the same volume of standard is injected into the FIS as sample)
- The incubation time and $OD_{600\ nm}$ of the bacterial culture is also useful if you want express the isoprene production as a rate ($mol_{isp}\ L^{-1}_{culture}\ OD^{-1}\ hr^{-1}$).

1. To open FIS data file first open this spread sheet and click on the FIS Raw Data tab, select open and then choose "Text Files" in the lower right hand corner of the pop up box, select your data file from the FIS
2. Choose "Delimited" click "Next" then choose "Comma" then click "Finish"
3. The data from the FIS will now appear in a new spreadsheet. Delete the columns labeled "time(hrs)" and ignore the right most column labeled "hrsminsec"
4. Copy the left 2 columns labeled "time(s)" and "photon_counts_per_second" and paste these into the corresponding left 2 columns in the "Isoprene Amount from FIS Data" spreadsheet
5. Fill out all the information in blue at the top right
6. Enter the sample names and starting time of each peak on the right
7. Enter the integration time for each peak. We typically use 30 seconds. Our peaks are normally about 10 seconds wide. Sometimes the spread sheet returns a unexpected number for isoprene this can be the result of noise in the baseline. Changing the integration time by 5 seconds can sometimes correct this.
8. Enter the OD of the sample at the time you collected the isoprene if you wish to normalize the data based on OD