Biogeosciences Discuss., 11, C166–C171, 2014
www.biogeosciences-discuss.net/11/C166/2014/

Biogeosciences
Discussions
Open Access

Interactive comment on "Optimizing sample pretreatment for compound-specific stable carbon isotopic analysis of amino sugars in marine sediment" *by* R. Zhu et al.

Anonymous Referee #3

Received and published: 3 March 2014

In this manuscript the authors describe a method development for 13C of amino sugars in marine sediments. Until now considerable work with 13C in AS has been done in soils, though in marine environments there application not extant. The paper is quite well written and the method tested relatively extensively. This method will open new opportunities in this domain.

The proposed method is definitely of great relevance FOR some scopes of Biogeosciences, though I am not aware if methodological papers are within the scope of biogeosciences, they are definitely not common in the journal. I personally think it is a positive thing to have both method papers as 'science' papers in the same journal, but this is a decision for the editor to be taken. The developed method was additionally

applied on a couple of sediment samples which nicely demonstrate the applicability of the method.

A quite high importance is given to MurA as "purely bacterial" marker, though according to Parsons et al. 1981, MurN should not be used as a marker for bacterial residues in sediments and estuarine soils as cell walls of blue-green algae contain muramic acid in concentrations up to 50% of the dry weight (Sharon, 1965; Drews, 1973). This, information might be obsolete, but should be discussed in the MS.

There are a couple of methodological tests In this MS the authors compare different hydrolisation, purification, derivatisation procedures described in literature, though in multiple cases the author adapted the original procedure in a way that, I expect, is likely to lesser the efficiency of the original procedure, the reason of the deviation should be discussed: In the hydrolisation tests, I do not understand why the H2SO4 samples where evaporated to dryness (P598, L16) (this drying step is commonly used to remove the volatile acids, HCl and TFA, but will only concentrate the H2SO4) before being redissolved in small amount of water to be neutralized with Ba(OH)2. This is not according to the referred method (Cowie and Hedges 1987). Dying the hydrolate have 2 potential problems 1) the increased H2SO4 might alter the liberated sugars and 2) the precipitation of BaSO4 in this small volume probably increases the amount of co-precipitated (amino)sugars. In the Neutralization and desalting part, in the description of method according to Zhang and Amelung (1996) the samples are brought to pH (6.6-6.8) by adding this was mainly intended to remove the Fe, Mg by precipitation, centrifugation. Though here the precipitate did not appear to be separated by centrifugation? Starting on page 599 L13 the authors describe a purification using a cation exchange resin. It is a bit strange to refer to Amelung, 1996. In that paper Amelung et al. used the cation exchange column re move cationic impurities, which are retained on the resin while there analytes NEUTRAL AND ACIDIC sugars are not retained. Amino sugars are retained on the resin together with the salts. And will be eluted together with the salts when eluted with 2M NH4+ . So this procedure is definitely not a desalt-

ing procedure. It is a clean-up procedure though as it will remove neutral and anionic (acidic) contaminants. It would make much more sense to refer to Indorf et al., 2013 (also a method paper on purification of AS extract) or Bodé, 2013 who also used the same resin to purify AS in soil extracts for 13C determination. The author should also mention that the cation resin was in "H+" now the reader has to get the pre-conditioning in Amelung, 1996 to know this.

The definition of the lower limit for isotopic measurement seems rather odd to me P607 L 7 "< 20 ng indistinct peaks precluded proper evaluation of the isotopic composition" from my experience much more than "distinctive peaks" are needed to have an reliable isotopic measurement (usually peak hight of 50 or 100 mV are used as limit of isotopic determination, though it is better t to determine it experimentally looking at the deviation of isotopic measurement and increase of sdev). It would also be interesting to have a limit of isotopic determination for sediment samples (expressed as mass per mass DW) with and without the prep HPLC purification method. It would be good to have this data in a table (e.g. table 4).

Minor remarks:

I am quite surprised not to find references to Amelung 2001 ("Methods using amino sugars as markers for microbial residues in soil", in "Assessment Methods for Soil Carbon, Advanced Soil Science" (an extensive review of methods for amino sugars ion soils.

All the uncertainties on measurements are given as standard errors. For a method evaluation I expect to see standard deviation, as here we are not interested in the values of the measurement, but on the precision of the method (except for the results of the of the selected marine samples. . ...), leaving the choice of the number of replicates to the user of the method.

The term "Hexosamine" is used to indicate GlcN, GalN and ManN, and make the differentiation with MurA. Indeed these three are hexosamines while MurA not strictly

speaking, though it is also derived from a hexose. Using this term to make the discrimination give the expectation that if the other AS (not hexosamine) are derived from a sugar with another C nr. While the difference is that there is a acidic group attached for MurA. I would recoment to talk about basic AS and MurA. Section 2.5 to 2.7 should be restructured, it is very difficult to follow, and titles of the sections are very confusing. The author should first describe the "Purification by prep HPLC", stating that this is only used in cases where the concentration was to low or matrix effect to strong, this part should also include the description of the instrumentation used for this. Next the "Quantification and compound specific stable isotope analysis of amino sugars" with description of the GC-MS and GC-IRMS method and including the part about determining the range.

Specific comments: P594 L14: Not clear, rewrite in the sort of; "Compound specific 13C analysis of amino sugars obtained from extractions of selected marine sediment samples indicated that. . .. . .

P595 L 1: The sentence that starts with "As amino sugars. . ." is not clear, is not because they are present as biopolimers that they are used as mic contribution to organic matter. It is because they are present as biopolymer that they are preserved in soil, and are used as indicators of contribution of microbial residues to OM. The reason that they are used as microbial proxys is that the contribution of meso/macro organisms is considered to be very small see Simpson et al., 2004.

P596 L 1: The comparison between LC-IRMS and GC-IRMS for AS is rather limited. It would be nice to expand this a bit. It should also be stated that the need of derivatisation increases the uncertainty on the isotopic value due to the need of correcting for the added C atoms and fractionation during derivatisation. . ...

P600 L 25, It is strange to have the NH4OH expressed as a volume ? (should be in masses or mole)

P600 L 25: Totally not clear what is meant with the sentence which starts with " NH4OH

and formic acid were included. . .. . .”

P601 L17: what is meant with ”online detection” here?

P602 L27: equation, description and R of reff are wrong it is: δ13C =(Rsample/Rstandard-1)x1000‰ with Rsample and Rstandard being the ratio’s 13C/12C for sample and reference standard respectively. The reference standard was the international reference Vienna Pee Dee belemnite (RVPDB = 0.011180$\pm$0.000028).

P603 L6: Equation for correction should be given, “F is a compound-specific correction factor for fractionation due to the derivatisation, and was determined experimentally using ANA derivatives of seven. . .. . .

P603 L20 should be referred to Amelung et al. 1996 for the loss of neutral sugars with harsh HCl conditions.

P604 L17: When it is evaporated it is NH3 not NH4OH. . ..

P605 L 20: Do the author mean “irreversible adsorption” ?

P605 L21: I do not think that “particularly sensitive” is right here, “hampered by” is probably what is meant here? Though this was not observed in the test with the Dowex 50x resin wher much higher ammonia concentration were used ? So I do not belive this is a valid hypothesis.

P606 L23: Do the HPLC prep separation of really help when GalN is high ? Looking at Fig 2,b it appear that MurA is very well separated from GalN, ManN is not well separate so here it might help but the Prep separation do not separate these two compounds. . .. . .

P608 L25 add a reff about low 13C in mehtanotrophs Table 2: The DOWEX 50WX8 H2O procedure is not described in the text, I assume it is elution with water? If the resin is in H form I would really not expect any AS to elute, so I wonder why this was

taken a one of the possible procedures?

Table 3: should also give the LoD for isotopic composition

Fig 1: Not really needed

Fig2 Why Is the GC-Method not stopped after 1000 min ?

Fig 5: The way the AS concentration are presented, require the use of color, I do not believe this really needed here as it could easily be presented in another way

---

Interactive comment on Biogeosciences Discuss., 11, 593, 2014.