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Biogeosciences Discussions

Interactive comment on "Improving the Strength of Sandy Soils via Ureolytic $CaCO_3$ Solidification by *Sporosarcina ureae*" *by* Justin Michael Whitaker et al.

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Author's Response – Review #3

(1) Author's Response

Dear referee,

We thank you for the important, thorough and helpful remarks made on the manuscript. The following is our reply to the remarks and suggestions.

For ease of following our reply to each comment, we have combined the, 'Comments' and 'Manuscript changes' sections together. Each 'comment' is followed by a reply with

the specific line-by-line changes made in the revised manuscript.

Please note that the line numbers (i.e. L21, etc) refer to line numbers in the original, unrevised manuscript. Revised line numbers (i.e. RL25) refer to the line numbers in the new, revised manuscript which have been added / changed in response to the remarks made on the unrevised manuscript.

For your convenience, we have also highlighted the sections in the revised manuscript in yellow which reflect these changes. In addition, we have provided additional comments throughout the revised manuscript for added clarity. Finally, we have made editorial changes in addition to those provided for the revised manuscript.

We do welcome any further comments regarding how we may make the revised manuscript stronger and if the rationale provided below can be made more clearly.

Thank you again for your time and kind efforts in review.

(2) Comments from Referee #3 / Changes in Manuscript

Comment 1 "The manuscript by Whitaker, Vanapali and Fortin describes work comparing several Bacillus- and Sporosarcina-species regarding their ability to consolidate (cement) a 'poorly graded' sand. The work presented presents interesting data but lacks a clear direction or message as well as easily comparable data, both within the manuscript and relative to other published literature. For instance, it is unclear how the activities (enzyme units per mL) can be truly compared with each other. The number of units is a function of the amount of urease and thus (assuming a constant urea fraction in each cell) a function of the number of cells (or the biomass (weight)) the experiment was amended with. Without normalizing the data to the amount of biomass (e.g. grams of biomass) and thus reporting the activities as U/g of biomass a direct comparison between the different cell-types is impossible."

We agree with this important, necessary clarification needed in the manuscript. The manner to which data can be compared is dependent on the number of cells. We

would expect an increase in the ability to produce ammonia-ammonium, overtime, from a higher density of cells compared to a lower density of cells. The cellular density can be commonly measured in terms of cell counts, biomass (dry weight), protein content as well as optical density. In our study we used the optical density as a measure of cell density to normalize values reported for a cell type and to compare values between cell types. The optical density values used for normalization were those measured and reported in the study (Fig. 2). This technique assumes that an increase in optical density corresponds to an increase in the number of cells and that for each cell type a given optical density value corresponds to the same amount of biomass. We have addressed this issue in the revised version of the manuscript by better clarifying the units of measure from 'mL of culture' to 'mL solution normalized to cultural density' as the optical density (i.e., turbidity) value (OD600).

In the revised manuscript please see: RL154-159 and RL331

Comment 2 "The authors should clearly state, which strains were investigated in this work and should also explicitly discuss the fact that Sporosarcina spp. used to be classified as Bacillus spp. See e.g. Yoon, J. H., et al. (2001). "Sporosarcina aquimarina sp. nov., a bacterium isolated from seawater in Korea, and transfer of Bacillus globisporus (Larkin and Stokes 1967), Bacillus psychrophilus (Nakamura 1984) and Bacillus pasteurii (Chester 1898) to the genus Sporosarcina as Sporosarcina globispora comb. nov., Sporosarcina psychrophila comb. nov. and Sporosarcina pasteurii comb. nov., and emended description of th." Int J Syst Evol Microbiol 51(Pt 3): 1079-1086."

We agree with this suggestion and have made the appropriate citation regarding the classification of Sporosarcina pasteurii as previously Bacillus pasteurii. In addition, we have also clarified the previous designation of Lysinibacillus sphaericus as Bacillus sphaericus.

In the revised manuscript please see: RL108 and RL110

Comment 3 "Also, the group of Michael Harbottle at Cardiff University has been doing

work on Sporosarcina and have provided clear evidence of S. ureae being about as efficient as S. pasteurii in regards of ureolytic activity? – see e.g. Harbottle, M., Mugwar, A.J., Botusharova, S., 2016. Aspects of Implementation and LongTerm Performance of Biologically Induced Mineralisation of Carbonates in Porous Media. Goldschmidt 2016, Yokohama, Japan."

We agree with this literature suggestion and thank the reviewer for this very helpful contextual literature source. We have also included the work of, 'Sarmast et al., 2014', which while cited in the original manuscript was not appropriately incorporated into the discussion section as regards work previously done comparing S. ureae and S. pasteurii. In this study we used S. ureae based on a random selection of the 7 species of Sporosarcina distinct from Sporosarcina pasteurii and that were urease positive. It was deemed that there is a lack, though not non-existent, amount of investigation present in the literature using the species as a model ureolytic bacteria in MICP. We believe this investigation does provide a novel understanding of the use of S. ureae as a model ureolytic species in MICP as regards to comparing it to not only S. pasteurii but also other species of Bacillus. Also, it has been applied in MICP treatment in a somewhat unique sense under environmental simulation testing. Finally, we believe that the strain employed (BGSC 70A1) has a potentially improved ability in MICP relative to the strains used in previous studies as both Harbottle et al. (NCIMB9251) and Sarmast et al. (PTCCi 1642) found that the ureolytic activity of S. ureae was less than S. pasteurii. For Harbottle et al. please see: pg 116 – 117 http://orca.cf.ac.uk/108519/1/Thesis%20Stefani%20Edited.pdf. Our results indicate a much more comparable level of ureolytic activity of S. ureae compared to S. pasteurii alongside its improved cell viability during MICP treatments in sands. Harbottle et al., also found that S. ureae spores could survive long term while encapsulated in CaCO3 while S. pasteurii could not. Our work, along with Harbottle et al., suggest that S. ureae has promising characteristics that may warrant future study by researchers as regards its application in ureolytic MICP.

In the revised manuscript please see: RL579-583

Comment 4 “Furthermore, the mineralogical characterizations are weak, for instance it is impossible for this reviewer to agree with the assessment of rhomobohedral[ly] shaped crystals as indicated in L 414/415; also, the statement in L418 that only calcium peaks were present in the rod shaped formations cannot be followed. This reviewer clearly sees Ca, C, and O peaks in the EDS spectra in Fig 5.”

We have addressed this issue in several sections of the revised manuscript and realize that the interpretation of the current data and figures as presented was incorrect.

In the revised manuscript please see: RL415-419, RL455, RL599-616

Comment 5 “The following detailed comments will support the assessment above as well as provide examples of sections and approaches, that make this manuscript hard to review and demonstrate at least some of the deficiencies that have to be remediated prior to publication.”

We realize that some important information is unclear in the original text and that some information is incorrect. We thank the reviewer for such a detailed and thorough comment section. We have addressed these issues as follows with replies to the line-by-line comments that reference the relevant section(s) of the revised manuscript.

L21 units need to be defined

Please see: RL21

L 143 unclear whether the OD measurements were taken in systems in which CaCO3 precipitation occurred – if so, the authors need to explicitly discuss the possible influence of CaCO3 precipitation on OD measurements

Please see: RL128-133

Note: Where OD measurements were taken, they were from media system where no CaCO3 precipitation was expected (i.e. UB-1 and UB-2 media).

L 147 the authors assume a SG of 1 for the fluids – this might or might not be a good assumption depending on the concentrations of urea and calcium used.

Please see: RL156-158

Note: An SG of 1 was assumed only for making dilutions necessary for the ammonia-ammonium measurements. As dilutions were between 50-1000X and diluted with ddH2O this was a very good estimate relative to error that would be caused by volume delivery by pipette (which could be as high as 5%).

L 151 – unclear what HACH assay was used

Please see: RL154-156

L 163 – should this sand indeed be described as ‘uniform’?

Please see: RL167

L 166 unclear how OD was measured (what was the blank, what was the pathlength – all this must be stated 189 ‘3 times each at 24 h periods’ – unclear)

Please see: RL175 and RL128-133

L 220-224 the authors need to check whether their statements are clear and not contradictory.

Please see: RL190-195

L 230/231 the authors will have to discuss the effect of drying at 65 deg C

Please see: RL223-226

L 236 the authors should discuss to what extent a 1 month duration can be considered ‘long-term’ – this reviewer thinks years to decades would be considered long-term for building materials and soil stabilization

Please see: RL240

L 274 onward – as indicated above, these data are not really easily comparable since bacterial cell densities (or biomass) were not accounted for. – culture density will play an important role in the ureolytic activity

Please see: RL154-159 and RL331

L 344-346 AND L 355-357 it is unclear what is being compared and what the p-values are indicating (or not)

Please see: RL342-347, RL354-356 and RL357-360

Note: The statistical tests utilized for testing statistical significance are outlined in section 2.6 of the manuscript (RL266-271).

L 360-364 the authors should discuss why acidity might increase and discuss & compare these parameters and treatments in more detail.

Please see: RL557-560

L 371-373 the discussion in these lines is weak and not well organized. – This reviewer is unable to understand what is being discussed and compared

Please see: RL366-376

L 488 'Destruction of MICP sands'? – what does that mean?

Please see: RL491-498

L490 'increase'? compared to what?

Please see: RL491-498

L 491-493 again, this section and statistical analyses are not clear – this reviewer might be able to see that some of the specimen retained their strength once exposed to water or freezing but strength did not 'increase' – did it?

Please see: RL491-498

Note: The statistical tests utilized for testing statistical significance are outlined in section 2.6 of the manuscript (RL266-271).

L 521/522 this author agrees that monitoring ammonium concentrations is a mediocre way to assess urea hydrolysis rate since among other factors, ammonia volatilization and ammonium uptake can affect concentrations. Hence, many groups working on urea hydrolysis-induced CaCO3 precipitation are not using direct urea measurements using either spectrophotometric assays (e.g. Jung assay) or HPLC-based analyses. – The authors must discuss in more detail how the ammonium/ammonia-based estimates of urea hydrolysis rate might have affect their assessments

We agree that other robust methods for measuring MICP capability, such as measuring dissolved Ca2+ levels overtime, are also appropriate for the assessment of the ureolytic activity of an organism. However, each assay has its limitations. For example, the use of dissolved calcium ions as a measure of ureolytic activity is limited by the adsorption of calcium to solid matter (i.e. sand particles, soil, glass surfaces, etc). This technique has been used by Harbottle et al. (see reference link above – pg. 116) who has noted these limitations. Likewise, the measurement of ureolytic activity as ammonia-ammonium measurements overtime is limited too. While a quantitative urea hydrolysis rate is unable to be determined, as discussed in the manuscript the data does indicate broad bacterial activity in ammonia-ammonium production. We believe this is reasonable evidence for modest conclusions that the use of urea as a nitrogen source is medium dependent for certain strains of Bacillus but not Sporosarcina. Also, that the activity is higher in Sporosarcina then in Bacillus and that the levels of activity are similar between S. ureae and S. pasteurii. We do agree that no completely quantitative urea hydrolysis rate can be determined from the current data of this study as has been measured by other groups (see: Lauchnor et al. (2015) Journal of Applied Microbiology 118: 1321-1332.)

Please see: RL526-530

L 549-553 incomprehensible section

Please see: RL553-563

L 566 ‘S. urea may use the proton gradient’ – unclear what ‘proton gradient’ – also unclear what ‘may’ means here

Please see: RL570-571 and RL573-577

L 572-573 what are ‘co-capable candidates’?

Please see: RL581-583

L 581 why ‘only’ – only compared to what?

Please see: RL590

L 585 – without showing replicates and applying proper statistical tests, any statement comparing strengths of specimen will remain highly speculative

Please see: RL591-593

L 594 – still don’t agree with ‘rhombohedral’ statement

Please see: RL602-619

L 597/598 ‘Media and S. subtilis treated sands gave no discernable CaCO3 formation’ – where are those data? Please see: RL621

L 600 what was discussed in this sentence is not supported by Figure 4 (or is it?)

Please see: RL602-619

L 600-604 these lines make little sense to this reviewer

Please see: RL602-619

L 606-610 these lines make little sense to this reviewer

Please see: RL602-619

L 615/616 the authors should consider and discuss that dead cells can also function as nucleation points. Plus all the sand surfaces can as well. Thus, more cells might not result in a significantly increased number of nucleation points

Please see: RL632-641

L 621 ‘gave rise to non-significantly’ – again, the statistical tests used by the authors are unclear to this reviewer and it is something different to ‘not statistically significantly different’ – this section once again does not make much sense to this reviewer

Please see: RL642-645

Editorial comments (not complete, just the ones that I spent the time on noting)

Urea hydrolysis equation CO(NH2)2 not CO(NH3)2

Please see: RL63

L 79 “spahericus”

Please see: RL79

L92 “alterative”

Please see: RL92

L134 “run” vs. “incubated”

Please see: RL139

L180 “fresh sample inoculate” – what is that?

Please see: RL182

L 186: ‘Each mould had equipped” !?

Please see: RL188-189

L 188 ‘Silica’ or ‘Silica sand’?

Please see: RL189-195

L 228 “Visualization . . . was carried out . . .’???

Please see: RL 232

L 244 ‘the trials’ do not ‘withstand’

Please see: RL247

L 386 Fig 3 ‘subtilis’ not ‘Subtilis’

Please see: RL390

L 509: ‘It was chief to understand’?

Please see: RL514

L 512: ‘regardless of source nitrogen availability as yeast extract or urea’? – can’t Follow

Please see: RL517

L518 one medium, two (or more) media

Please see: RL523

L 527 and 532 why ‘see also’?

Please see: RL535

L 561 ‘Returning to s. ureae’ ?? what does that mean?

Please see: RL570

L 565 ‘Whiffin’

Please see: RL576

L 605 ‘cell viability of inoculates’ – what is an inoculate?

Please see: RL620

L 616-619 – language issues – this is almost incomprehensible

Please see: RL635-656

L 625/626 – language issues – this is almost incomprehensible

Please see: RL635-656

L 635 ‘may prove’? or may not prove . . .

Please see: RL655-657

L 639 – ‘ would be quite proximal’? – what does that mean?

Please see: RL658

L 647 ‘remain’ ? – maybe ‘maintain’? References have random (or not so random) spaces in them that make no sense. Please see: RL666-667

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-517/bg-2017-517-AC3-supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-517, 2018.