

Interactive comment on “Improving the Strength of Sandy Soils via Ureolytic CaCO₃ Solidification by *Sporosarcina ureae*” by Justin Michael Whitaker et al.

Anonymous Referee #3

Received and published: 6 June 2018

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

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between the different cell-types is impossible.

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.

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.

Furthermore, the mineralogical characterizations are weak, for instance it is impossible for this reviewer to agree with the assessment of rhombohedral[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.

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.

Abstract

L21 units need to be defined

BGD

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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

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.

L 151 – unclear what HACH assay was used

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

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

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

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

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

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

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

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

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

L490 'increase'? compared to what?

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?

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

L 549-553 incomprehensible section

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

L 572-573 what are 'co-capable candidates'?

L 581 why 'only' – only compared to what?

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

L 594 – still don't agree with 'rhombohedral' statement

L 597/598 'Media and S. subtilis treated sands gave no discernable CaCO₃ formation' – where are those data?

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

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

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

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

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

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

Urea hydrolysis equation $\text{CO}(\text{NH}_2)_2$ not $\text{CO}(\text{NH}_3)_2$

L 79 “spahericus”

L92 “alterative”

L134 “run” vs. “incubated”

L180 “fresh sample inoculate” – what is that?

L 186: ‘Each mould had equipped’ !?

L 188 ‘Silica’ or ‘Silica sand’?

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

L 244 ‘the trials’ do not ‘withstand’

L 386 Fig 3 ‘subtilis’ not ‘Subtilis’

L 509: ‘It was chief to understand’?

L 512: ‘regardless of source nitrogen availability as yeast extract or urea’? – can't follow 518 one medium, two (or more) media

L 527 and 532 why ‘see also’?

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

L 565 'Whiffin'

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

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

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

L 635 'may prove'? or may not prove ...

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

L 647 'remain' ? – maybe 'maintain'? References have random (or not so random) spaces in them that make no sense.

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

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