Review report hessd-11-9829-2014

General comments

This paper presents an interesting novel experimental setup to study the effects of flow velocity on biofilm development and denitrification rates. This is an important and relevant topic especially to better understand denitrification in fractures. The developed setup indeed may have wider applicability to study the effects of flow velocity on biogeochemical reaction rates. Although the study delivered interesting and useful data, I see numerous issues with interpretation and perhaps over interpretation of the experimental results. Furthermore, the readability of the manuscript would profit from professional editing. I found the paper difficult to read and this distracted me from the otherwise interesting study. I recommend a major revision to resolve all issues with interpretation of the data and writing followed by another reviewing round to better appreciate the discussion and conclusion. Below a list of specific comments with the major ones indicated in bold.

Specific comments

1. **Page 9832, line 13:** explain and discuss in paper how biofilm development will be different between plastic and natural aquifer material. For example, do microorganisms attach better to plastic than to sand grains and how will this affect the translation of the lab results to field implications?
2. **Page 9832, lines 20-21:** 'slow flow velocities of 6-35 mm/min'. This is equivalent to 3-18 km per year. These are unrealistically high flow velocities for matrix flow. Of course, the flow will be much faster through the fractures as 'studied' here but representative flow velocities are not discussed in the paper. Therefore, substantiate these velocities with literature references on representative flow velocities through fractures. Later on I feel this is only partly done at page 9833, line 5.
3. Page 9833, line 9: how exactly is denitrification enhanced by the flow dynamics?
4. **Page 9833, line 19:** as DOC is produced how does this affect the experimental interpretation and mathematical model where it seems assumed that only immobile organic carbon reacts?
5. **Page 9833, line 24:** what about bacterial detachment: what is the biomass concentration flowing out of the tubes?
6. Page 9834, line 7: what about N2 gas formation and it potential effects on biofilm development and hydraulic properties? Gas bubbles were absent?
7. Page 9834, line 11: explain if the system is entirely anoxic or whether aerobic degradation of organic matter may also have occurred? Can oxygen penetrate through the tube walls?
8. Page 9834, line 25: explain why this range and specific velocities were chosen. What do they represent?
9. Page 9835, line 9-10: explain 'independent of flow velocity'
10. Page 9835, equation 1/Figure 3: I find it confusing that nitrate consumption is defined without stating the per time unit. As the flow velocities are different the incubation times are also different and thus also the amount of nitrate consumed. I think it is easier to understand if the denitrification rates are plotted as function of time.
11. Figure 1: also plot TIC and relate the stoichiometric decrease of nitrate?
12. Page 9836, line 8: also initially the rate differs on flow velocity with a higher rate with lower flow velocity which makes sense as the reaction time is longer with lower flow velocity.

13. Page 9836, line 10: isn't it remarkable that the intermediate velocities show a different behaviour than the extreme ones? This is not well discussed in the paper.
14. Page 9836, line 16: unclear: the first phase is the black dashed curve? But this is for the total experimental duration?
15. Page 9836, line 17: I would not state that the result is similar (see before).
16. **Page 9836, line 18:** agree, but the concentrations are not identical at the outlet initially. With this figure it cannot be stated that the reactivity is initially higher with higher flow velocity.
17. Page 9836, line 25: initially there are no differences in denitrification rate.
18. **Page 9836, line 25:** I disagree or I do not understand what the authors mean here: the reaction times differ among the 4 velocities thus the masses of nitrate consumed should be different (and not equal) as Fig 3 shows but the rates more similar as Fig 5 shows.
19. **Page 9837, line 17:** it remains unclear to what extent denitrification is coupled to solid OC oxidation and till what extent to dissolved OC oxidation. I think this is relevant for biofilm development.
20. Page 9837, line 13: how is this 'maximum nitrate consumption' defined? Unclear.
21. Section 4.1.1. Here it is assumed that the proportion of cells produced per gram of nitrate consumed is constant and independent of flow velocity. However, could it be that if flow velocity impacts biofilm development it also affects the reaction stoichiometry which may differ 50%?
22. **Section 4.1.1.** The authors seem to assume silently that biomass is fully retained in the tubes. But what is biomass is flushed out? How does this affect the calculations?
23. **Section 4.1.1.** Equation 4 is not well explained. It seems to suggest that biomass is not created in the nitrate to nitrite step only if denitrification is complete to N2. This assumption should be explained and substantiated.
24. **Figure 6:** it is unclear why the biofilm development is slowest for the lowest velocity and how to get from Fig 5 to Fig 6: what is the role of nitrite here? Does the proportion of nitrite produced differs between velocities and may explain these results? Possible to add error bars?
25. Page 9837, line 18: indeed is biomass loss due to detachment not very relevant in this setup?
26. **Page 9837, line 18:** but biomass loss cannot explain why the measured mass is lower than the calculated mass!
27. **Figure 7:** I am surprised to see that the order is reversed with Fig 6 whereas I would expect that a higher mass also translates to a higher thickness. Is this caused by the parameter 'nutrient input' which meaning is not well explained?
28. **Page 9842, line 5:** I disagree with the statement 'fast velocities result in thinner biofilm' based on these calculations as time is not clearly considered in Fig 7. For example, at a total nutrient input of 20 mg, the slow velocity setup had a much longer time available to digest this 20 mg and thus had the time to develop a larger biomass and thickness than the high velocity setup. Such a statement can only be drawn if the same time scale is considered and with the parameter nutrient input the times scales are different.
29. **Section 4.2:** considering all the issues above I wonder if this part is not an over interpretation of the results.
30. **Figure 8:** As I see it the nitrate consumption rate is not clearly different as function of biofilm thickness or flow velocity.

31. **Section 5.1**: difficult to follow as it is not referred to figures. The reader is getting lost here. I wonder if this all is not overinterpreted.
32. **Sections 5.2-5.3.** I think first the issues before should be resolved before this discussion can be appreciated.
33. **Conclusions:** I cannot agree with the main conclusions before the issues before are clarified.

Technical corrections

1. Title: a nice title but too general. Make it more specific. For example, change 'biogeochemical processes' to 'denitrification' and 'a laboratory experiment' to 'plastic tubes laboratory experiment'.
2. Line 5: explain already shortly here what is original to this lab experiment
3. Line 6: replace 'biogeochemical reactions' with the precise process simulated 'denitrification'
4. Line 12: add 'inorganic' before 'carbon'?
5. Line 16: explain 'assimilation'
6. Line 17: define 'significant'
7. Line 18: are these kinds of experiments to biofilm development not always dynamic? Later on this is explained but it is not directly clear here.
8. Abstract: specific conclusions are missing; they are stated as too general.
9. Page 9831, Lines 18-21: clearly written and explained. Use this also in the abstract?
10. Introduction: I miss a clear statement of the research objectives.
11. Page 9832, line 12: explain also in introduction that this 'model' applies also for pyrite.
12. Page 9833, line 19: and what about 'inorganic carbon'?
13. Page 9833, line 23: unclear 'water compounds'
14. Page 9835, line 4: 45 micrometer filter? Divide by 100?
15. Page 9837, line 1: It helps the reader if first the various phases are roughly defined and indicated.
16. Page 9837, line 1: first time it is stated the rate increases with time. Should be stated earlier.
17. Page 9837, line 8: these different phases are not indicated in Fig 3.
18. Page 9840, line 16: is the unit mg correct? It seems so low considering the mass of nitrate passed through the system.
19. Page 9838, line 17: unclear what precisely is meant here with nutrient input. Equation 8 tells this is nitrate. Take other word for nutrient?

END OF REVIEW REPORT