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Discussions

Interactive comment on “Impact of flow velocity on biochemical processes – a laboratory experiment” *by* A. Boisson et al.

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The revised version of the manuscript including figures was uploaded as a supplement

Dear Reviewer,

We thank you very much for your careful consideration of our manuscript. We have made a concerted effort in our revisions to address your comments, which we found to be very constructive and helpful. It appears that most of the disagreements were due to a lack of explanations in the first version of our manuscript. We have made as well specific efforts for improving the readability of the manuscript with the help of a native English-speaker. In the revised version, extensive explanations have been added and some parts of the manuscript have been rewritten. Please find our detailed responses

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to your comments in text below.

Sincerely, A. Boisson, D. Roubinet, L. Aquilina, O. Bour, and P. Davy

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?

RESPONSE: In order to discuss the differences expected between synthetic and natural material, we have added the following sentences in the second paragraph of section I (Introduction): “Since this experiment is not conducted on natural aquifer material, results and interpretations cannot be directly translated to field applications. For example, it has been established that microorganisms attach more rapidly to hydrophobic and nonpolar surfaces, such as Teflon and other plastics, than hydrophilic materials, such as glass or sand [Donlan, 2002]. It implies that the duration of the attachment period might be shorter in the proposed experiment than in natural environments where the simple geometry of the system might impact attachment as well." In addition, we justify that these differences are very difficult to characterize and that our study rather focuses on the biofilm-growth period of the experiments with the following sentences: "As attachment is known to be very difficult to characterize [Cunningham et al., 1991] and remains an unknown to be estimated for each specific case [Donlan, 2002], we do not aim to obtain conclusions concerning this process that occurs on a very short period in comparison to the biofilm-growth period [Singh et al., 2006]. Our study focuses on the biofilm-growth period of long-time experiments and aims at characterizing. . ."

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.

RESPONSE: "Slow flow velocities" is mentioned here in comparison to the flow velocities used in existing open channel experiments. In terms of reproduction of the natural environment, these flow velocities correspond to forced hydraulic conditions as enhanced denitrification has been observed under these conditions in the Ploemeur site. In the modified version of our manuscript, we clarify our description of the flow velocities and justify the chosen values as follows: The flow velocities and size of the systems used in existing open channel experiments are mentioned in the first paragraph of section I (Introduction): "where the latter experiments are conducted on conduit reactors at the centimeter scale (length and diameter/thickness) with flow velocities with orders of magnitude of 102-103 mm/min." We clearly mention that the flow velocities used in our experiment are slow in comparison with existing open channel experiments in the second paragraph of section II.1 as follows: "As our experiment is conducted with slow flow velocities (from 6.2 to 35 mm/min) in small diameter tubes (2 mm) in comparison to existing open channel experiments. . .” We justify the chosen values of the flow velocities in relation with the forced hydraulic conditions observed in the Ploemeur site in the third paragraph of section II.1 as follows: “The water used in the static and flow-through experiments presented in the following section has been collected in the Ploemeur site (Brittany, France). Since 1991, this site provides water to the city of Ploemeur at a rate of 106 m3 per year [Jiménez-Martínez et al., 2013; Leray et al., 2012] thanks to a contact zone between granite and schist [Ruelleu et al., 2010]. As this water extraction started, an increase of nitrate reduction and sulfate release has been observed in areas where the pumping conditions modified the flow dynamics, whereas concentrations of nitrate remain high in other areas of the system. From these observations, Tarits et al. [2006] concluded that natural denitrification due to a heterotrophic denitrification reaction with pyrite was enhanced by forced hydraulic conditions in this site. In order to reproduce this phenomenon at the laboratory scale, the presented experiments are conducted with flow velocities in the range of those estimated in the Ploemeur site under pumping conditions [Tarits et al., 2006]. Flow velocities with the same order of magnitude are considered as well for remediation applications [Li et al., 2010] and reactivity assessment [Boisson et al., 2013] in natural environments where reactivity and biofilm development usually occur where the highest velocities are observed.”

3. Page 9833, line 9: how exactly is denitrification enhanced by the flow dynamics?

RESPONSE: In the reply of the previous comment, we justify the high flow velocities used in the presented experiment. At the same time, we give a more detailed description of the study that concludes in an enhanced denitrification under forced hydraulic conditions with the following sentences in the third paragraph of section II.1: “As this water extraction started, an increase of nitrate reduction and sulfate release has been observed in areas where the pumping conditions modified the flow dynamics, whereas concentrations of nitrate remain high in other areas of the system. From these observations, Tarits et al. [2006] concluded that natural denitrification due to a heterotrophic denitrification reaction with pyrite was enhanced by forced hydraulic conditions in this site.”

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?

RESPONSE: The produced DOC corresponds to carbon initially present as a solid and immobile phase, which is then released due to plastic degradation by microorganisms. This phenomenon does not impact our mathematical model and interpretation as they are not based on specific assumptions regarding the production of organic carbon and they do not assume that only immobile organic carbon reacts. Our model is based on the quantity of consumed nitrate and assumes that denitrification is not limited by the availability of carbon. In order to clarify these assumptions, we added the following sentence at the end of the third paragraph of section 4.1: “In addition, the previous formulation considers that biofilm formation is not limited by the availability of carbon, as we observe that this reactant is in excess during the whole experiments.”

5. Page 9833, line 24: what about bacterial detachment: what is the biomass concentration flowing out of the tubes?

RESPONSE: As explained now in the modified version, the water volumes sampled during the experiments were too small to evaluate the biomass concentration flowing out of the tubes. As our objective was mainly to measure and interpret nitrate concentration variation, the presented experiments were not set up for evaluating biomass concentration flowing out of the tubes. This is explained in section II.4 of the modified manuscript with the following sentences: “The limited amount of sampled water prevented us from quantifying gas production in the reactive process (NO, N2O, and N2) and biomass concentration flowing out of the tubes.” However, we agree that this information would help to improve our interpretation. For this reason, an additional

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parameter has been added to our analytical model (equation 5 in the revised version) in order to take into account potential loss of biofilm. In our study, this parameter is set to 0, as the proposed model tends to underestimate biomass in the tube as explained in the reply of comment 22 (in the list of specific comments). In addition, we propose in conclusion to improve the experimental set up in order to evaluate biomass concentration flowing out of the tubes for future work.

6. Page 9834, line 7: what about N2 gas formation and it potential effects on biofilm development and hydraulic properties? Gas bubbles were absent?

RESPONSE: Although gas bubbles were observed during the experiments, we assume that their presence did not impact biofilm development and hydraulic properties as: (i) Measurements of the velocity of some bubbles show that their traveling time was the same as the theoretical mean flow velocity based on water weight measurements. It implies that these gas bubbles were not trapped into the biofilm, and, consequently, did not modify the biofilm permeability. (ii) The flow velocity measured at the outlet of the tubes was constant, and thus not modified by the presence of bubbles. However, these observations are limited to bubbles that were big enough to be observable by the human eye and the impact of micro-bubbles was not considered. As reported in existing studies, micro-bubbles formed during denitrification (or other reactions) can be trapped in the media and modify its permeability and hydraulic properties. However, these phenomena are less likely to occur in media characterized by large pores [Istok et al., 2007] and the observations (i) and (ii) validate this assumption. In addition, we are not aware of studies reporting effects of N2 on biofilm poisoning. The potential effects of gas bubbles and our justifications for neglecting these effects are now mentioned in the second paragraph of section II.4 as follows: “Concerning the assumptions related to bubble formation, the impact of the presence of bubbles has been verified by measuring the velocity of bubbles that are big enough to be observable by the human eye. These velocities are the same as the theoretical mean flow velocity based on water weight measurements and the flow velocity measured at the outlet of the tubes is constant. We thus consider that these bubbles are not trapped into the biofilm and have a negligible impact on biofilm and hydraulic properties. For the same reasons, we assume as well that potential micro-bubbles (not observable by the human eye) have a negligible impact on these properties. This assumption is coherent with existing studies that show that an impact of bubbles on biofilm and hydraulic properties is less likely in media characterized by large pores [Istok et al., 2007]."

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?

RESPONSE: According to the tube manufacturer, PVC double manifold tubes have a very low permeability to gas (as indicated at page 46 of the documentation available at http://www.watson-marlow.com/Documents/knowledge-hub/Brochures/gb%20-%20UK/Product/Watson%20Marlow%20UK/b-OEM-gb-02.pdf). In addition, measurements done at the inlet and outlet of the tubes during the whole experiment show that the concentration of oxygen is below the detectable measurement threshold. Consequently, we consider that the system is anoxic and thus no aerobic degradation occurs in the system. We added further explanations in the modified version of the manuscript to justify that the system can be considered as anoxic in the third paragraph of section II.3 as follows: "Prior to experimental use, the water is deoxygenated by Argon bubbling and then maintained in anoxic conditions under an argon atmosphere in a high-density polyethylene tank (whose non reactivity is controlled). The entire system is considered as anoxic since no oxygen enters the system either at the inlet or through the tube walls that have a low gas permeability (as indicated by the manufacturer of PVC tubes at page 46 of the documentation available at http://www.watson-marlow.com/Documents/knowledge-hub/Brochures/gb%20-%20UK/Product/Watson%20Marlow%20UK/b-OEM-gb-02.pdf). In addition, the anoxic condition has been verified by measurements of oxygen concentration in water at the tube outlet. As these concentrations remain below the measurable threshold for the whole experiment, we consider that no aerobic degradation occurs in the system."

8. Page 9834, line 25: explain why this range and specific velocities were chosen. What do they represent?

RESPONSE: Please refer to comment 2 (in the list of specific comments) where we explain the chosen range of flow velocities.

9. Page 9835, line 9-10: explain 'independent of flow velocity'

RESPONSE: In this part, we simply meant that the analyzed volume is the same for all the experiments. In the modified version of our manuscript, we clarify this part by removing the definition of pore volume and by simply mentioning the quantity of water analyzed during the experiments (in section 2.4): "For all the experiments, the volume used for analyses is equal to 5 ml."

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.

RESPONSE: We agree that the results related to equation 1 and Figure 3 are impacted by incubation time that is different for different flow velocities. However, we think that it is important to present these results as it provides the raw measured variations of nitrate concentrations (without data post-processing) and shows directly the effect of the reaction on these concentrations. Plotting the denitrification rates as a function of time would lead again to results difficult to compare, as the quantity of water used into the system until a given time t is flow-velocity dependent. In order to compare the reactivity for similar quantities of water used in the system, we prefer to plot the denitrification rates as a function of the pore volume number, which corresponds to the number of tubes filled up with water until a given time and for a given flow velocity. In order to clarify this representation and its advantages, we added the following explanations in the

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third paragraph of section 3.2: For the definition of the nitrate degradation rate: “The previous observations are based on the temporal evolution of nitrate consumption for experiments conducted with several flow velocities. As the residence times within the tubes are flow-velocity dependent, these results might be difficult to interpret. For example, similar values of nitrate consumption correspond to a greater reactivity for a higher flow velocity. In order to take into account the impact of various residence times, we define the nitrate degradation rate R_(ãĂŰNOãĂŮ_3ˆ-) (in mg m-2 s-1) as. . .” For the definition of the pore volume number: “In addition, as the quantity of water passing through the system until a given time is flow-velocity dependent as well, we define the pore volume number P_vol (-) as” . . . “which corresponds to the volume of water used into the system until time t divided by the tube volume V (m3). In other words, the pore volume number enables us to evaluate the number of tubes that are filled up until a given time for a given flow velocity.” For the advantage of plotting the nitrate degradation rate as a function of pore volume number: “Studying the evolution of the nitrate degradation rate R_(NO_3ˆ-) with the pore volume number P_vol enables us to compare the reactivity observed for different flow velocities considering similar quantities of water used in the system.”

11. Figure 1: also plot TIC and relate the stoichiometric decrease of nitrate?

RESPONSE: Figure 1 has been modified and now includes the evolution of IC. The variation of IC is very limited and only a small increase is observable. As the exact constitution of the carbon molecule is unknown, the stoichiometric relation cannot be provided. However, the measurements show that, before 100 hours, 1.42 to 4.2 mg/L of nitrates are reduced per mg of carbon produced. After this, the nitrate concentration is zero and no further calculation is possible.

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.

RESPONSE: Although this observation is true for the first measurement, it is not verified for the following measurements. In addition, considering the variability of the system, we think that it is difficult to compare each measurement for different flow velocities. For this reason, we prefer to discuss general tendencies of the observed data along the whole experiment and between different flow velocities. Our objective to discuss general tendencies has been clarified in the first paragraph of section 3.1 as follows: “We wish here to identify phases characterized by specific behaviors of nitrate consumption and to determine which processes are responsible for these behaviors. For this purpose, we interpret general tendencies of the results presented in Figure 3a, and we focus on the evolution of nitrate consumption along the experiment for each flow velocity and on the differences observed between the experiments conducted with different flow velocities.” Considering general tendencies as defined above, the data observed in phase 1 present small differences for different flow velocities in comparison with the large differences observed in phase 2. This is now clarified as follows: In the first paragraph of section 3.2: “In the first phase identified in Figure 3a (denoted phase 1 in Figure 3b), the nitrate consumptions observed for the four flow velocities tend to follow a linear increase in comparison to the general variations observed during the whole experiment.” In the second paragraph of section 3.2: “In comparison to the large variations observed during the whole experiment, we consider that nitrate consumptions observed for the different flow velocities present a small range of variation during phase 1. For example, when t=67 h, the experiments conducted with a flow velocity of 6.2 mm/min, 11, 17 and 35 mm/min are in phase 1 and the values of nitrate consumption range from 0.9 to 2.9 mg/L. In opposition, when t=187 h, the experiment conducted with a flow velocity of 35 mm/min is in phase 2 and presents a value of nitrate consumption of 1.2 mg/L, whereas the experiments conducted with a flow velocity of 11 mm/min, 17 and 35 mm/min are in phase 1 and present values of nitrate consumption that range from 4 to 4.8 mg/L.” In the fourth paragraph of section 3.2: “These results show that strong variations of the nitrate degradation rate are not observed during phase 1 and are rather observed after this phase. These observations are again relative to the general behavior along the whole experiment, as nitrate degradation rate differs for the flow velocity 35 mm/min from the values observed for the three lower flow velocities. However, these variations are small in comparison to the variations observed during the rest of the experiments."

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.

RESPONSE: Considering general tendencies as described above, the intermediate velocities show a different behavior during phase 2. This is now clearly mentioned: In the second paragraph of section 3.1 as follows: "After these transition times, nitrate consumption clearly differs from the previous general linear behavior, as we observe (i) a "relative" stabilization with small variations for the slower (full blue curve) and higher (dotted red curve) flow velocities, and (ii) a general decreasing tendency for the intermediate flow velocities (dashed green and dashdot magenta curves)." In the first paragraph of section 3.3 as follows: "In the second identified phase (phase 2 in Figure 3), the nitrate consumption is characterized by either (i) a "relative" stabilization with small variations for the slower (full blue curve) and higher (dotted red curve) flow velocities, or (ii) a general decreasing tendency for the intermediate flow velocities (dashed green and dashdot magenta curves). Concerning the fastest velocity v_4, the previously named "relative stabilization" corresponds to a succession of decreases and increases oscillating around a "relative threshold"."

14. Page 9836, line 16: unclear: the first phase is the black dashed curve? But this is for the total experimental duration?

RESPONSE: The definition of phase 1 and phase 2 has been clarified as follows: Before section 3.1, the division into two phases is mentioned as follows: "In order to understand which processes impact on nitrate consumption for these experiments, we consider that the evolution of nitrate variation can be roughly decomposed into two phases (Figure 3b). The identified phases are defined and described in detail below. . .” Section 3.1 (entitled “Definition of the identified phases”) has been added to the manuscript in order to clearly define phase 1 and phase 2. The duration of these phases is now clearly indicated in Figure 3. The duration of phase 1 is now clearly indicated in Figure 5. A comment has been added in the first paragraph of section 3.2 concerning the duration of phase 1 relative to the duration of the whole experiment: “As previously described, the duration of this phase depends on the flow velocity, and last, for example, for 92% of the experimental duration for the slower flow velocity and only 19.8% of the experimental duration for the higher flow velocity.”

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.

RESPONSE: For comments 15-18, please refer to comment N°12 (in the list of specific comments) where we explain that we aim at studying general tendencies of the measurements and where we explain how we clarify this in the revised version. In addition, the observation concerning higher reactivity for higher flow velocity has been rewritten as follows: “As the residence times within the tubes are flow-velocity dependent, these results might be difficult to interpret. For example, similar values of nitrate consumption correspond to a greater reactivity for a higher flow velocity.”

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.

RESPONSE: With the system we used, it is indeed difficult to know to what extend denitrification is coupled to solid or dissolved oxidation. As explained in comment N°4: The source of the DOC produced is in fact the carbon release from the tubes, which is initially present as a solid-immobile phase. DOC release comes from the degradation of the plastic by microorganisms taking it from the solid part and as well from the mobile part when the reaction becomes important.

20. Page 9837, line 13: how is this ‘maximum nitrate consumption’ defined? Unclear.

RESPONSE: The section that described phase 1 and phase 2 has been rewritten and the definition of these phases is now clarified in the text and the figures. The term “maximum nitrate consumption” is not used anymore in the modified version of the manuscript.

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 that may differ 50%?

RESPONSE: Indeed the flow velocity could affect the reaction stoichiometry and explain why the proposed model underestimates biofilm mass. However, it is not possible to evaluate this impact with the presented experiment. In the last paragraph of section 4.1.1 of the revised version, we added a comment to mention the uncertainties on our biomass estimates related to the potential impact of the flow velocity on the reaction stoichiometry as follows: “Uncertainties remain as well concerning the relation linking cells produced per quantity of consumed nitrate and the impact of flow velocity on the reaction stoichiometry.”

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?

RESPONSE: The biofilm weight evaluated in section 4.1.1 corresponds to the cumulative biofilm weight created along the whole experiment. Consequently, potential detachment and biomass flowing out of the tubes are not taken into account in these calculations. In a similar manner, the biofilm thickness defined in section 4.1.2 corresponds to the cumulative biofilm thickness as it is evaluated from the previous cumulative biofilm weight. In order to clarify these definitions, (i) the titles of these subsections have been modified for “Cumulative biofilm weight” and “Cumulative biofilm thickness”, (ii) “cumulative” has been added every time that “biofilm thickness” is mentioned in section 4.1.2 (and it was already present in the submitted version for “biomass”), and (iii) the assumptions regarding the processes that could lead to an overestimation of the biofilm properties have been extended in the last paragraph of section 4.1.1 as follows “we assume that processes such as detachment and decay are negligible in the present study. Regarding the previous assumptions, biofilm properties such as biomass and thickness are referred and interpreted as cumulative properties along the experimental time.”. However, we agree that this information would help to improve our interpretation. For this reason, an additional parameter has been added to our analytical model (equation 5 in the revised version) in order to take into account potential loss of biofilm. In our study, this parameter is set to 0 as the proposed model tends to underestimate biomass in the tube as explained in the last paragraph of section 4.1.1 as follows: “As the proposed model tends to underestimate the biofilm weight, the parameter k_bio that represents potential loss of biofilm is set to 0”.

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.

RESPONSE: Equation 4 (renamed equation 6 in the modified version) assumes that the number of nitrate moles that have been consumed and not transformed to nitrite is then transformed to biomass. Consequently, it does not assume that the denitrification step has to be complete to N2. It only assumes that the quantity of produced gasses is negligible as justified in the second paragraph of section 4.1. In order to clarify the meaning of equation 6, the following sentence has been added below this equation:

“Note that equation 6 assumes that the quantity of nitrate used for biofilm formation corresponds to the quantity of consumed nitrate that is not transformed to nitrite. As stated before, it implies that only a small portion of the consumed nitrate is reduced to gas and that this quantity can be neglected.”

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?

RESPONSE: In order to understand the biofilm development presented in Figure 6, it is easier to relate the observed behavior to the results presented in Figure 3 as both results are presented as a function of time. The relation between Figure 3 and Figure 6 is not obvious either, as the nitrate consumptions measured for the flow velocities v1, v2 and v3 during phase1 present small differences (Figure 3 at time t=200h, for example), whereas clear differences of the biofilm weight are observed for the same experiments and same time. However, this behavior can be explained by considering the equations used to evaluate the biofilm weight (equations 5-8 in the modified version). From these equations, biofilm weight depends on nitrate consumption and flow rate where the flow rate regulates the quantity of nitrate that has entered into the system until a given time. Considering a given value of nitrate consumption, smaller values of the flow rate lead to smaller values of the biofilm weight. It implies that biofilm weight estimated at a given time increases when the flow velocity increases, as observed in Figure 6 for the flow velocities v1, v2 and v3. The previous behavior is not observed for the flow velocity v4, as the nitrate consumption in this case differs from the values observed for the three lower flow velocities after 100 hours (Figure 3). These observations are now explained in the sixth paragraph of section 4.1 as follows: “Concerning the flow-through experiments, differences are observed for the different flow velocities (Figure 6). For a given time, the biofilm weight increases when increasing the flow velocities for the experiments conducted with the flow velocities v_1, v_2, and v_3. In relation to the results presented in Figure 3, nitrate consumption for these three flow velocities present small differences during phase 1 in contrast to the large differences observed during the rest of the experiment. These small differences are observed for 266 hours, as it is the smallest duration of phase 1 for the flow velocities v_1, v_2, and v_3. As biofilm weight estimates are based on the values of nitrate consumption and flow rate (equation 5-8), the differences observed before this specific time are mainly due to the different flow velocities of these experiments. Knowing that these velocities regulate the quantity of nitrates entering into the system, a higher flow velocity results in a larger biofilm weight, as observed for the flow velocities v_1, v_2, and v_3 from t=0 to t=266 hours. After this specific time, although nitrate consumption in Figure 3a is higher for the slower velocity, these differences with the other flow velocities are not large enough to modify the previous behavior. Note that the previous observations are not valid for the experiment conducted with the highest velocity v_4, as nitrate consumption in this case differs from the nitrate consumption observed for the three lowest velocities after 100 hours (Figure 3a). We observe then in Figure 6a that the small values of nitrate consumption combined with the high flow velocity v_4 lead to a biofilm weight comparable to the estimate obtained for the flow velocity v_3.” In order to study the role of nitrite, we present the temporal evolution of nitrite produced along the experiments in the figure below. This figure does not show a specific evolution of nitrite that could explain the differences in biofilm weight observed in Figure 6. It thus reinforces the explanation provided in the previous paragraph.

As the behavior observed in Figure 6 is not related to an issue of repeatability of the experiments, it seems that adding error bars will not provide additional information that could help for interpreting our experiments. However, if the reviewer considers that this is required for a full understanding of our interpretation, this information could be added easily.

25. Page 9837, line 18: indeed is biomass loss due to detachment not very relevant in this setup?

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RESPONSE: Please refer to comment 22 of the list of specific comments.

26. Page 9837, line 18: but biomass loss cannot explain why the measured mass is lower than the calculated mass!

RESPONSE: This part of the manuscript has been modified as follows: (i) first, the assumptions that could result in an underestimation of biomass are listed as follows: “This difference is likely due to the simplifications of the proposed model where addition of suspended materials and Extracellular Polymeric Substances are not considered”; and (ii) secondly, the assumptions that could result in an overestimation of biomass are listed.

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?

RESPONSE: Indeed a higher mass represented as a function of time results in a smaller thickness represented as a function of “nutrient input”. In order to clarify this part, additional explanations are provided concerning the definition of “nutrient input” in the second paragraph of section 4.1.2 as follows: “For batch experiments, N_input corresponds to the mass of nitrate present in the tank at the beginning of the experiment and is defined as. . .” and “For the flow-through tube experiments, N_input (t) corresponds to the mass of nitrate introduced into the system until time t and is defined as. . .”.

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.

RESPONSE: Indeed, the statement “fast velocities result in thinner biofilm” and all the observations done in this paragraph are based on the evolution of the biofilm thickness with the quantity of nutrient used in the system. These observations are thus not valid for the evolution of the biofilm thickness with time. In order to clarify this, the term “per nutrient input unit” has been added after the statement “fast velocities result in thinner biofilm”. (Note that the term “per nutrient input unit” was already present for other observations in the same paragraph.) In addition, the following comment has been added at the end of this paragraph in the revised version: “Note that the previous observations are valid for the evolution of the biofilm thickness along the quantity of nutrient injected into the system and not along the experimental time.” Note that, in addition, as mentioned at the end of the first paragraph of section 4.1.2, biofilm thickness gives the same quantitative information as biofilm weight. Consequently, for obtaining information on the temporal evolution of biofilm properties, the reader can refer to Figure 6, as this figure represents the evolution of biofilm mass with the experimental time.

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.

RESPONSE: Extensive justifications and modifications in the text have been provided for comments 1-28. We hope that these justifications and changes reply as well to comments 29-33. Concerning comment 31, section 5 has been partially rewritten in order to improve its readability.

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’.

RESPONSE: The title has been modified and is now: “Impact of flow velocity on denitrification - A plastic tube laboratory experiment”

2. Line 5: explain already shortly here what is original to this lab experiment

RESPONSE: “an original laboratory experiment” has been replaced by “an original plastic-tube laboratory experiment”.

3. Line 6: replace ‘biogeochemical reactions’ with the precise process simulated ‘denitrification’

RESPONSE: “Biological reaction” has been replaced by “denitrification”.

4. Line 12: add ‘inorganic’ before ‘carbon’?

RESPONSE: As explained in comment 12 of the technical correction list, inorganic carbon presents only small variations during the experiment. Consequently, “organic” has been added before “carbon” in the abstract.

5. Line 16: explain ‘assimilation’

RESPONSE: This is now explained as follows: “These main behaviors of the reactivity rate are related to phases of biofilm development through a simple analytical model based on the assumption that nutrients are incorporated to cells (assimilation).”

6. Line 17: define ‘significant’

RESPONSE: As the meaning of “significant” is too broad, this term has been removed and replaced by detailed results explained as follows: “It results in observing various dynamics of nitrate transformation associated with biofilm development where flow velocity appears to be a key factor, as (i) the experiments conducted with the largest flow velocities are characterized by a fast increase of the reactivity rate until reaching a threshold where strong oscillations are observed; and (ii) experiments conducted with a small flow velocity lead to a slow increase of the reactivity rate until reaching a stable threshold value."

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.

RESPONSE: As the term "dynamic" in opposition to "static" can be confusing, it has been replaced by the term "flow-through", which is used in existing open channel experiments (e.g., "Garny, K., Neu, T. R., and Horn, H. (2009), Sloughing and limited substrate conditions trigger filamentous growth in heterotrophic biofilms-Measurements in flow-through tube reactor, Chemical Engineering Science, 64, 2723-2732"). It implies that the terms "static and dynamic experiments" are replaced by "static and flow-through experiments" in the modified version of the manuscript.

8. Abstract: specific conclusions are missing; they are stated as too general.

RESPONSE: Specific conclusions have been added to the abstract as follows: "It results in observing various dynamics of nitrate transformation associated with biofilm development where flow velocity appears to be a key factor, as (i) the experiments conducted with the largest flow velocities are characterized by a fast increase of the reactivity rate until reaching a threshold where strong oscillations are observed; and (ii) experiments conducted with a small flow velocity lead to a slow increase of the reactivity rate until reaching a stable threshold value."

9. Page 9831, Lines 18-21: clearly written and explained. Use this also in the abstract?

RESPONSE: These sentences have been added at the end of the abstract.

10. Introduction: I miss a clear statement of the research objectives.

RESPONSE: We now clearly mention the objectives in the second paragraph of the introduction as follows: "Our study focuses on the biofilm-growth period of long-term experiments and aims at characterizing the impact of hydraulic properties on (i) the

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efficiency of denitrification along the biofilm-growth period, and (ii) the stability of this biological reaction for bioremediation applications."

11. Page 9832, line 12: explain also in introduction that this 'model' applies also for pyrite.

RESPONSE: Autotrophic denitrification with pyrite is now referred as an example of biological reaction that can be considered by our model at the end of the first paragraph in section I (Introduction) as follows: "This aspect is critical as flow velocity may be a key-controlling parameter in systems where mobile water interacts with a growing non-mobile biological phase (e.g., autotrophic denitrification with pyrite)."

12. Page 9833, line 19: and what about 'inorganic carbon'?

RESPONSE: Inorganic carbon evolution has been added to Figure 1. The evolution of organic carbon is described in the revised manuscript as follows in section 2.2: "Inorganic carbon shows small variations with a small increase at the beginning of the experiment."

13. Page 9833, line 23: unclear 'water compounds'

RESPONSE: In order to clarify this section, a subsection entitled "Static experiments" has been added (section II.2) and the sentence containing "water compounds" at the end of this subsection has been replaced by "PVC tubes are thus the carbon source of the observed denitrification reaction that does not occur without the presence of these tubes."

14. Page 9835, line 4: 45 micrometer filter? Divide by 100?

RESPONSE: Indeed, the filters are 0.45 μm and not 45 μm. This mistake has been corrected at the beginning of section 2.4 (which was section 2.3 in the first version of the manuscript).

15. Page 9837, line 1: It helps the reader if first the various phases are roughly defined

and indicated.

RESPONSE: In the modified version of our manuscript, the various phases are roughly mentioned in the third paragraph of section 3 and subsection 3.1 (entitled “Definition of the identified phases”) has been added to clarify the definition of these phases before their description in section 3.2 (phase 1) and section 3.3 (phase 2).

16. Page 9837, line 1: first time it is stated the rate increases with time. Should be stated earlier. RESPONSE: In the modified version of our manuscript, this is mentioned at the beginning of the description of phase 1 in the first sentence of the second paragraph of section 3.2.

17. Page 9837, line 8: these different phases are not indicated in Fig 3.

RESPONSE: In the revised version of the manuscript, we have improved Figure 3 and Figure 5. The different phases are now described in these figures in order to clarify the description and interpretation of these phases.

18. Page 9840, line 16: is the unit mg correct? It seems so low considering the mass of nitrate passed through the system.

RESPONSE: Yes, the unit mg is correct. The estimates of biofilm thickness, related to biomass, enable us to verify that the order of magnitude of the estimates is comparable to existing studies.

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?

RESPONSE: In the case of this experiment the nutrient is nitrates. The general term “nutrient” is used because this equation could be valid for other elements in other contexts.

Added References

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Please also note the supplement to this comment:
http://www.hydrol-earth-syst-sci-discuss.net/11/C5174/2014/hessd-11-C5174-2014-supplement.pdf

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