In this document we indicate where changes have been to the manuscript following the comments made by the referees. Line numbers refer to the tracking changes document version. A short description of the changes made is emphasized with **blue text**.

Response to Comments and Edits to Manuscript

Dear Authors,

The revised version of the manuscript addresses appropriately many of the comments raised by the two Reviewers. However, Reviewer #1 still raises some points concerning mainly i) the lack of information on how well the model is simulating substrate consumption for the reference experiment; ii) the need to compare modelled and measured water fluxes and/or hydraulic heads; iii) the need to clarify the "additional comparisons between the experimental results with our numerical simulations".

I recommend "Moderate Revision" and asks the Authors to respond to all points raised by Reviewer #1.

Kind regards,

Roger Moussa

Overall Response

Dear Dr. Moussa,

Thank you for handling our manuscript and for highlighting the areas where further clarification is needed. We also appreciate the valuable comments from the Reviewers, which have helped us improve the quality of our submission. At the core of this work is our mathematical model, which couples three key components: unsaturated flow, nutrient consumption and fate and transport, and biomass growth. The core novelty lies in the coupling of these three components with the explicit calculation of inactive and active biomass, and resulting impacts on performance of soil aquifer treatment systems. This advance provides a more rigorous representation of the biological processes occurring within the system that is critical to system clogging. This new representation is the reason why we must use the unique biomass characterization data obtained from this particular column experiment, as such detailed experimental data for both active and inactive biomass is rare.

We acknowledge the experimental data are not specific to the model parametrization, in fact, these experiments were performed in 2011 without this particular modeling effort in sight. Beyond the contributions to simulating clogging processes, our manuscript aims to motivate the development of experimental methods that separate active and inactive biomass in porous media, as our results show that this is an important process but generally has not been considered in either experimental or modeling studies to date.

To address your and Reviewer #1's concerns regarding the substrate consumption and unsaturated flow components of the model, we have added model-data comparisons and clarified the limitations of the experimental data.

Detailed Response

- Regarding **point i)** concerning substrate consumption: Samples from the column feed solution and effluent were taken only at the experiment's conclusion. These measurements showed 89% removal of the substrate, which is comparable to the 99% removal of DOC predicted by our model at the same time point.

- Regarding **point ii)** about unsaturated flow: We have added a comparison between the modeled and measured water content profiles. New plots are included comparing the water content sensor data and simulation results over time at various depths.
- Finally, **point iii)** regarding additional comparisons between experimental results and numerical simulations: As requested by Referee #2, we have made several additions throughout the revised manuscript to highlight and clarify all comparisons made between the experimental results and our numerical simulations. The specific changes addressing these comparisons are detailed in our individual response to Referee #2, which accompanies this submission.

We want to reiterate that while we have addressed the concerns regarding the substrate consumption and unsaturated flow components, our primary focus in this manuscript remains on presenting and validating the novel aspect of our model related to the explicit treatment and calculation of active and inactive biomass, and its key role in bioclogging and implications for soil aquifer treatment systems, which are emerging as a critical approach for wastewater reuse. We hope these revisions fully address your remaining points. These revisions have strengthened the manuscript, and we appreciate the time and effort invested in evaluating our work.

Referee #1

Nothing to change.

We thank the reviewer for his positive evaluation of the revised version of our paper.

Referee #2

2.1) *The revised version of the manuscript "Continuum modeling of bioclogging of soil aquifer systems segregating active and inactive biomass" by Cifuentes et al. (hess-2024-251) addresses many of my original comments appropriately. There are however some points where I am not satisfied by the replies/revisions of the authors. My main concern is that there is still no information on how well the model is simulating substrate (and electron donor) consumption for the reference experiment. To my opinion, this is one of the core criteria for the accuracy of a model applied to a biodegradation setting and I am somewhat surprised that I need to argue for that.*

Samples from the column feeding solution and effluents were taken only at the end of the experiment for total organic carbon analysis and that analysis showed 89% removal of the substrate. In the simulations, 99% of the input DOC is consumed. There was no continuous monitoring of effluent substrate during the experiment, but this end-point measurement provides a reasonable check, especially considering SAT systems generally lead to near-complete DOC consumption. **We added Lines 226 to 231 to the Results section addressing this comparison and acknowledging the limitations of the data. We also added a plot of simulated DOC concentration over time as Supplemental Figure A16.**

Similarly, I do not find a comparison between modeled and measured water fluxes and/or hydraulic heads. The only data shown as indication for the accuracy of the model simulations are the biomass data in Fig. 3 and the water contents in Fig. A13. In their reply the authors claim to have added "additional comparisons between the experimental results with our numerical simulations" but I can not identify and futher comparisions than those mentioned above. This challenges consequently all conclusions drawn from the model scenarios presented in the manuscript.

We added an additional comparison between water content sensor data with the simulation results as Supplemental Figure A14. This complements the end-state comparison presented before between simulated and water content. The agreement is reasonable for the upper layers of the column but it is poorer on the deeper layers. We argue that the model's ability to capture the general trends of water content fluctuations is sufficient to say that it provides a fair representation of the internal hydraulic conditions in the system. We considered that the tensiometer sensor data and the simulated matric head are not readily comparable because it is expected that unsaturated flow parameters (i.e., the van Genuchten-Mualem model parameters) will change as biomass accumulates. Our proposed model does not capture or solve for such changes and it is one of the simplifications we made in the model conception. **We added Lines 86 to 88 in the Methods section to detail this consideration in the proposed model.**

Some further minor points (comment number refer to the reply list provided by the authors):

Comment 2.13: Clarify, in the reply you mention DOC concentrations of 10 mg/L in the manuscript it is 100 mg/L. Also, are such concentrations comparable to the values considered for SAT systems?

DOC concentrations on a SAT system are lower than the concentration used in the experiment. The DOC source in a SAT system is the effluent of a wastewater treatment plant and those concentrations are around 15-20 mg/L (Idelovitch et al., 2003). In the column experiment, the DOC source is a growth broth which is characterized with a concentration of 100 mg/L in the simulations. We actually ran simulations with other influent concentrations and found that the system reached the same end-state: accumulation of biomass near the top clogging the system. In terms of experimentation, running a column with actual wastewater treatment plant effluent would be interesting because DOC bio-availability is much lower than that of the growth broth. This kind of column experiments are rare and a note for future directions with these considerations is added in the manuscript. ***To clarify this point, we added Lines 392 to 398 to the Discussion section.***

Comments 2.21: If the yields are expected to be "considerably lower" in the SAT systems that in the column experiments, what does this mean for extrapolating the results to the SAT systems. Less clogging, slower clogging or …? Here and at for some other comments I think the manuscript would benefit if arguments/clarifications/etc. are not only given in the reply but also find their way into the manuscript.

Relative to the column experiments, lower yields are expected in SAT systems primarily due to the nature of the dissolved organic carbon (DOC) typically present in secondary-treated wastewater effluent. This DOC is generally more refractory and less readily metabolized by microorganisms compared to the growth substrate used in the controlled column experiments. This difference in substrate bio-availability would not necessarily lead to less clogging in SAT systems in the long term, but rather to slower clogging initially. The accumulation of refractory fraction of inactive biomass that we found to be a key driver of bioclogging will still occur: accumulation of recalcitrant material will eventually dominate the clogging process, leading to the clogged end state we observe and simulate in our model. ***We have added this information to the Discussion section of the manuscript in Lines 398 to 404.***

Comments 2.23: If rho_x is the most sensitive parameter in clogging simulations (which I agree on) than one should argue carefully if the presented value is meaningful. The low value for the density provided here is similar to values other authors have used for "bulk biomass". If the bulk biomass mainly consists of EPS such low values can be justified. If the bulk biomass mainly consists of bacterial cells this is not possible. When comparing result with the literature it is thus necessary to be more careful than what is done here.

While bulk biomass (ρ_x) has a physical basis in Equation 11, its treatment as a calibrated parameter can obscure this significance. The composition of bulk biomass in the simulation changes over time: at very early times, the bulk biomass is dominated by the active fraction, but inactive biomass accumulates in the porous medium and becomes the dominant biomass fraction later. Therefore, the accumulation of inactive biomass means that lighter-weight EPS become a larger fraction of the total biomass over time, and the cell density within the biomass correspondingly decreases over time. ***We added this insight into the manuscript in Lines 368 to 373 in the Discussion section.***

Comment 2.25: In addition to my comments above: What is the corresponding value predicted by the model?? There should be changes made to the manuscript!!

The value of ρ_x used was mentioned briefly in Section 2.6 of the original manuscript. ***For clarity, we added it to Table 2 of the revised manuscript to make the information easier for readers to find.***

Comment 2.27: I do not find the mentioned comparison between observations and numerical results.

To clarify this point, ***we added plots (Supplemental Figure A14) that explicitly compare the water content sensor data with the simulation results.*** These new plots complement the end-state water content comparison presented previously.

Comments 2.32: I acknowledge that the definition is take from the literature. However, I think one should spend some words on the fact that the relative order of the values for the different scenarios depends on the (arbitrary length) of the experimental length. At the end the initial behavior of the systems differs from the behavior at later times.

Thanks for the suggestion. Yes, a characteristic time scale was needed for the definition of long-term hydraulic loading. We chose the length of the experiment for this, as it accounts for both the initial high flows into the clean column and the later low flows after clogging occurs. For SAT applications, this time scale should be chosen to span system resets, meaning it should cover the dry periods when there is no water inflow and until the system infiltration capacity is restored either by a much longer drying time or some mechanical intervention. **We added this consideration to the Discussion section in Lines 347 to 351.**

References

Idelovitch, E., Icekson-Tal, N. , Avraham, O., Michail, M.: The long-term performance of Soil Aquifer Treatment (SAT) for effluent reuse. *Water Supply,* August 2003; 3 (4): 239–246. https://doi.org/10.2166/ws.2003.0068, 2003.