Reviewer 2

The article titled "Optimization of Dissolved Silica Removal from Reverse Osmosis Concentrate by Gedaniella flavovirens for Enhanced Water Recovery" represents the treatment of reverse osmosis concentrate (ROC) using photobiological treatment (brackish diatoms).

> **General Response:** We appreciate the thorough review provided by the reviewer. Their insightful comments have provided valuable perspectives for improving our manuscript. We have carefully considered each point raised and have made revisions accordingly. While we may not agree entirely with all the comments, we acknowledge their importance in strengthening the quality of our work. Thank you for your time and constructive feedback.

1. I found that the concluding remark "The results of this study suggest the importance of light and temperature management in this photobiological process" in Lines 23-25 is very general and well-known; authors are encouraged to show the novelty of their study!!

> **Response:** We appreciate and agree with this comment. This statement has been modified as follows to make it more specific.
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> > The results of this study are being used in the subsequent pilot-scale investigations and full-scale technoeconomic analysis and will contribute to the further development of this sustainable water technology.

2. The study objectives in Lines 70-81 are not clear

> **Response:** We appreciate and agree with this comment. This paragraph has been modified as follows to clarify our research objectives and improve the flow.
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> > Although previous research has highlighted the potential of this innovative green technology using a diatom photobioreactor (PBR) followed by secondary RO, there remains a gap in understanding the impact of light-related parameters (such as intensity, color, and duration) and temperature on dissolved silica. These operational parameters play a critical role in optimizing the growth of photosynthetic microalgae, including diatoms [21-23], and in the efficient operations of PBR systems [24-27]. Therefore, this study aimed to systematically investigate the optimum treatment conditions concerning light and temperature. Identifying these optimal conditions is crucial for subsequent research and development activities, including continuous flow pilot testing and technoeconomic analysis. Additionally, this study also evaluated the removal of NDMA from AWPF ROC via the photobiological process. This assessment is important giving the regulatory limits on NDMA, particularly for certain AWPFs involved in indirect and direct potable water reuse [28-30].

3. In Table 1, the authors are requested to additional column for the water quality standard for comparison

> **Response:** We appreciate the suggestion to include water quality standards in the table. However, we believe that doing so might lead to confusion among readers. The water

quality data presented pertains to RO concentrate (ROC) before photobiological treatment, which undergoes additional treatment before it becomes drinkable. Furthermore, the quality of the final product water is primarily influenced by the performance of the secondary RO system. Given these factors, we have chosen not to incorporate water quality standards in the table as it may not accurately reflect the intended purpose of the data.

4. For Lines 133-134; add a separate section for this ANOVA statistical method

   Response: We appreciate the suggestion to expand on our explanation of ANOVA. However, given that ANOVA is a well-established and widely understood statistical method, we opted to summarize its application in a single sentence. We believe that most of our target audience will be familiar with the basics of ANOVA, making further explanation in this paper redundant. Nonetheless, we are open to adding a reference or a brief note for readers less familiar with the method, if deemed essential.

5. What are the devices used to measure the water quality parameters in Table 3

   Response: We appreciate this question. The information on analytical instruments was overlooked in the original manuscript. We thank the reviewer for pointing out this oversight. The following sentences were added:

   > A Hach DR-1900 spectrophotometer (Loveland, CO, USA) was used for colorimetric analysis, while Hach Digital Titrators were used for titration analyses. A Hach IntelliCAL ISENA381 probe with a Hach HQ40d meter was used for sodium analysis.

6. What are the biochemical reactions used to describe the effect of light in section “3.1. Impact of Light”

   Response: We appreciate the feedback on the section title. While the primary parameter optimized in this research, as indicated in the article title, is silica uptake, we acknowledge that the original section title might have been too abstract for some readers. To improve clarity, we have revised the section title to “Impact of Light on Reactive Silica Uptake”.

7. At what operational time the images in Fig. 5 were captured.

   Response: We appreciate this comment. It is indeed important to indicate the time when these images were taken. We modified the Figure 5 caption as follows:

   > **Figure 5.** Photomicrographs of *G. flavovirens* Psetr3 incubated at (a) 40 and (b) 23 °C in GWRS ROC for three days (96 hr in Figure 4).

8. What are the biochemical reactions used to describe the effect of temperature in section “3.2. Impact of Temperature”

Response: We appreciate the feedback on the section title. Similar to Section 3.2, we have revised the section title to “Impact of Temperature on Reactive Silica Uptake”.

9. At what operational time the images in Fig. 7 were captured.

Response: We appreciate this comment. It is indeed important to indicate the time when these images were taken. We modified the Figure 7 (renumbered to Figure 8) caption as follows:

> **Figure 8.** Photomicrographs of *G. flavovirens* Psetr3 exposed to (a) direct sunlight in the outdoor experiment Run 1 (after 120 hr), and (b) high temperature in Run 2 (after 70 hr).

10. In section “4. Discussion” the authors are requested to draw a schematic diagram illustrating the effects of light and temperature on RO performance

Response: We appreciate this feedback. While it is true that the performance of the secondary RO system can be influenced by the operation of the diatom photobioreactor, factors such as UV light and temperature primarily pose risks within the diatom PBR process itself. Establishing a direct relationship between these parameters and secondary RO performance in a scheme could prove challenging. Instead, we have included additional discussion in the manuscript to emphasize the importance of risk mitigation in the PBR for ensuring the effectiveness of downstream RO processes. We believe this approach is more suitable for addressing the reviewer's concerns.

> Fluctuating temperatures, both high and low, along with UV radiation, represent potential major risk factors for seamless continuous operation of the diatom PBR-RO process and must be effectively mitigated. Disruption or poor performance of the PBR due to suboptimal temperature and UV exposure could impact the secondary RO system, resulting in reduced freshwater recovery.

11. The Conclusion section is very lengthy!!

Response: We appreciate this feedback. We have thoroughly revised the Conclusions section to improve its clarity and readability.

> This study investigated optimal light and temperature conditions for maximizing silica uptake by the brackish diatom *G. flavovirens* Psetr3 in ROC from OCWD GWRS AWPF. While color temperatures (2,700–5,000 K) had no significant impact on silica up-take (39 ± 2 mg/L/day) at a PAR of 200 µmol m^{-2} s^{-1}, blue-colored LED bulbs slightly im-proved uptake (+22%) despite impractical light output. Increasing PAR to 200–300 µmol m^{-2} s^{-1} enhanced silica uptake, while intermittent light slowed uptake by 34% compared to continuous illumination. Optimum temperature ranged from 23–30 °C, with uptake slowing at 10 °C and diatom mortality at 40 °C.
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> Outdoor experiments confirmed the impact of light and temperature on silica uptake, with high temperatures (>40 °C) and UV radiation from sunlight

negatively affecting up-take. Outdoor uptake rates (15–27 mg/L/day) were generally lower than indoor rates (40–50 mg/L/day), primarily due to fluctuating temperatures. These findings underscore the importance of light management and temperature control in the diatom PBR-RO process using sunlight. Sunlight-based photobiological treatment effectively removed NDMA from AWPF ROC, likely through direct photolysis rather than biological degradation. This highlights the potential of sunlight-based process for ROC treatment, especially given NDMA's significance in potable reuse projects.

These results inform ongoing pilot testing and full-scale lifecycle cost analysis, demonstrating the technical and economic feasibility of this green technology. Ultimately, the diatom PBR-RO process promises to enhance freshwater recovery from reclaimed and brackish water, supporting sustainable desalination and potable water reuse projects in arid and semi-arid regions.