A Novel PstS Protein-Enabled 3-Tier Biofiltration Device for Wastewater Phosphorus Capture and Reuse

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Problem and Solution

- Agricultural runoff and outdated wastewater treatment plants contribute to severe phosphorus pollution, with 37 plants discharging over **54,000 kg of pollutants** into local waterways daily.
- These excess nutrients fuel harmful **algal blooms**, devastating marine life, disrupting economies, and degrading **water quality**.
- We developed a **novel PstS protein-enabled 3-tiered biofiltration device** — a **sustainable, cost-effective** alternative for phosphorus capture and reuse.
- Each of the 3 layers has different strengths, and put together, it increases the amount of phosphorus absorbed from the water.

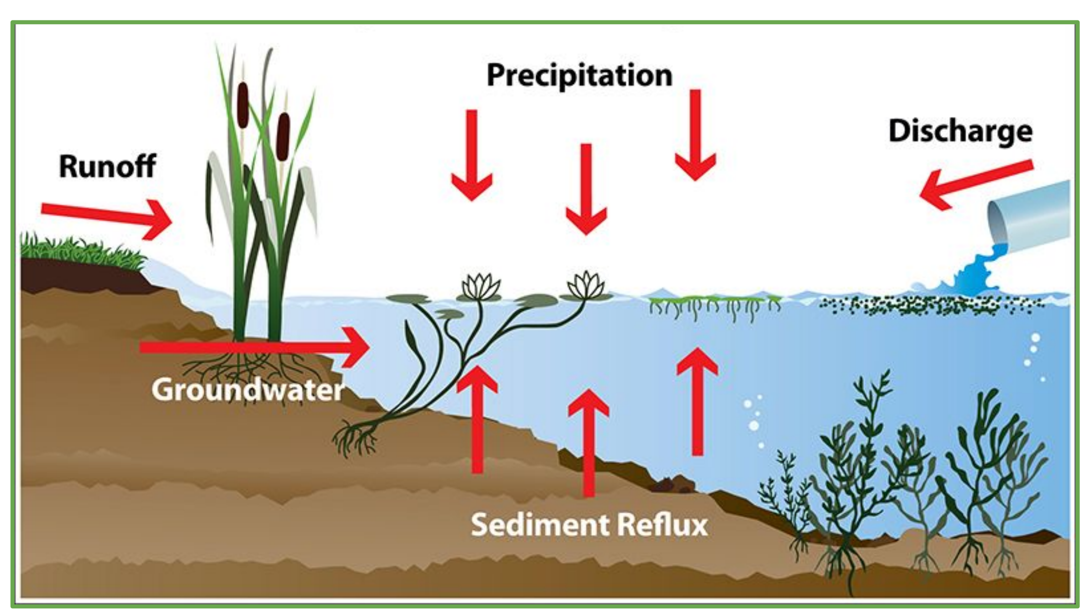


Figure 1. Sources of phosphorus loading (SOLitude Lake Management)

History and Alternatives

- Current phosphorus removal techniques, such as chemical precipitation and physical filtration, are often **costly and produce secondary pollutants**.
  - Ex: 2022 San Francisco Nutrient Bill estimated **$11.2B.**

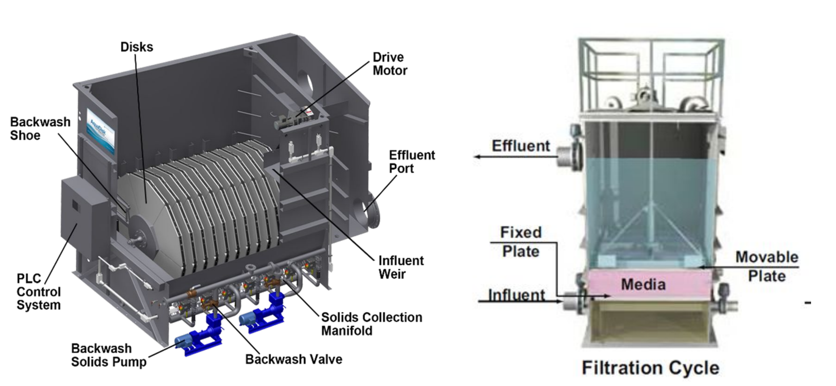


Figure 2. Current wastewater phosphorus filtration systems (Ruekert & Mielke, Inc.)

- Despite its value, **less than 12% of removed phosphorus is recycled** and never reused.
  - New research (Venkiteshwaran et al., Choi et al.) is exploring *E. coli* based bioremediation as a form of phosphorus absorption for reuse

Detailed Solution and Sketches

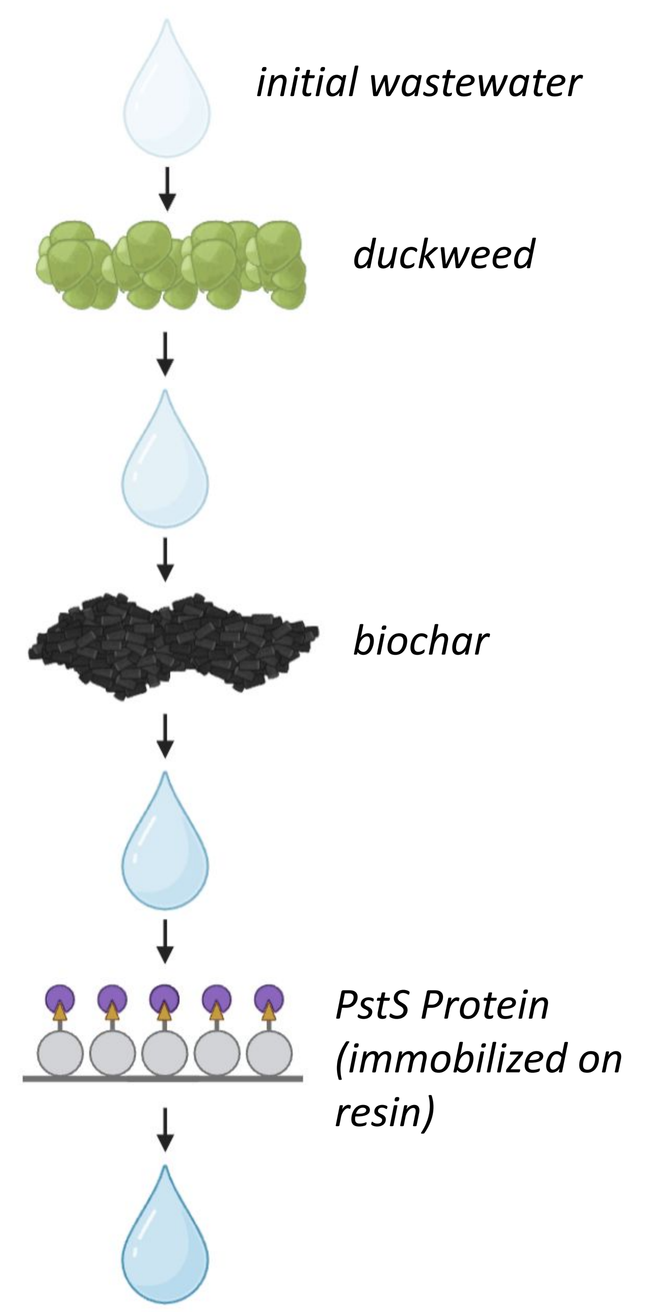


Figure 3. Filter layers (Made with BioRender)

The three layers of our filter:

1. *Lemna minor,* duckweed, an aquatic plant capable of absorbing and storing excess phosphorus
2. Biochar, black carbon produced from biomass, enhances phosphorus adsorption through surface interactions
3. PstS protein, expressed in *E. coli* and immobilized on Nickel-NTA resin affinity beads

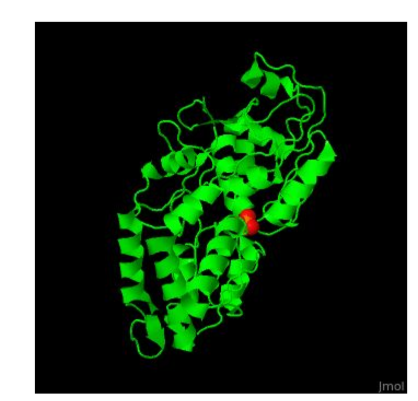
Figure 4. Our wastewater filtration columns and controls

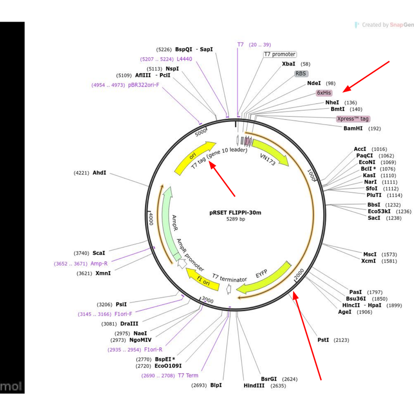
The structure of our filter:

- Our filter will be housed in 3d-printed gravity columns, which are easy to customize based on the situation.
  - Adjust surface area/volume ratio for more effective filtration.
  - Simple to swap out layers when needed.
- Each layer is separated by filter paper.

Figure 5. PstS Protein
Figure 6. Our PBP plasmid pRSET FLIPPi-260n (Gu et al.)

PstS proteins play a crucial role in phosphate transport, **binding phosphate ions with high affinity** to facilitate their uptake. We will take advantage of this property in layer 3 and use it to absorb phosphorus from the wastewater.





Testing, Benchmarking, & Comparison to Alternatives

Testing Procedure:

Layer 1 (PstS Protein)

- Plasmid pRSET FLIPPi-260n ordered from Addgene; miniprepped and isolated.
- BL21 DH3 *E. coli* grown via overnight culture; plasmid transformed into cells.
- IPTG added to induce expression; PstS purified and immobilized on Nickel-NTA resin affinity beads.

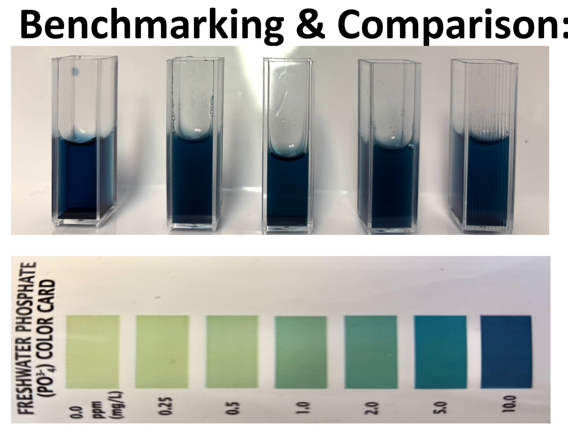
Layer 2 (Biochar)

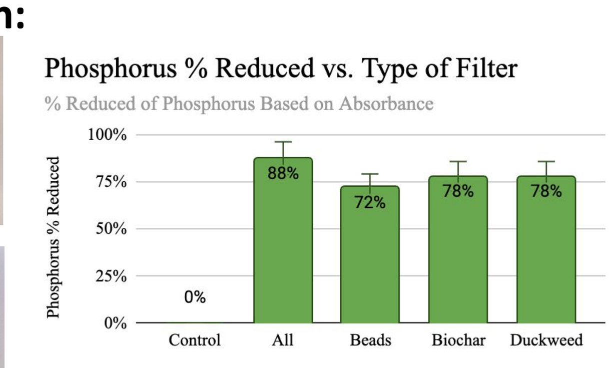
- Biochar obtained from NatureSolutions; homogenized to consistent particle size for adsorption.

Layer 3 (Duckweed)

- Duckweed grown on Murashige & Skoog media; placed in agar disk and added to filter.

Benchmarking & Comparison:





Phosphorus concentration in water was compared before/after filteration via UV/VIS, where **blue color change** was detected in presence of phosphorus. In comparison to alternatives, our filter is energy-free, and virtually **just as effective** in removal!

Figures 7, 8, 9. Cuvettes of filtered wastewater and results from experiment

Technical Challenges

- **Reusing phosphorus:** Our PstS protein releases phosphorus when pH is changed; we must determine the most efficient process for elution and reuse.
- **Material degradation:** Wastewater contains chemicals that could impede the filter — for example, duckweed not getting enough nutrients.
- **Layer order optimization:** Test different arrangements to maximize efficiency while keeping resin beads accessible.
- **Filter size & shape:** Optimize the column design for maximum phosphorus removal and reusability.

Ethical and Societal Concerns

- **Leaching of PstS proteins into water:** If proteins detach from the resin, they may enter waterways, posing unknown ecological risks to aquatic life and human health.
- **Duckweed as an invasive species:** Duckweed reproduces rapidly and could become an ecological nuisance if it escapes into natural waterways beyond the filter.
- **Metal waste from Nickel-NTA resin:** The Nickel-NTA resin used for protein immobilization may introduce metal waste if disposed of improperly, potentially contributing to environmental toxicity.
- **Limited absorption capacity over time:** Biochar and duckweed reach saturation, reducing effectiveness and necessitating replacement.
- **Degradation of PstS protein:** Reduced efficiency over time may require frequent replacement, increasing maintenance costs and waste.
  - Creating additional layers will cost time and manpower beyond a traditional filter.
- **Financial accessibility:** Though overall cost is low, maintenance costs in rural areas could limit accessibility for smaller communities.

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Acknowledgements

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