

Coupled *Azolla*-*Spirulina* Bioregenerative Life-Support System — A Closed-Loop Model for Energy, Water, and Nutrient Recovery in Space Habitats

Concept Paper

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1 Executive Summary

Every bioregenerative life-support architecture that recycles waste nitrogen for food production, including ESA’s MELiSSA, hits the same problem: ammonium is toxic to the organisms meant to eat it, so it has to be converted first — typically by a dedicated bacterial nitrification reactor that produces no food and no energy of its own. We’re proposing to replace that single-purpose reactor with *Azolla*, a nitrogen-fixing aquatic plant that absorbs ammonium efficiently and, once digested, yields usable ammonium, biogas, and bicarbonate alkalinity — three outputs instead of one. Because existing *Spirulina* growth models assume nitrate as the nitrogen source and don’t account for ammonia’s toxicity regime, we are extending the standard model with a Haldane-type inhibition term and a temperature-dependent pK_a correction to handle ammoniacal feeding directly; this extension is still in development and has not yet been calibrated against real data. This document lays out the gap we found, the model we are building to address it, and the three candidate architectures (direct dilution, *Azolla*-mediated extraction, and *Azolla* as pre-treatment) we’re weighing against each other — along with what we still need real data, not literature bounds, to resolve.

2 System Context & Mission Rationale

This work sits within the broader effort to design closed-loop bioregenerative life-support sys-

tems (BLSS) for long-duration space habitats — systems where food, water, and air are regenerated internally rather than resupplied from Earth. MELiSSA (ESA) remains the most mature reference architecture in this space, and its *Spirulina* compartment is the closest existing analog to what we’re proposing.

Coupling *Azolla* and *Spirulina* specifically matters because it collapses two separate engineering problems — nitrogen conversion and food/energy production — into a single biological subsystem. Where MELiSSA treats nitrification as a dedicated, single-purpose process, our approach treats it as a byproduct of biomass that is itself useful. This is directly relevant to mass and volume efficiency, which are the tightest constraints in any real space habitat design.

3 The Gap We Found

Every major bioregenerative life support system that’s dealt with this problem has hit the same wall we’re looking at: ammonium from waste is toxic to the organisms you eventually want to feed it to, so you have to convert it into something safer first. MELiSSA — the most developed BLSS program out there, run by ESA for over 30 years — solves this with a dedicated bacterial compartment: a bioreactor running an axenic (pure) culture of *Nitrosomonas* and *Nitrobacter* that oxidizes ammonium into nitrate, which is then fed to their *Spirulina* (*Limnospira*) compartment. It works, but it’s a purely engineered fix — a whole extra bioreac-

tor whose only job is converting nitrogen from one chemical form to another. It doesn't produce food, doesn't produce energy, and the culture has to be kept pure and carefully maintained, which is its own operational burden.

What nobody's tried, as far as we can tell, is doing that same conversion with a nitrogen-fixing plant instead of a bacterial monoculture — specifically *Azolla*, which is already known for absorbing ammonium efficiently and fast. The difference matters: unlike a nitrifying bioreactor, *Azolla* is itself edible biomass, and once you digest it, you get usable ammonium, biogas, and (as we found later) bicarbonate alkalinity as byproducts. So the same conversion step MELiSSA needs a dedicated single-purpose reactor for could, in principle, be replaced by something that gives back three usable outputs instead of one converted one.

That's the system-level gap. The reason it hasn't been modeled properly yet comes down to the math: the standard *Spirulina* growth models (Cornet-Dussap 2009, and the later Poughon et al. work) are built around nitrate as the nitrogen source, because that's what MELiSSA-style architectures feed it. They don't account for what happens when the nitrogen source is ammoniacal instead, coming from something like *Azolla* effluent — and that matters because free ammonia (NH_3) isn't just another nitrogen source, past a certain concentration it actually becomes toxic and inhibits growth instead of feeding it. None of the existing models capture that inhibition regime, because none of the existing BLSS architectures needed them to. So if we want to actually replace the bacterial nitrification step with an *Azolla*-based one, we need to extend the model ourselves — the standard framework simply doesn't apply once ammonium, not nitrate, is what's actually reaching the *Spirulina* culture. That extension work is currently underway and is described, in its planned form, below.

4 What We Are Adding to the Model

We are extending the base *Spirulina* growth model by introducing a Haldane/Andrews-type inhibition term for free ammonia, so that the model will eventually capture both sides of the story: NH_3 as a nitrogen source at low concentration, and NH_3 as a toxin past a threshold. Alongside this, we plan to add a temperature-dependent pK_a correction (based on Emerson et al., 1975), because the split between NH_4^+ and NH_3 — which is what actually determines toxicity, not just total ammoniacal nitrogen — depends on temperature and pH, not just

on how much nitrogen is in the water. The intended implementation is in Python (`scipy solve_ivp`, RK45), with this document tracking the design as it develops. To be clear, this model is meant as a design tool for us, not a source of results we'd publish as validated fact — the structure is still being worked out and the parameters still need real calibration before any output from it can be treated as reliable.

5 How Do We Actually Get Nitrogen to Spirulina Without Shocking It

This ended up being a bigger question than we expected, and we found three real directions worth comparing, not just one obvious answer.

Direction A — just dilute the urine and feed it straight to Spirulina. Turns out this isn't a bad idea on its own. We found a paper (Feng & Wu, 2006) where they cultured *Spirulina* directly in diluted human urine — no *Azolla*, no pre-treatment — and by diluting it somewhere between 140x and 240x, they avoided ammonia toxicity completely and got successful growth. So “ammonia shock” by itself is a solvable problem just by controlling dilution. That's important to admit honestly, because it means we can't justify *Azolla* purely as a way to “prevent toxicity” — that problem already has a simple answer.

But that same paper showed a different problem: at that dilution, the biomass ended up low in protein (32% instead of 67% in standard Zarrouk medium) and high in lipid instead. The reason wasn't nitrogen at all — it was a lack of inorganic carbon. Diluted urine has way too little carbon relative to nitrogen (roughly 20:21.6:1 C:N:P instead of Zarrouk's 74.66:10.25:1), so even with enough nitrogen around, *Spirulina* can't actually build protein without enough carbon backbone. There was also way less iron in the biomass (about 6 mg per 100g dry weight vs 37 mg in Zarrouk). So Direction A avoids toxicity, but it doesn't give us good protein yield, and it doesn't solve the iron gap either — carbon and iron have to be supplemented no matter which direction we choose.

Direction B — extract ammonium from digested Azolla and dose it into Spirulina gradually. The original idea here: grow *Azolla* in urine (*Azolla* is very good at absorbing $\text{NH}_4\text{-N}$ from urine — one study showed it dropping ammoniacal nitrogen from about 25 mg/L to under 2 mg/L in five days), then anaerobically digest the *Azolla* biomass, which releases the nitrogen it absorbed back as ammonium (since living *Azolla* stores nitrogen mostly as organic nitrogen, and digestion min-

eralizes it back to NH_4^+), and at the same time gives us biogas as an energy byproduct. Then we would dose that ammonium into the *Spirulina* culture in a controlled, gradual way instead of dumping it all at once — this is also why the growth model needs the ammonium input to be a function of time, not a fixed constant, since we intend to control the feed rate deliberately rather than assume a static concentration.

There's a fourth output from this same digestion step that we didn't originally account for: anaerobic digestion naturally produces significant bicarbonate alkalinity as a byproduct (typically 4000-5000 mg/L as CaCO_3 in comparable systems), generated by the digester's own bacterial community as part of buffering the process against the volatile fatty acids produced during fermentation. That matters a lot, because it directly addresses the carbon shortage problem we found in Feng & Wu's direct-dilution results (Direction A) — diluted urine on its own is carbon-poor relative to nitrogen, which is why protein synthesis stalled even with enough nitrogen present. So the same digester built for Direction B wouldn't just give us ammonium and energy, it would also give us the carbon/alkalinity source that Direction A structurally lacks.

Direction C — use *Azolla* as a pre-treatment stage rather than a separate extraction step. Instead of diluting urine all the way down to 140-240x for *Spirulina* directly, we would dilute it much less (say, around 50x) and let *Azolla* itself absorb the ammonium down to a safe level before that water ever reaches *Spirulina*. If this works, we skip the extraction/digestion step for the water pathway entirely — *Azolla* just acts like a biological buffer stage. The open question is whether *Azolla* itself can survive at that starting concentration; the numbers we have so far are two single data points from two different studies (one showing *Azolla* failing above $\sim 5\text{mM NH}_4^+$, another showing it surviving fine at $\sim 25\text{ mg/L}$), not a real tolerance curve, so this needs actual testing on our specific urine and dilution setup, not just literature.

Why we think B/C are worth the extra complexity over just doing A. At first it looked like Direction A wins by default since it already solves toxicity and is simpler. But the real cost we were missing is water and reactor volume, not just toxicity risk. Diluting urine 140-240x means a much bigger bioreactor volume to process the same amount of nitrogen, and in a closed-loop space system, water and volume are not free — they're some of the tightest constraints there are. If *Azolla* lets us run *Spirulina* at a lower dilution ratio, that

would be a direct cut in the water/volume footprint of the whole system, not just a “nicer” way to avoid toxicity. On top of that, Direction A only gives us one output (treated water), while B/C would give us three: treated water, *Azolla* biomass itself as food/feed (roughly 22-27% crude protein by dry weight), and biogas as energy from digestion. So the real comparison isn't “which one avoids toxicity” (both can) — it's “which one gets us more usable output per liter of urine and per unit of system volume,” and that's the question we plan to answer with the math once the model is calibrated, not one we're assuming the answer to.

A note on B vs. C — sequence, not alternatives. We've framed B and C above as separate directions for comparison, but that's a simplification for this document, not our actual working assumption. Our real picture is closer to a sequence: *Azolla* first acts as the pre-treatment buffer (C), absorbing ammonium from moderately diluted urine, and then some portion of that same *Azolla* biomass — once it's done its absorption work — would be fed into the digester (B) to recover ammonium, biogas, and bicarbonate rather than being discarded or fed directly as biomass. What we haven't worked out yet is the split: how much of the *Azolla* biomass goes to digestion versus staying as harvestable feed/food, and on what cycle. This is one of the open questions we'd want to discuss with our advisor rather than resolve on our own.

6 Full Nutrient Budget for the Coupled Loop

Spirulina's value as the human-food anchor of this system comes from its exceptional nutritional profile: up to 60-70% crude protein by dry weight under standard Zarrouk medium, a complete essential amino acid profile, and high concentrations of phycocyanin, vitamins, and micronutrients — making it one of the most nutrient-dense biomass sources achievable in a closed-loop system. This is what justifies treating nitrogen delivery to *Spirulina* as the central engineering problem of the coupled loop, rather than treating *Azolla* as the end product.

We plan to build the full nutrient budget in three layers: (1) *Spirulina*'s own requirements, using Zarrouk medium as the baseline reference ($\text{C:N:P} \approx 74.66:10.25:1$); (2) *Azolla*'s own growth requirements, since its nutrient uptake for its own biomass isn't free; and (3) what the digestate can actually deliver after accounting for *Azolla*'s own consumption and any mineral precipitation losses (e.g., Ca/Mg-phosphate scavenging). The gap between (1) and (3) will be the real external supple-

mentation need, once this budget is built.

Based on literature to date, iron appears to be a likely gap regardless of nitrogen pathway chosen; carbon/bicarbonate and sodium may be covered internally by the digestion process and urine composition respectively, though literature values for *Azolla*'s elemental composition vary substantially between sources and will need refinement as the project proceeds.

7 Planned Calibration Work

The model extension described in Section 4 is intended to introduce two parameters that don't exist in standard *Spirulina* growth models: $K_{S,N}$ (half-saturation constant for ammoniacal nitrogen uptake) and K_{i,NH_3} (inhibition constant for free ammonia toxicity). Literature gives us starting bounds — an IC_{50} of approximately 44 mg-FA/L for *Arthrospira platensis*, and an *Azolla* failure threshold near 5 mM (~ 90 mg N/L) — but these values come from different strains, media, and conditions than ours, so they can only serve as provisional starting bounds for the model, not final parameters. Refining them against our own cultures and our own urine-based medium is a core part of the next phase of this work, and no numerical results from the model should be treated as final until that calibration is complete.

8 Proposed Mitigation Layer

As an additional buffering option between *Azolla* effluent and the *Spirulina* reactor, we're considering a zeolite (clinoptilolite) filtration stage. Clinoptilolite is a naturally occurring aluminosilicate with high selectivity for ammonium ions, and comparable digestate-pretreatment systems report 77-95% NH_4-N removal efficiency. This would serve as a passive safety margin — catching any batch where *Azolla*/digestion output runs higher in ammonium than intended — before that stream reaches *Spirulina*. This is a candidate mitigation layer to evaluate, not yet a committed design choice.

9 Validation Plan

Testing will proceed using the cultivation setup already underway (*Azolla* and *Spirulina* cultures maintained since August 2026), moving toward the three-direction comparison outlined in Section 5. The immediate priorities are: establishing *Azolla*'s real tolerance curve under our specific urine-dilution conditions (rather than relying on the two disparate literature data points cited in Section 5), measuring actual ammonium uptake and release rates through the digestion cycle, and using that data to replace the literature-bound parameters in Section 7 with calibrated ones once the model extension itself is complete.