



2022 MELISSA CONFERENCE
8-9-10 NOVEMBER 2022

CREATING
A CIRCULAR
FUTURE

Fertilizer production for soilless plant cultivation in closed life support system – lessons learned from 4 years study

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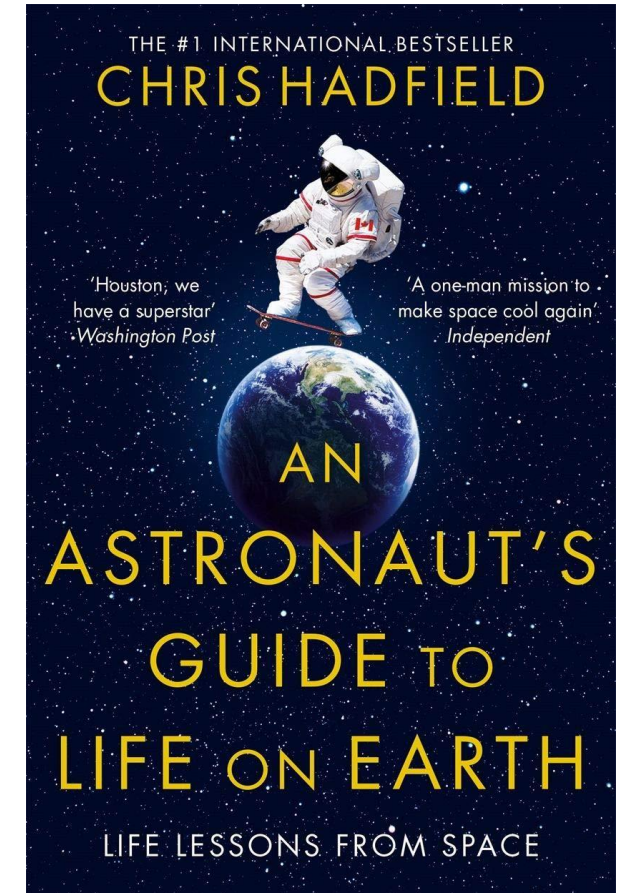
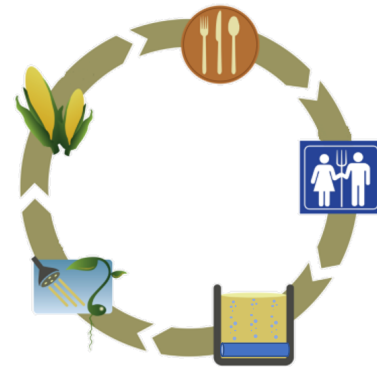




Aim of the study

Key issues:

1. characterization of the fertilizer production process based on the nitrification of urine and a mixture of urine and grey water in an activated sludge SBR reactor,
2. characterization of the fertilizers,
3. characterization of the plants cultivated using produced fertilizers (yield, quality parameters, organoleptic test).





Research phases

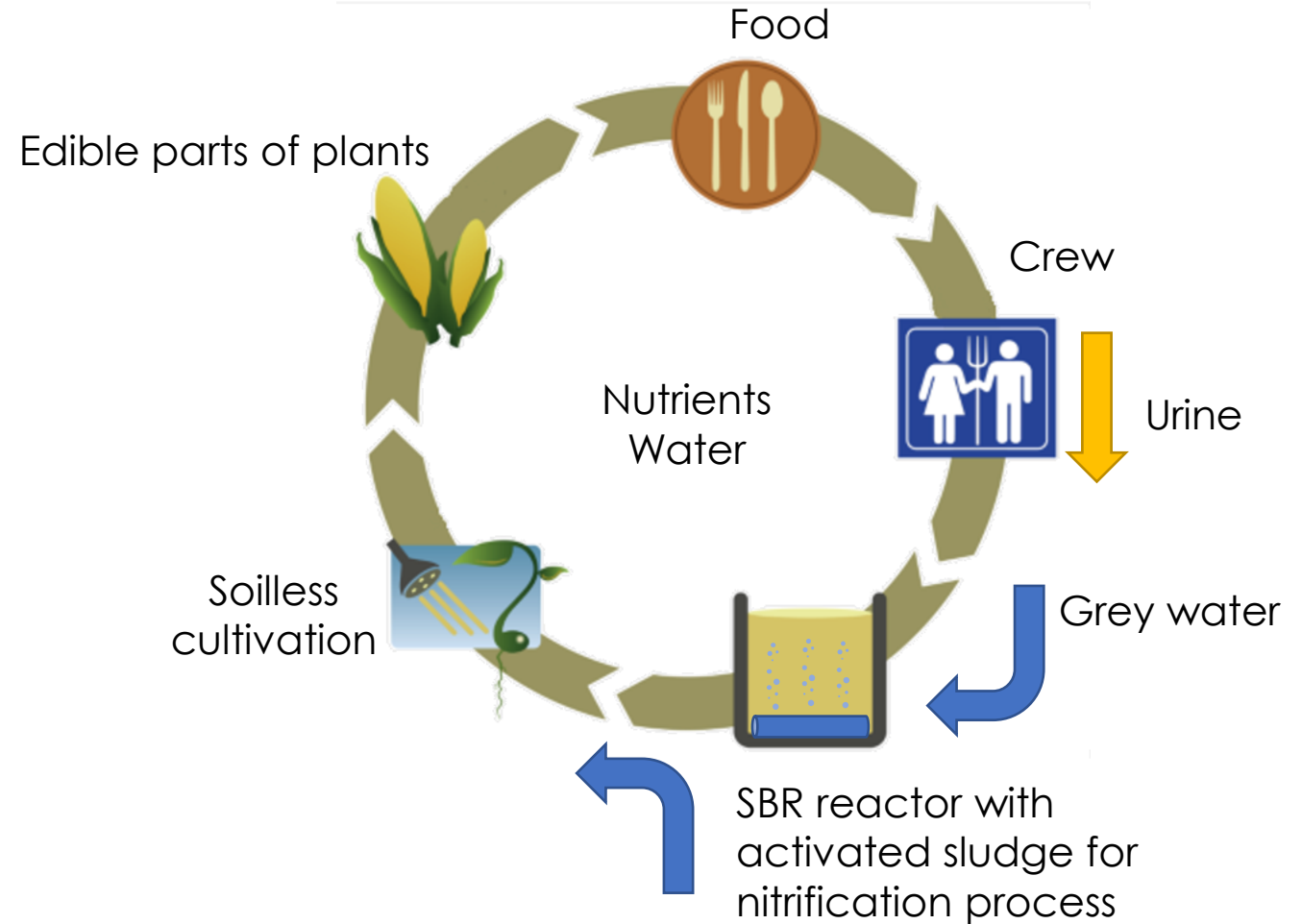
Phase 1: Urine nitrification in an SBR activated sludge reactor as production of liquid fertilizer for soilless cultivation

Phase 2: Lettuce hydroponic cultivation using diluted, nitrified urine

Phase 3: The influence of anionic surfactants on soilless lettuce cultivation

Phase 4: Urine and grey water nitrification in an SBR activated sludge reactor as production of liquid fertilizer for soilless cultivation

Phase 5: Lettuce hydroponic cultivation using diluted, nitrified urine and grey water





Nitrification process of urine and urine and grey water

Potential challenges of running the nitrification of urine and a mixture of urine and grey water in an aerobic activated sludge SBR include:

1. high salinity of urine,
2. high concentration of ammoniacal nitrogen, which can result in inhibition due high concentrations of free ammonia (FA) or nitrous acid (FNA),
3. high concentration of the anionic surfactant SMCT and its effect on activated sludge.



Image 1. Experimental set-up.

MATERIAL AND METHODS

1. Research conducted on artificial streams (Anderson et al, 2018; Feng and Wu, 2016; Verostko et al, 2004).
2. Experimental set-up placed in the Wroclaw Wastewater Treatment Plant.
3. Lowering the pH set-up to 5.5-5.9 due to plant preference in soilless cultivation.
4. The respiratory activity of activated sludge study in the absence or presence of substrate and in the presence of inhibitory substances (FA, FNA, salinity, anionic surfactants).
5. Periodic analytical control of treated effluent composition (N-NH₄, N-NO₃, N-NO₂, N_{tot}, Cl, COD, alkalinity, EC, anionic surfactants), sludge concentration, and nitrification rate.



Nitrification process of urine and urine and grey water

URINE NITRIFICATION

- Duration: 225 days.
- Reactor parameters during stable performance phase:
 - temperature: 30°C,
 - pH 5.9,
 - nitrogen load: 0.101 gN/gSS ·day
 - DO: 3.0 mgO₂/dm³,
 - alkalinity: NaHCO₃.

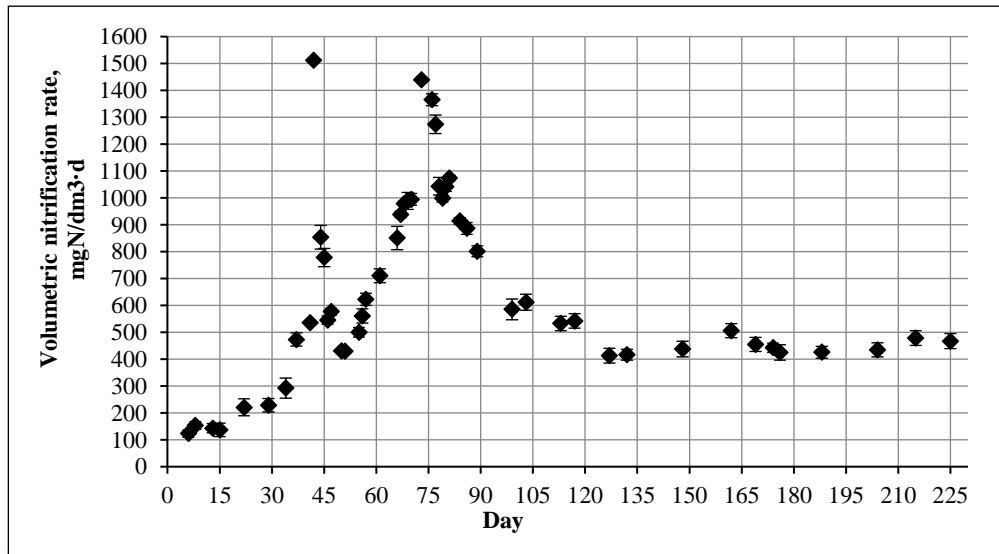


Fig 1. Nitrification rate during urine nitrification experiment.

URINE AND GREY WATER NITRIFICATION

- Duration: 140 days.
- Reactor parameters during stable performance phase :
 - temperature: 30°C,
 - pH 5.9,
 - nitrogen load: 0.027 gN/gSS ·day
 - DO: 3.0 mgO₂/dm³,
 - alkalinity: NaHCO₃.

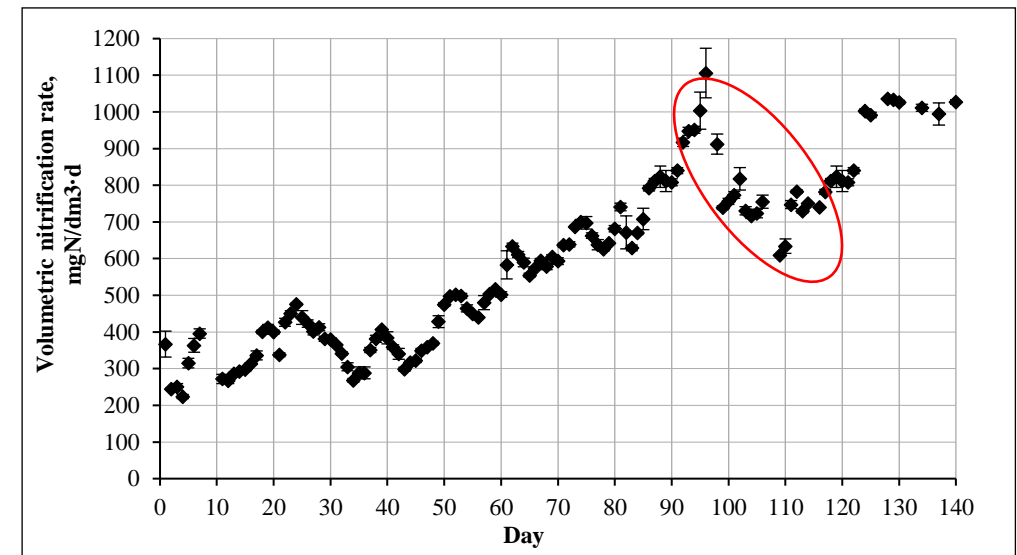


Fig 2. Nitrification rate during urine and grey water nitrification experiment..

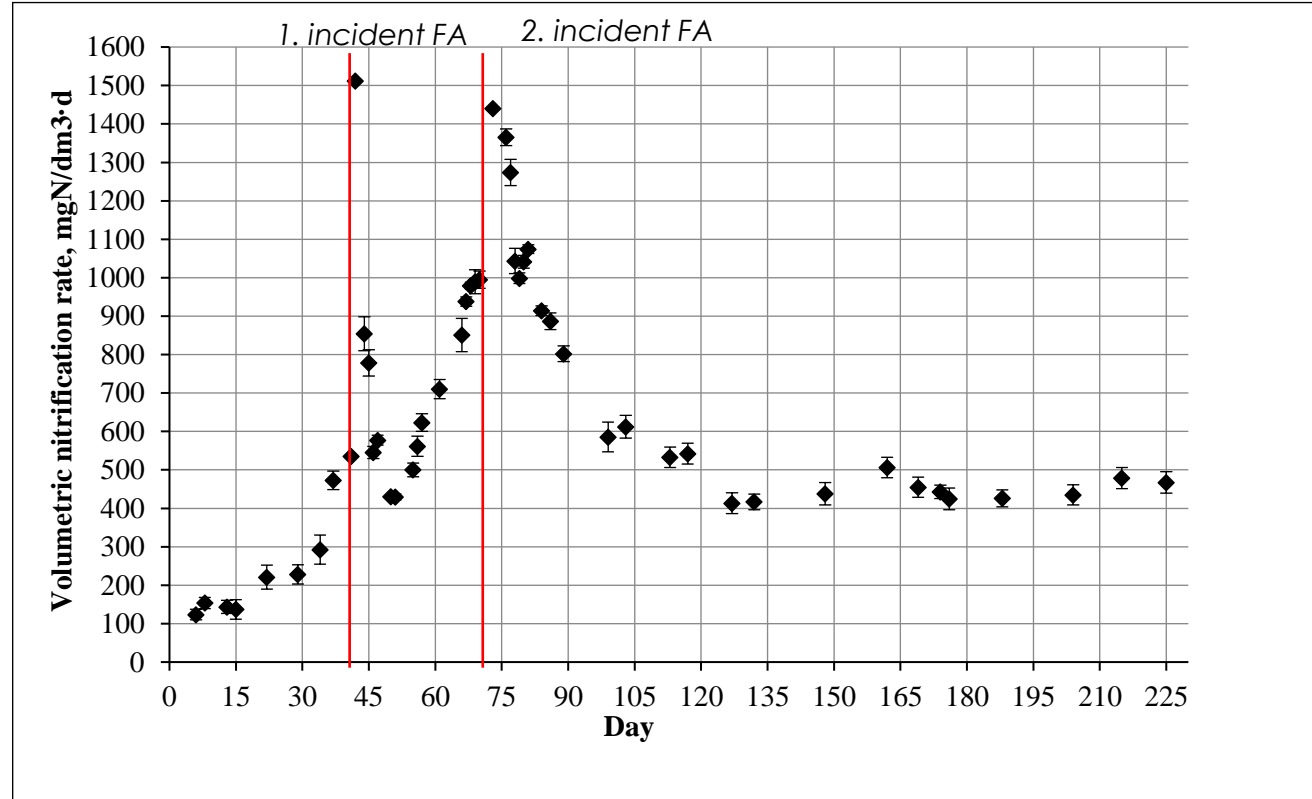


High salinity influence

T=19 h, pH 8.80,
NH₄-N: 807 mgN/dm³,
FA: 280 mgN/dm³

T=27 h, pH 8.19,
NH₄-N: 756 mgN/dm³,
FA: 84 mgN/dm³

URINE NITRIFICATION



Simple remedial action allowed the process to return to normal operation.

Reduced salinity (due to remedial action) and increased nitrogen availability were two factors contributing to the increased nitrification rate immediately after the failures.

Fig 3. Nitrification rate during urine nitrification experiment.



Urea hydrolysis

PCR TEST OF DOMINANT HETEROTROPHS IN ACTIVATED SLUDGE

- Dominance of Luteibacter, urease-negative heterotrophs.

RESPIROMETRIC TESTS

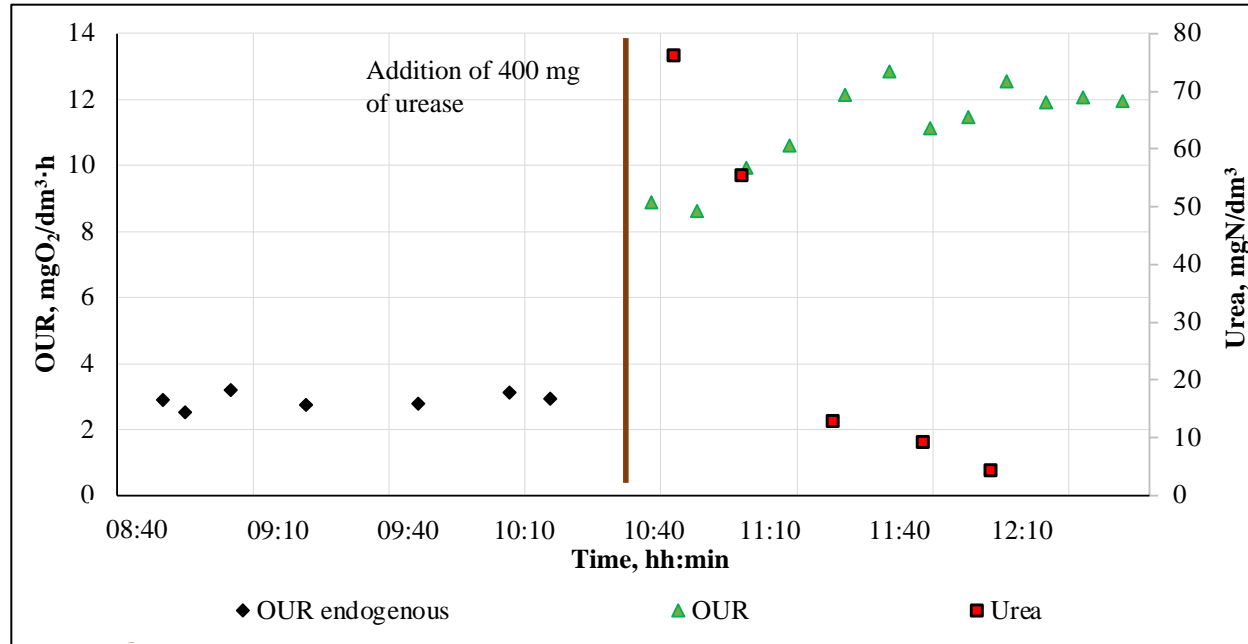


Fig 4. Respiratory activity of microorganisms in urea-containing solution before and after addition of urease.

The efficiency of urea hydrolysis in a nitrifying reactor depends on the presence of the urease enzyme (i.e., on the species composition of the activated sludge).

A key issue is to provide a suitable urease-positive inoculation, or external source of urease (i.e., extraction from the Jack Bean).



Anionic surfactant SMCT removal

- Previous studies regarding the influence of anionic surfactants on activated sludge have shown negative effects on the respiratory activity of the activated sludge bacterial consortium, on the morphology and properties of activated sludge flocs and on the population composition of activated sludge biomass. Thus, reducing the efficiency of nitrogen, organic and suspended solids removal.

(Dereszewska et al., 2015; Li et al., 2020; Liwarska-Bizukojc and Bizukojc, 2005, 2006; Othman et al., 2010; Wu et al., 2020).

In this study:

- No negative effect on nitrification efficiency (>95%).
- No negative effect on COD removal efficiency (>96%).
- No negative effect on suspended solids removal efficiency (>98%).
- No drastic changes in sludge respiratory activity with increasing concentration of SMCT surfactant in the influent.

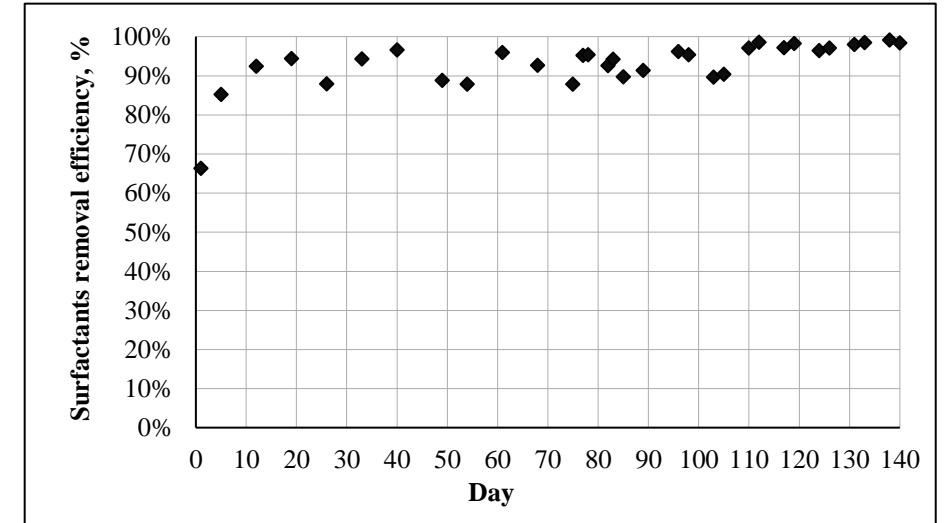


Fig 5. Surfactant removal efficiency during urine and grey water nitrification experiment

The anionic surfactant SMCT present in grey water does NOT affect the nitrification process of urine and grey water.



Soilless cultivation of lettuce

Potential 3 stress factors resulting from the use of diluted, nitrified urine or a mixture of urine and grey water:

1. Elemental deficiencies (P, K, S, Ca, Mg, trace elements).
2. Salt stress (resulting from the composition of the raw urine, and the use of NaHCO_3 as an alkalinity correction in the biological reactor).
3. Presence of the anionic surfactant SMCT (resulting from the composition of the grey water).

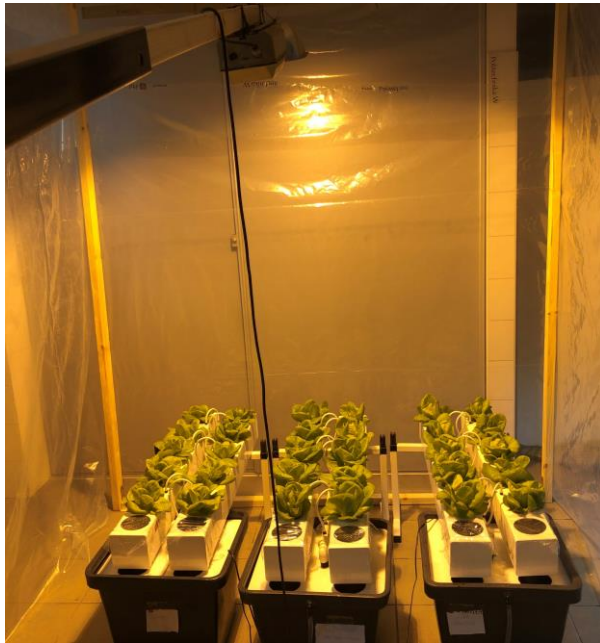


Image 2. A research set-up for soilless cultivation.

MATERIAL AND METHODS

1. Quantitative parameters determined by measuring wet weight, dry weight and water content of edible and non-edible parts.
2. Qualitative parameters determined by measuring elemental composition, photosynthetic pigment content, protein content, stress parameters, transpiration rate and organoleptic tests.
3. Cooperation with the Wrocław University of Life Sciences, the Poznań University of Life Sciences, and the University of Wrocław.

Experimental set-ups

PHASE 2: DILUTED, NITRIFIED URINE

- Elemental deficiencies
- Salt stress

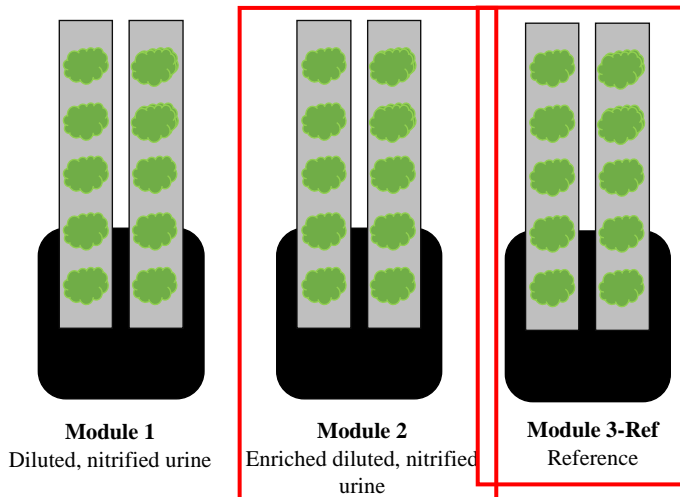


Fig 6. Experimental set-up in Phase 2.

PHASE 3: THE INFLUENCE OF ANIONIC SURFACTANTS ON SOILLESS LETTUCE CULTIVATION

1. Soilless cultivation on untreated grey water is not recommended due to high concentrations of anionic surfactants.
2. Soilless cultivation on fertilizers containing anionic surfactants at concentrations expected at the reactor effluent (phase 4) is possible.

PHASE 5: DILUTED, NITRIFIED URINE AND GREY WATER

- Elemental deficiencies
- Salt stress
- Anionic surfactants SMCT

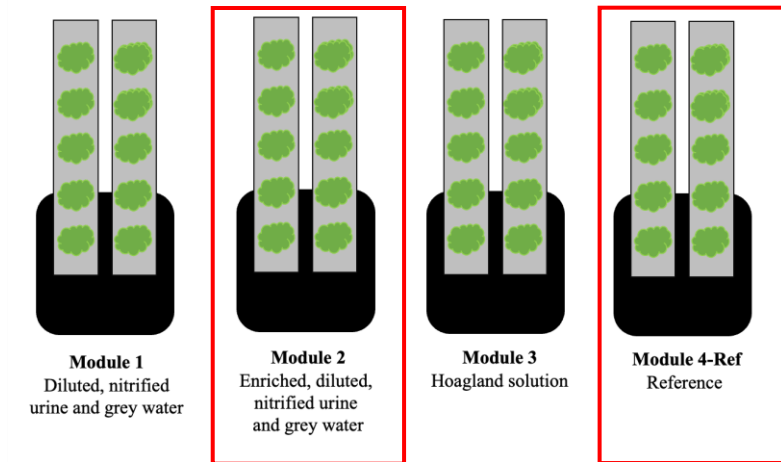


Fig 7. Experimental set-up in Phase 5.



Soilless cultivation of lettuce on produced fertilizers

DILUTED, NITRIFIED URINE

EDIBLE FRESH MASS

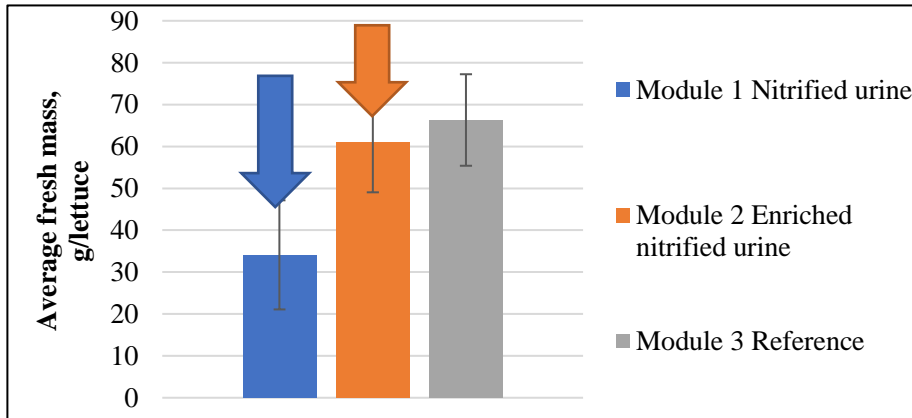


Fig 8. Average fresh edible biomass obtained in Phase 2.

DILUTED, NITRIFIED URINE AND GREY WATER

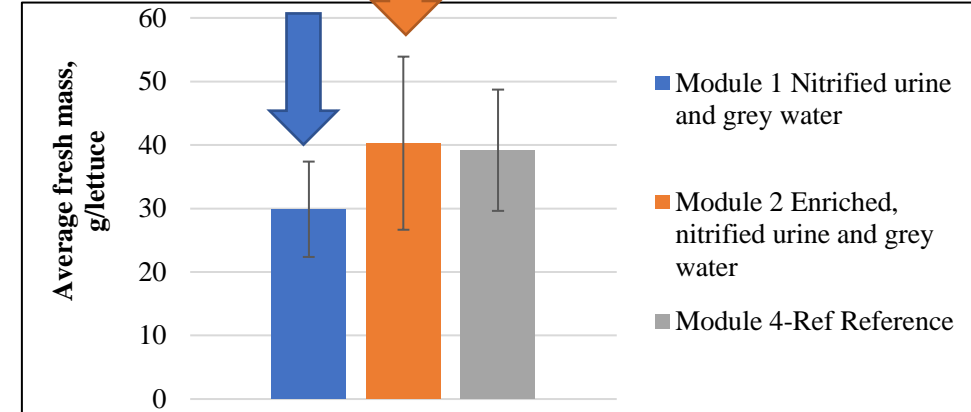


Fig 10. Average fresh edible biomass obtained in Phase 5.

PHOTOSYNTHETIC PIGMENTS

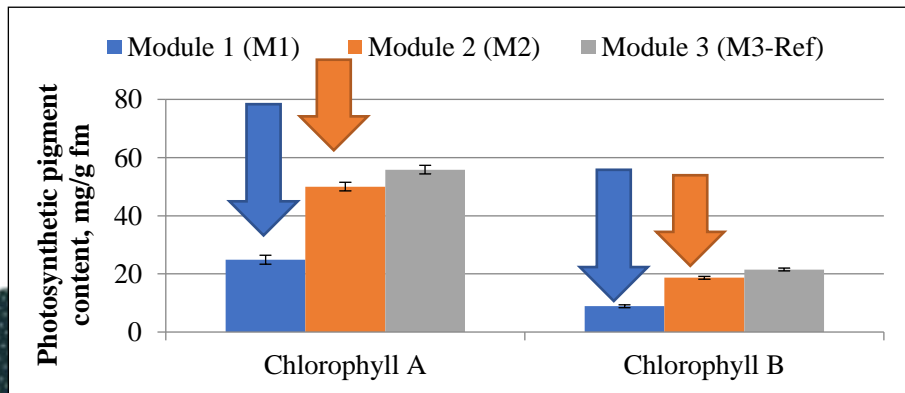


Fig 9. Photosynthetic pigment content in lettuce from Phase 2.

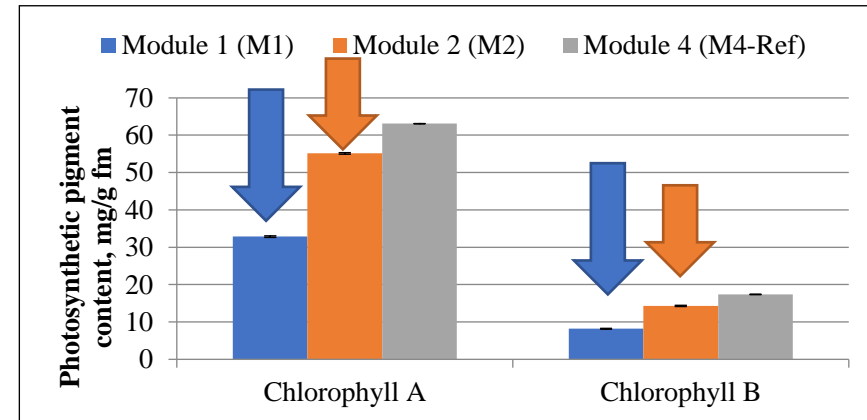


Fig 11. Photosynthetic pigment content in lettuce from Phase 5.



Soilless cultivation of lettuce on produced fertilizers

DILUTED, NITRIFIED URINE

ELEMENTAL COMPOSITION OF EDIBLE PARTS

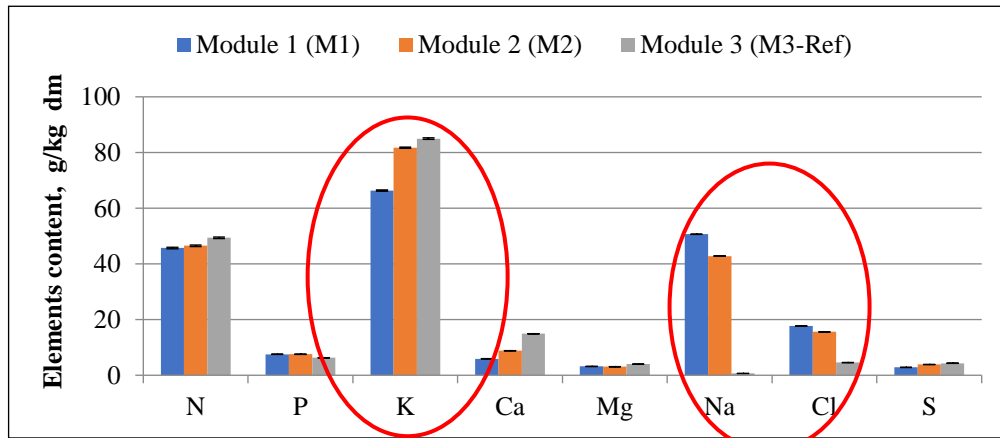


Fig 12. Macronutrient content in lettuce from Phase 2.

DILUTED, NITRIFIED URINE AND GREY WATER

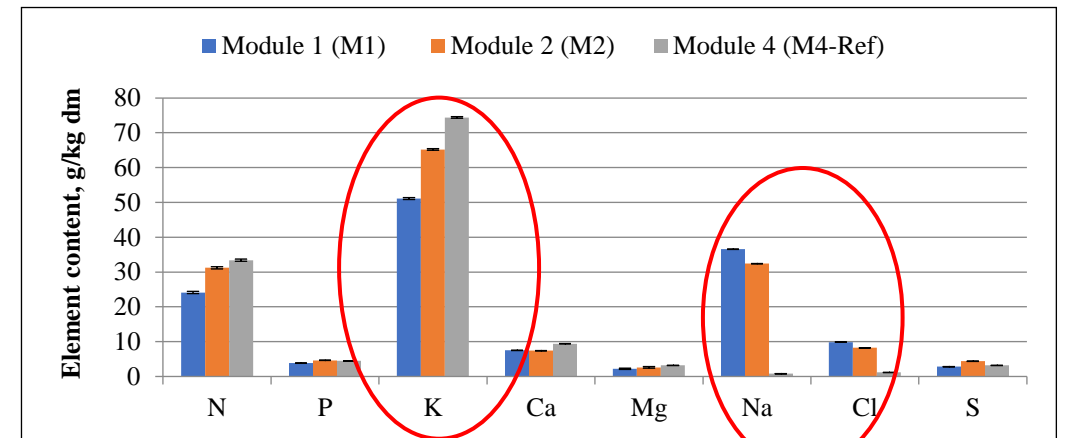
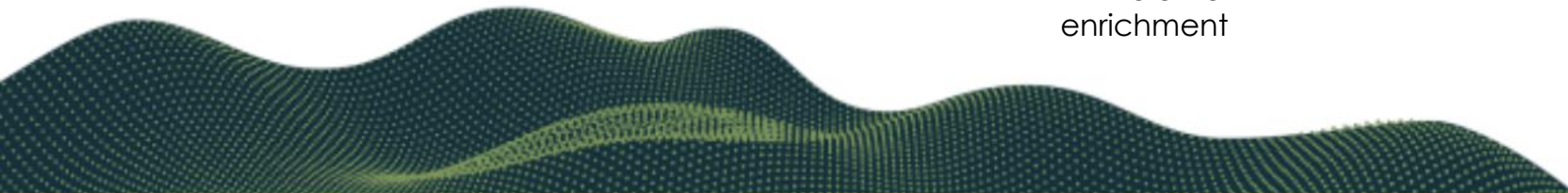


Fig 13. Macronutrient content in lettuce from Phase 5.

M1 – nutrient solution without element enrichment

M2 – nutrient solution with element enrichment





Soilless cultivation of lettuce on produced fertilizers

DILUTED, NITRIFIED URINE AND GREY WATER STRESS PARAMETERS

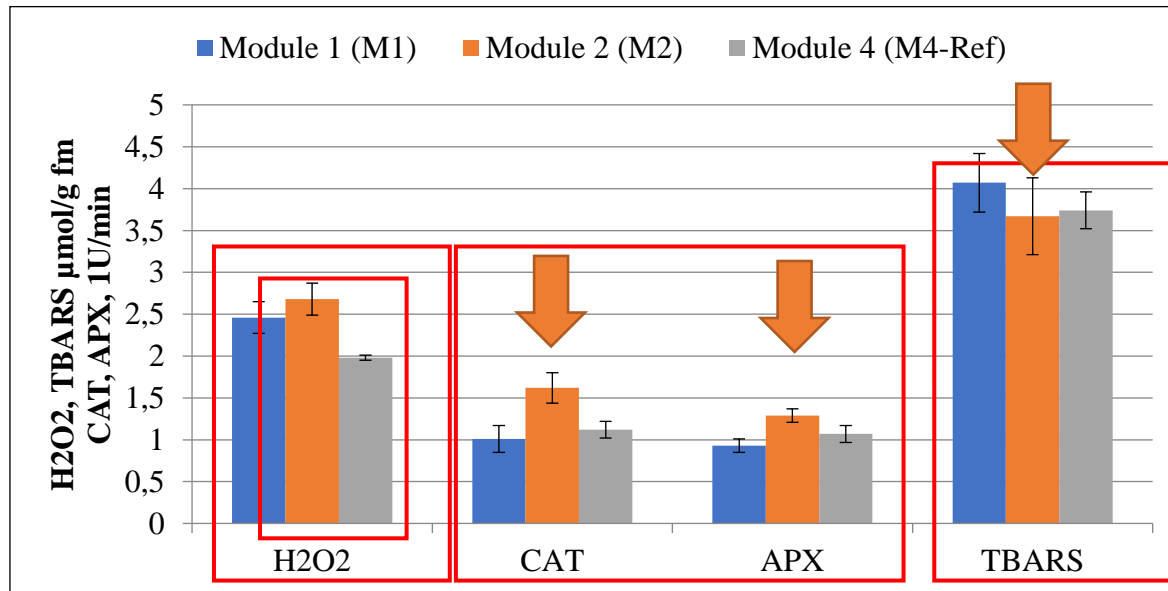


Fig 14. H₂O₂ level, CAT and APX activity and TBARS content in lettuce from Phase 5.

The enrichment of the nutrient solution with M2 resulted in the restoration of normal protein synthesis and an increase in antioxidant enzyme activity, which is reflected in a qualitatively and quantitatively better yield.

The main factor limiting lettuce growth is elemental deficiency, not the presence of the anionic surfactant (SMCT) in the nutrient solution or salinity of the medium.

M1 – nutrient solution without element enrichment, presence of SMCT surfactant

M2 – nutrient solution with element enrichment, presence of SMCT surfactant



Soilless cultivation of lettuce on produced fertilizers

DILUTED, NITRIFIED URINE

ORGANOLEPTIC TEST

- 10 volunteers.
- Evaluation in terms of taste, aroma, appearance, turgor.
- No apparent differences between lettuces grown on nitrified urine and enriched, nitrified urine.
- Lettuce grown on nitrified urine performed better than the reference.



Image 3. Photo of experimental set-up of soilless cultivation of lettuce on nitrified urine.

Lettuce grown on a fertilizer based on nitrified urine is organoleptically comparable to a lettuce grown on commercial fertilizer.



Summary

1. Both reactor configurations produced a fertilizer that was suitable for use in soilless cultivation.
2. Operation of the urine and grey water nitrifying reactor was more stable and had higher nitrification rates compared to urine nitrifying reactor.
3. It is necessary to provide a suitable urease-positive inoculation for the reactor, or to dose urease externally.
4. Soilless cultivation of lettuce on produced fertilizers is possible. However, quantity and quality parameters are reduced when using, unenriched medium.
5. Enrichment of the missing elements provides yield and quality parameters analogous to the reference.
6. The main factor limiting lettuce growth was elemental deficiency, not the presence of the anionic surfactant (SMCT) in the nutrient solution or salinity of the medium.



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THANK YOU.

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