MELiSSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

Coupling urine treatment and water recycling with *Limnospira indica* cultivation for air revitalization and food production under closed loop conditions

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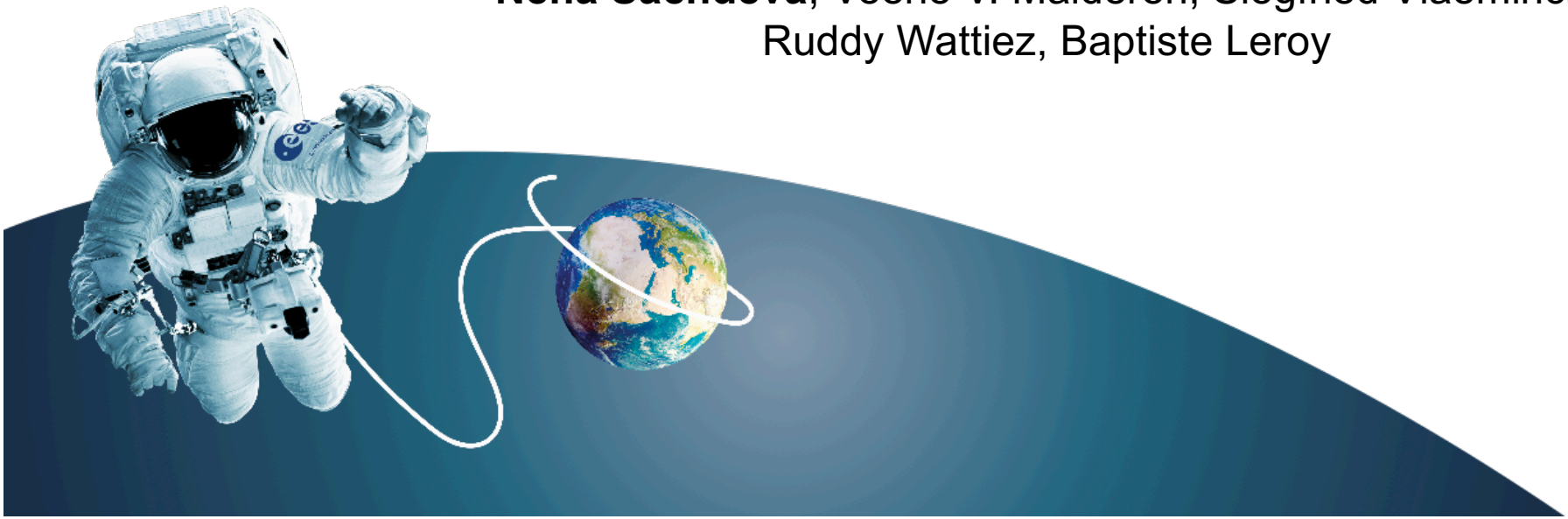

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Limnospira cultivation using Urine

Partially or fully ureolysed/ nitrified Urine from CIII:

N sources:
Individual and mix

High salinity

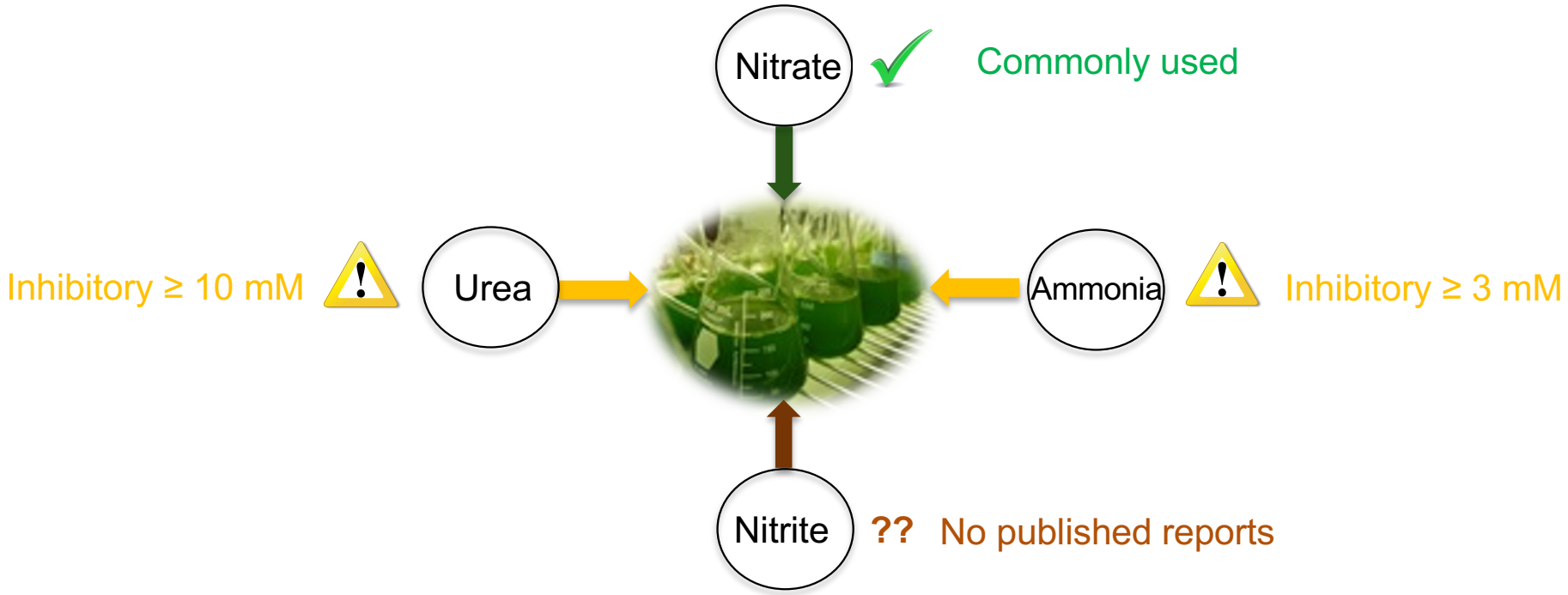
Organics

Limnospira indica (in CIVa)

1. Growth
2. Biochemical content
3. Oxygen Productivity

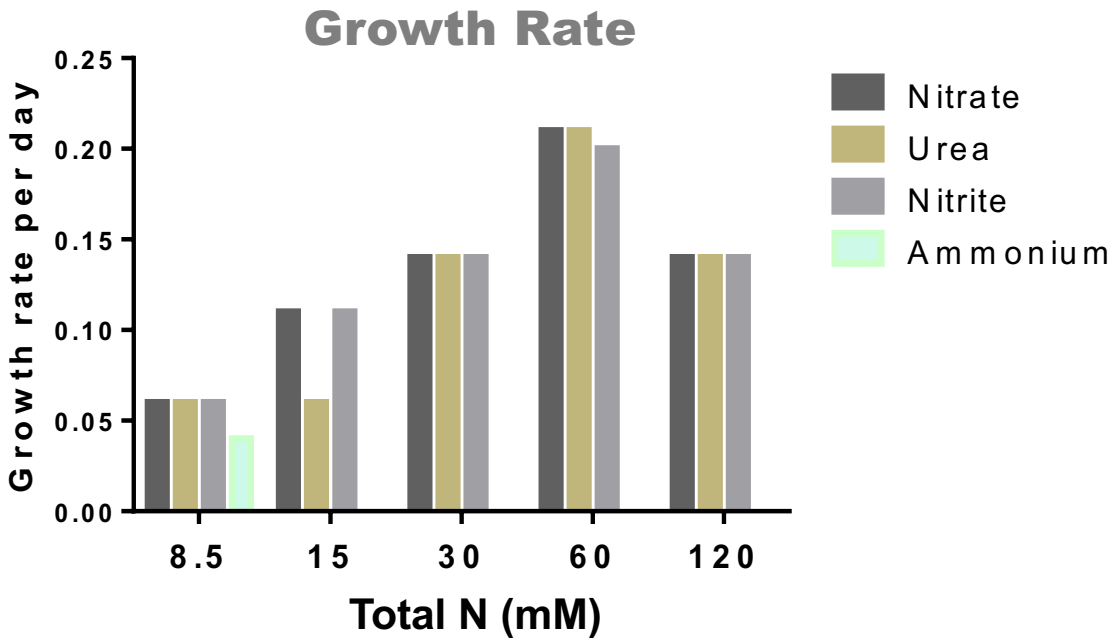
Effect of N sources

Treated Urine: CIII



Partially or fully ureolysed/ nitrified Urine

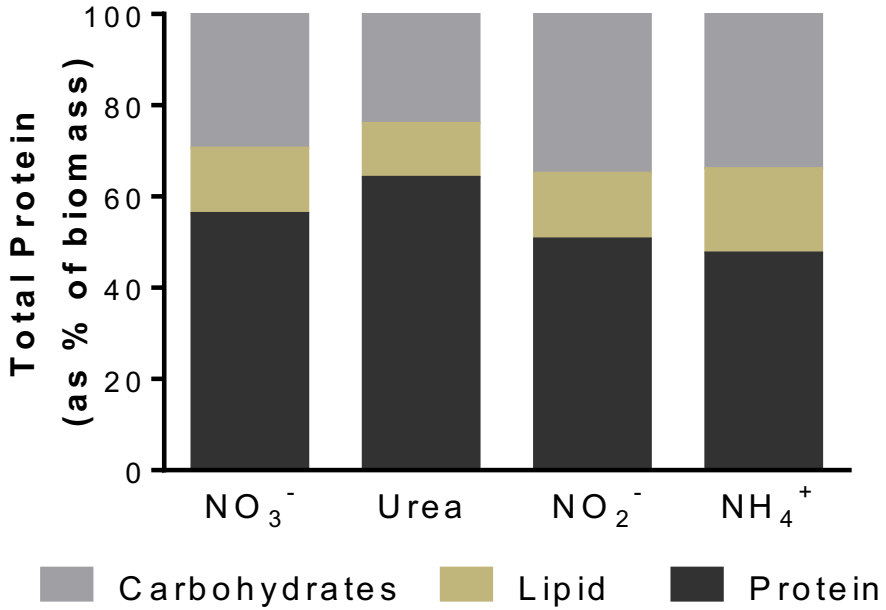
Effect of N sources



- No inhibition with Urea and Nitrite upto 120mM-N
  - ✓ *comparative growth rates as Nitrate*
- Ammonium: inhibitory at 15 mM and higher

Effect of N sources

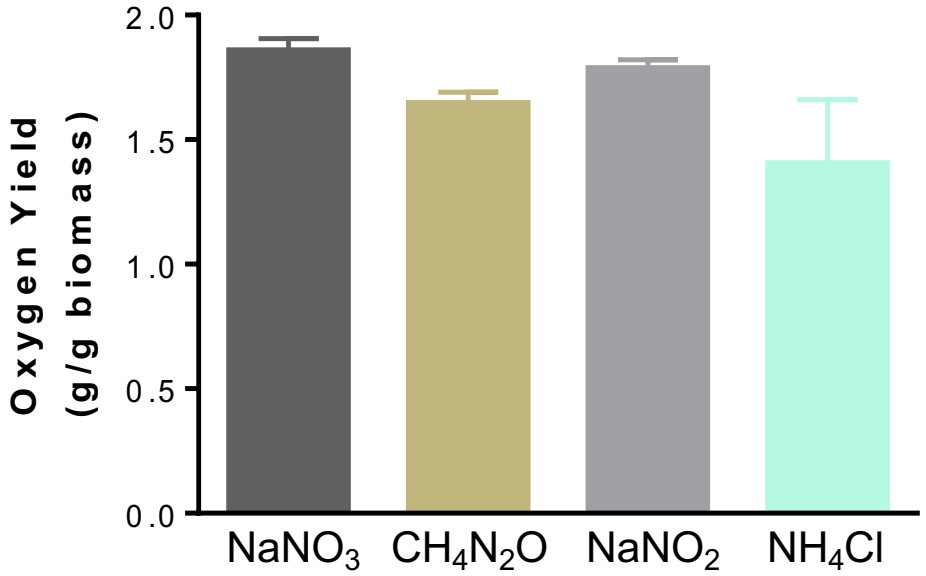
Biochemical Content



Urea a viable alternative to Nitrate

Effect of N sources

Oxygen Yield



O_2 yield varied in accordance with stoichiometric O_2 content of N-source

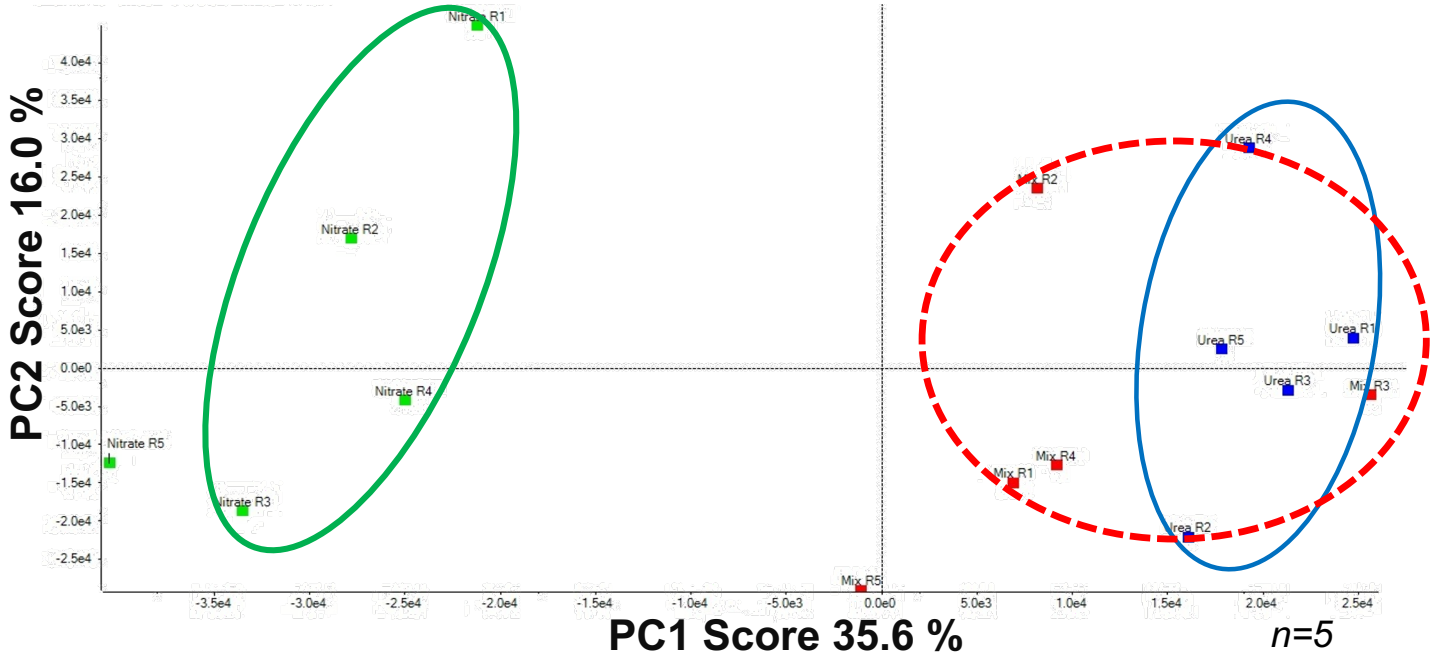
Effect of Mix of N sources

Effect of Mix of N sources

Proteomic Study

Unsupervised Principle Component Analysis: 1747 proteins identified

Nitrate 30 mM-N Mix (15 mM-N Nitrate+ 15mM-N urea) Urea 30 mM-N



n=5

Effect of Mix of N sources

Proteomic Study

N and Urea cycle Related		Fold Change		
Protein	**Function**	***Nitrate vs Urea***	***Nitrate vs Mix***	***Urea vs Mix***
H1W7Y5_9CYAN	Ferredoxin-nitrite reductase: NirA	N	N	⟷
H1W8M1_9CYAN	Nitrate transport ATP-binding protein: NrtC	N	N	⟷
H1W8M3_9CYAN	ABC Nitrate transport system, periplasmic component: NrtA	N	N	⟷
H1WBL9_9CYAN	Global nitrogen regulator: NtcA	⟷	⟷	⟷
H1W8D8_9CYAN	Ornithine carbamoyl-transferase	U	⟷	⟷
H1WM95_9CYAN	Cyanophycinase	U	⟷	⟷
H1WHQ6_9CYAN	Biosynthetic arginine decarboxylase	U	⟷	⟷

Urea better N-Source

n=5

N Higher in Nitrate

U Higher in Urea

⟷ No change

Effect of Salinity

Effect of High Salinity

	Ca (mg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)
Human Urine	**50-250**	**975-4875**	**60-200**	**920-5060**

CIII: MBBR

AAS analysis

Stabilization Method	Electrochemical stabilization	105.12	479.53	4.95	526.4
	Neutral pH	96.34	544.48	21.16	1273.53
	Alkaline Treatment	50.12	586.6	3.72	1037.00
	Natural Hydrolysis	251.77	518.1	15.87	738.10

CIVa: *Limnospira* cultivation

30 mM Nitrate

ULS: Urine Like Salinity supplemented in Zarrouk Medium	**Not tested *(HCO_3^- precipitation)***	**4900**	**200**	**5060**
TU: Zarrouk supplemented with average salinity level of Treated Urine samples		**550**	**15**	**650**

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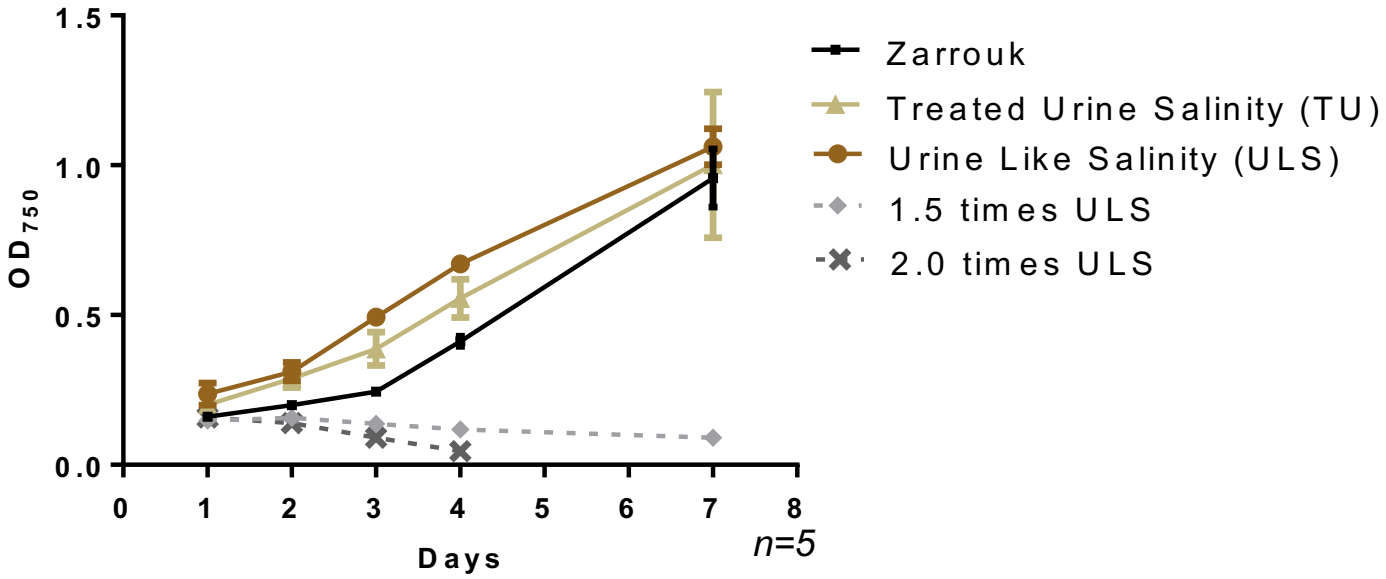
Effect of High Salinity

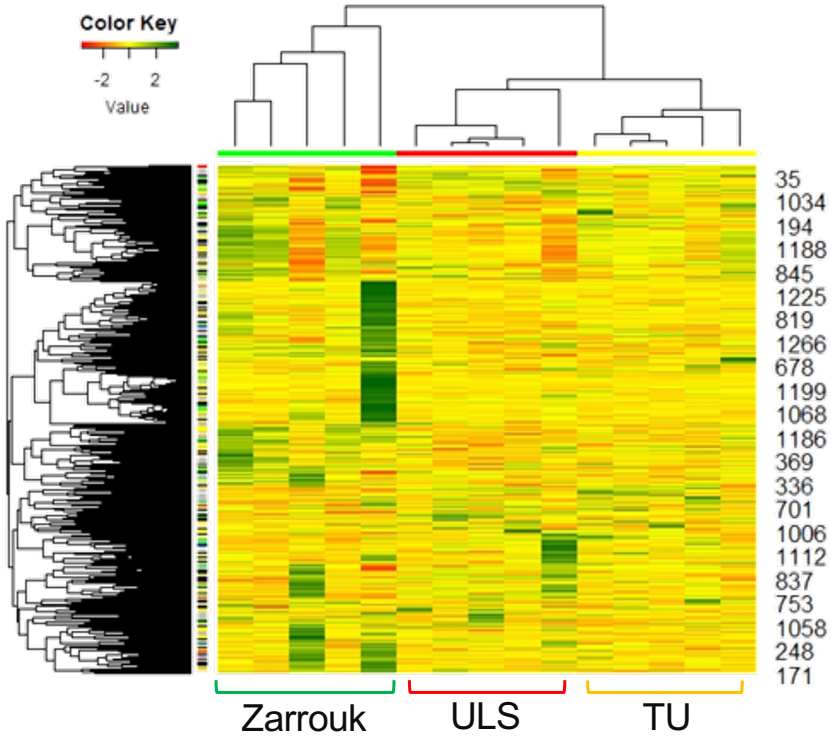
Growth Rate

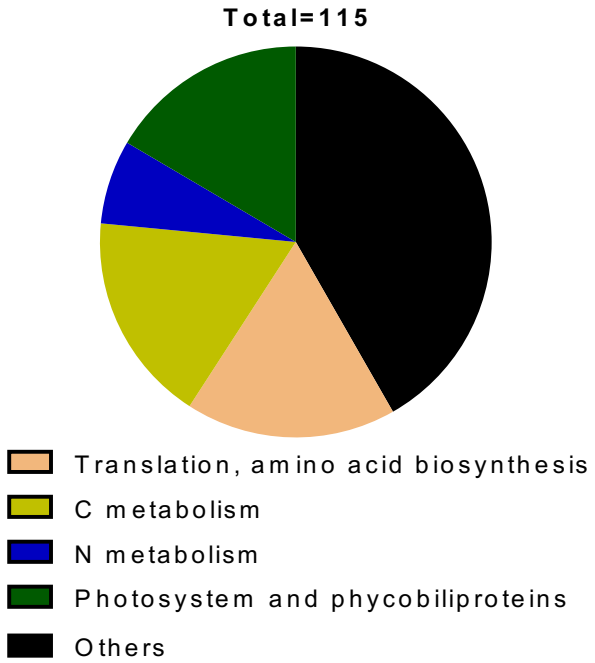


Limnospira is resilient to urine-like salinity

Effect of High Salinity

Proteomic Study



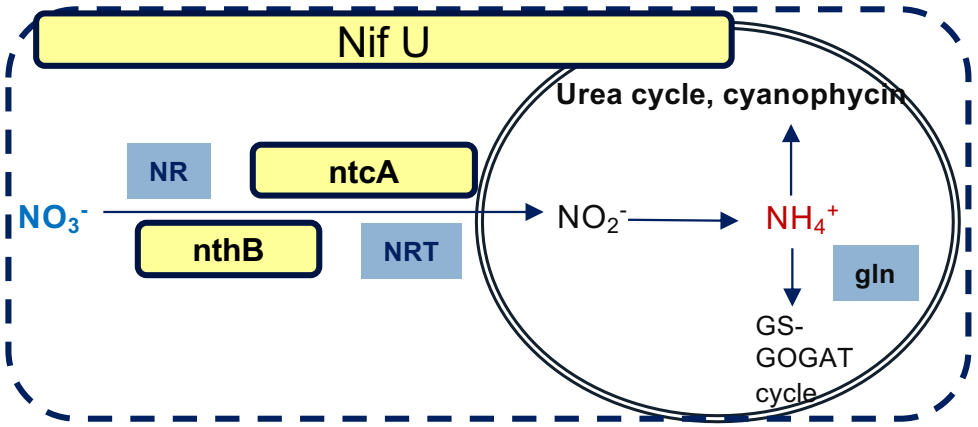


115 proteins "significantly up or down regulated"

- p value <0.05 and
- fold change is either >1.5 or <0.66

Out of 1291 identified proteins only 777 proteins had >1 peptide

Salinity and N metabolism



Protein Name	Protein Function	Fold change Z vs ULS	Fold change ULS vs TU	Fold change Z vs TU
H1WIV5_9CYAN	NifU domain-containing protein	↑	↑	↔
H1WJQ6_9CYAN	Arginine biosynthesis bifunctional protein ArgJ	↑	↔	↔
H1W7Q8_9CYAN	N-acetyl-gamma-glutamyl-phosphate reductase: *plays role in arginine biosynthesis*	↑	↔	↔
H1WM96_9CYAN	Cyanophycin synthetase	↑	↔	↔
H1WM95_9CYAN	Cyanophycinase	↑	↔	↔

n=5

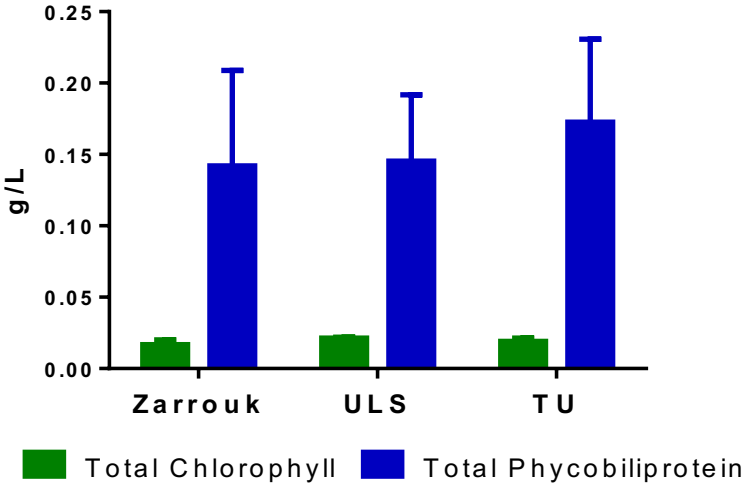
↑ Higher in ULS ↑ Higher in Zarrouk ↔ No change

Salinity and Photosystem

Protein	Protein Function	Fold change Z vs ULS	Fold change ULS vs TU	Fold change Z vs TU
H1W8H4_9 CYAN	Allophycocyanin beta-18 subunit	↑	↔	↔
H1W703_9 CYAN	Phycobilisome polypeptide	↑	↔	↑
H1WAX7_9 CYAN	Photosystem I reaction center subunit III (PSI-F)	↑	↑	↔
H1WMT5_9 CYAN	Photosystem II reaction center Psb28 protein	↑	↔	↑
H1WMM8_9 CYAN	Photosystem I P700 Chlorophyll-a (PsaB)	↑	↔	↔

n=5

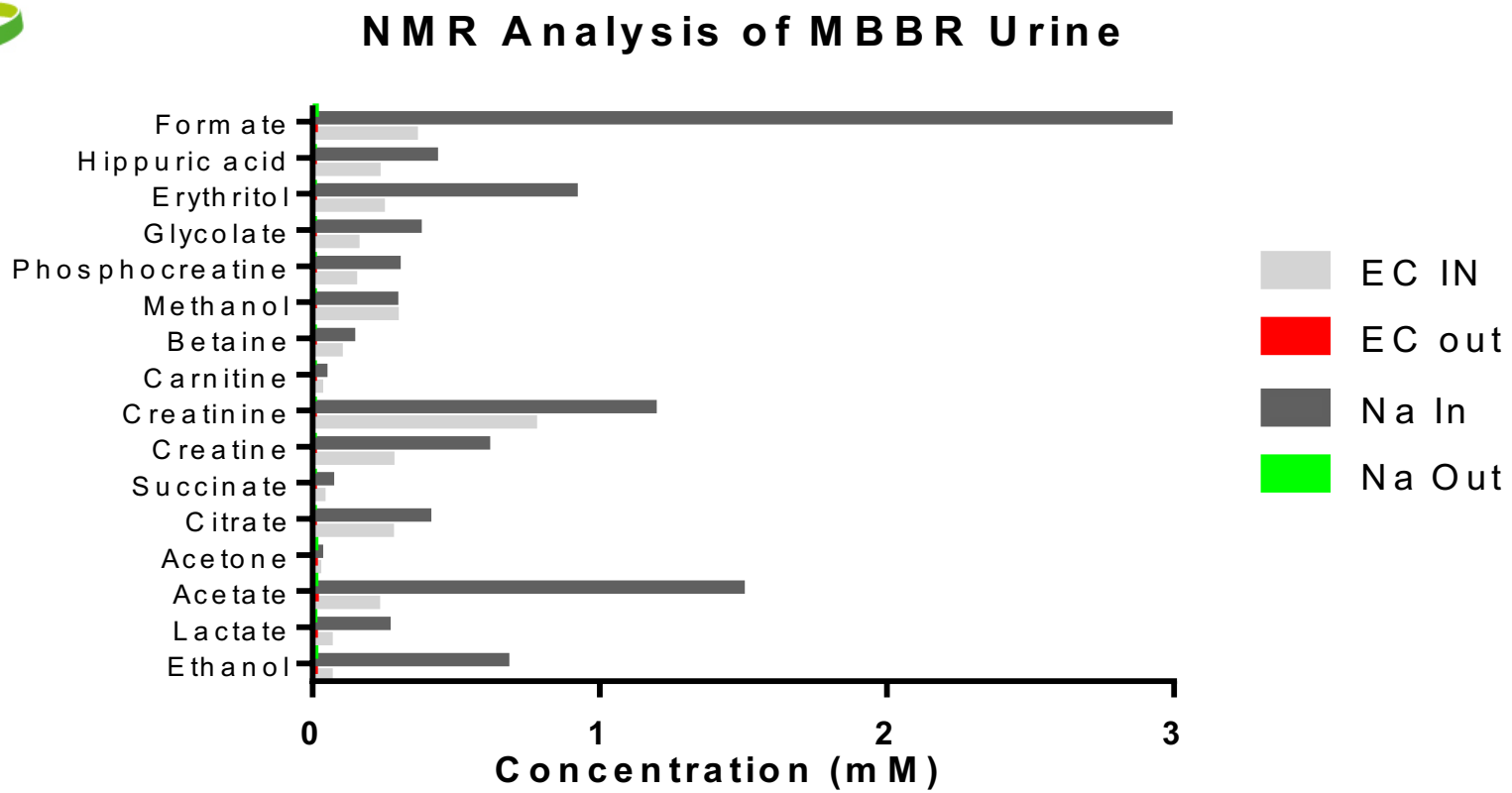
↑ Higher in ULS ↑ Higher in TU ↔ No change



No significant effect of high salinity on Photosystem

Effect of Organics

Organic Composition: Treated Urine



Effects of organics to be studied

EC: Electrochemical Stabilization

Na: Alkaline Treatment

In: MBBR Influent

Out: MBBR Effluent

Conclusions

Treated Urine from CIII

1. N sources:
Individual and mix

Other N mixes to be tested

2. High salinity

High resilience to high salinity

3. Organics

To be investigated

Limnospira CIVa

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THANK YOU.

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Medium/ Condition	Remarks	Conductivity (mS/cm)
Zarrouk	Modified Cogne Zarrouk	~25
ULS	Modified Zarrouk supplemented with Na, Mg, K to match human urine like salt concentration	~60
UZ	ULS diluted 10X with Modified Zarrouk	~15-17
1.5X concentrated ULS	ULS with 1.5 times salt concentration of human urine	~90-95
2X concentrated ULS	ULS with 2 times salt concentration of human urine	~120