

Design and validation of a synthetic urine recipe with special focus on the organic composition (in prep)

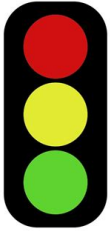
Toward a More Representative Synthetic Urine:

Inclusion of Organic Compounds and Validation of their Conversion During Anaerobic Storage

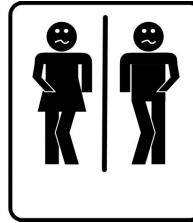
POMP III



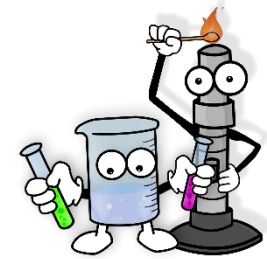
Urine is everywhere... where is the problem?



Ethical approvals



Lack of infrastructure or WTP
(willingness to pee)



Experimental design

An urgent call for using real human urine in decentralized sanitation research and advancing protocols for preparing synthetic urine



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Examples of Recipes in Literature of «Fresh Urine»

	pH	Urea	Creatinine	Uric acid	Hippurate	Citrate	Other	
Ipe (2016)	5.5-6	16800	1020	2270		650		<div><div>High concentrations</div><div>Large concentration range of 36%</div><div>Synthetic solutions are not representative</div><div>Composition varies substantially</div><div><div><div>• Proposed for medical UTI studies</div><div>• Not tested in the lab</div></div><div></div><div></div><div></div></div></div>

3 Synthetic Solutions based on Real Urine Collection

	Synthetic 24-hour 10 organics	Synthetic 24-hour 4 organics	Real 24-hour “pooled collection”	Synthetic Daytime 10 organics	Real Daytime “pooled collection”	
Nsol			4850		4230	10 Organics
TAN			232		184	
PO ₄ ^{3--P}			328		219	
SO ₄ ²⁻			709		514	
Cl ⁻			2190		2510	
Na ²⁺			1290		1240	
K ⁺			1100		1360	
Mg ²⁺			81		77	
Ca ²⁺			54		40	
DOC			4500		4030	
COD			5690		4940	COD representation no need for pH adaptation Recipe for pooled collection
COD/N			1.2		1.2	
COD/DOC			1.3		1.3	

No COD

Urea
Creatinine
Citric acid
Hippuric acid
Formic acid
Trigonelline
Glycine
Creatine
Lactic acid
Alanine

4 Organics

Comparison ≠ Validation of Synthetic Urine

Synthetic 24-hour 10 organics	Synthetic 24-hour 4 organics	Real 24-hour “pooled collection”	Synthetic Daytime 10 organics	Real Daytime “pooled collection”
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Research Questions

- ? Are the synthetic solutions **representative** of real urine?
- ? Does the synthetic urine behave the same way during **anaerobic storage**?
- ? Does it matter which **organics** are added?

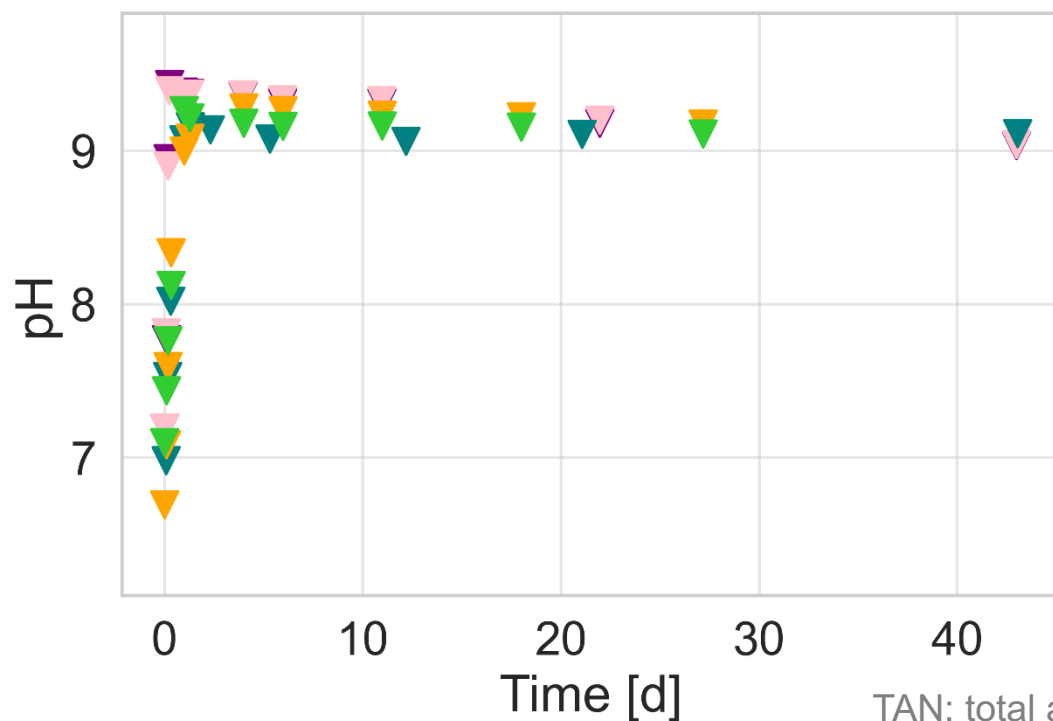


Urea Hydrolysis → pH increase & TAN increase

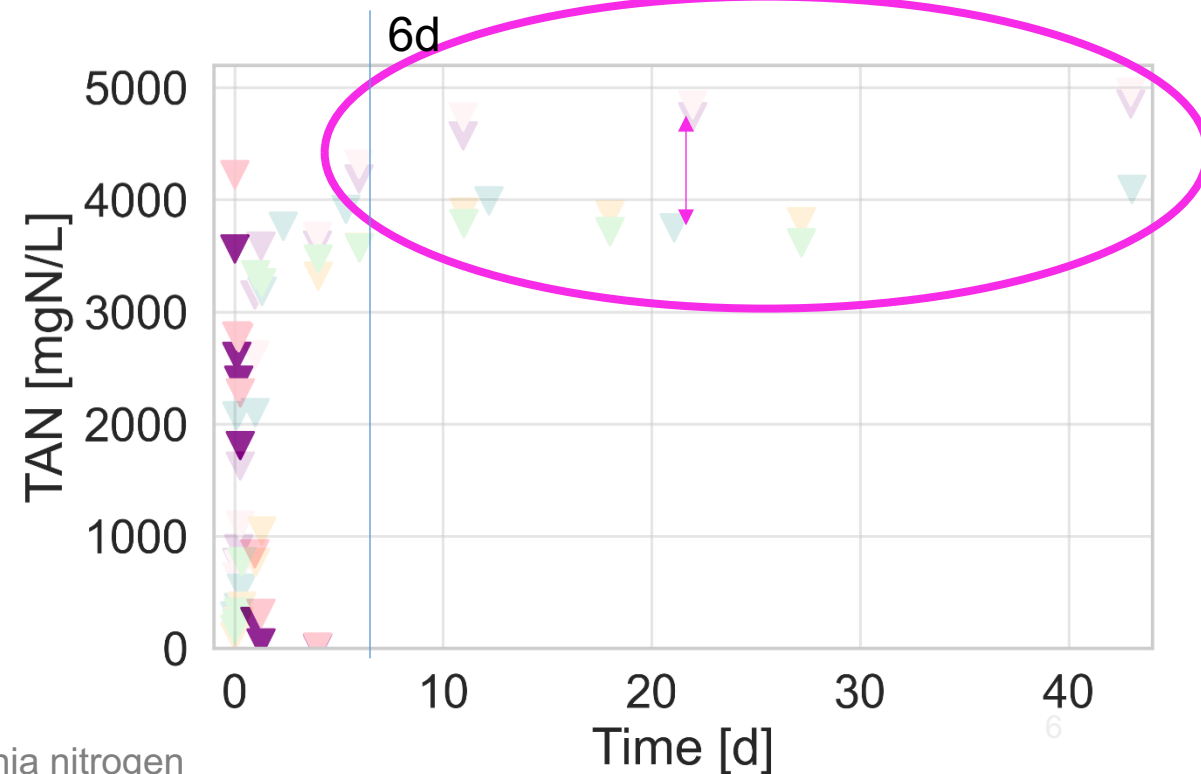
- ▼ Synth 24h 10 org
- ▼ Synth 24 h 4 Org
- ▼ Real 24h
- ▼ Synth Daytime 10 Org
- ▼ Real Daytime

Urea is hydrolyzed equally in synthetic and real urine

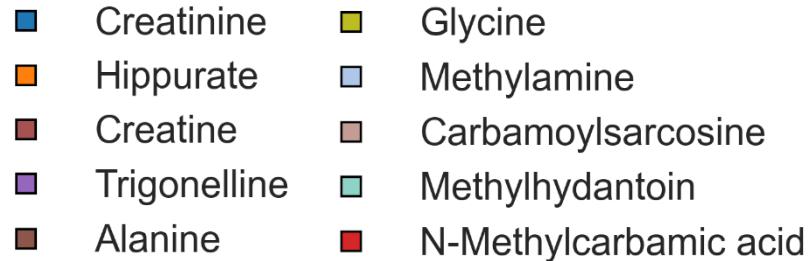
Further increase of the total ammonia nitrogen?



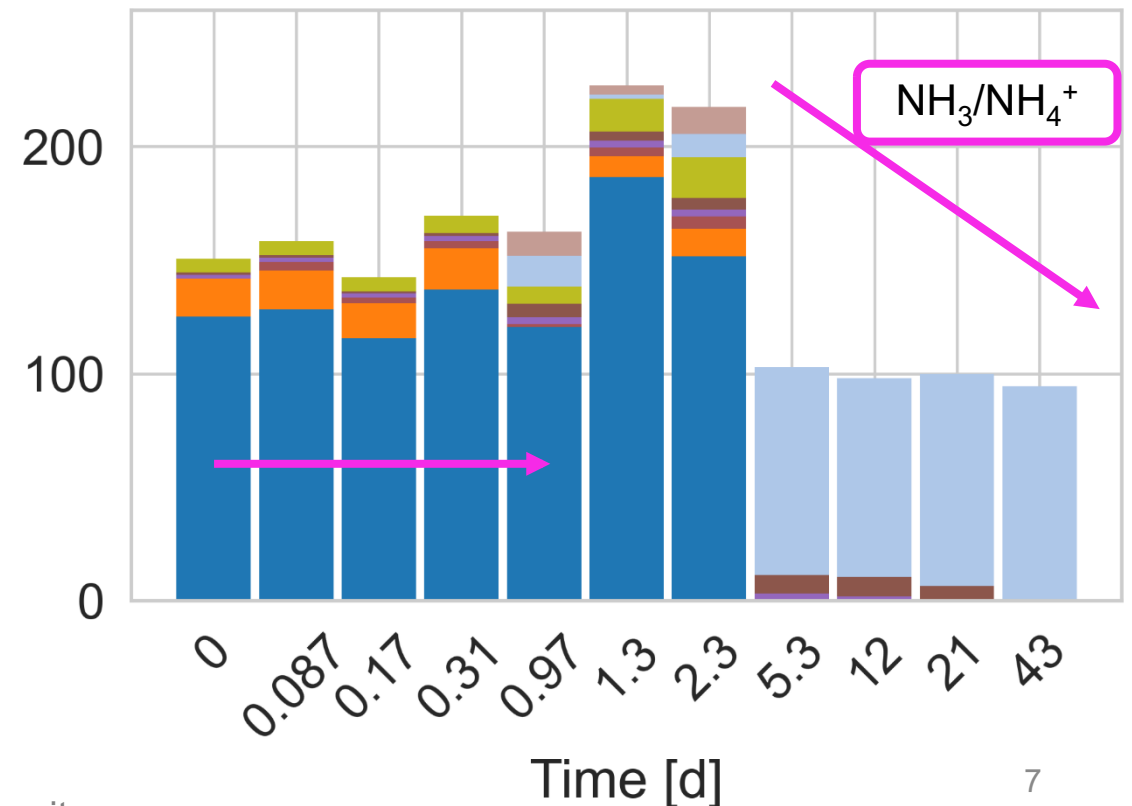
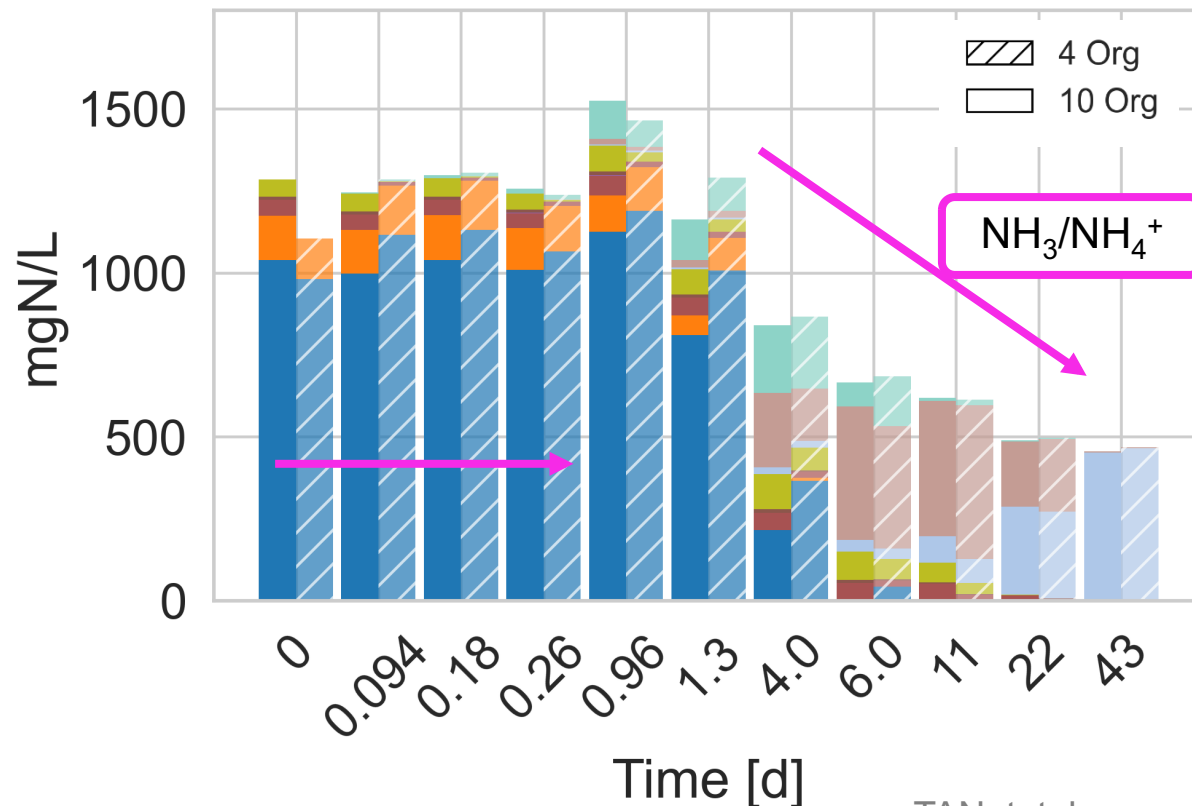
TAN: total ammonia nitrogen



Can Organic N contribute to the additional TAN?



1. Organic N is converted to TAN
2. Creatinine and Methylamine dominant



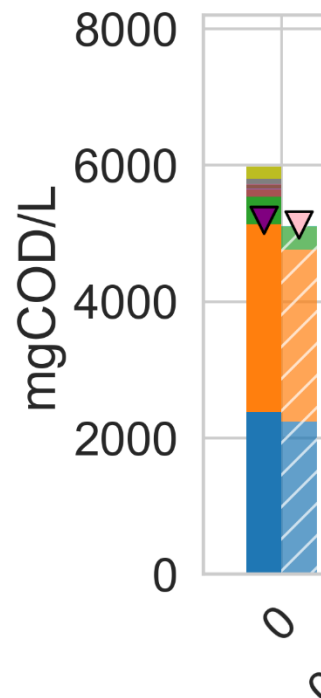
TAN: total ammonia nitrogen

Carbon during Anaerobic Storage as COD

Creatinine
 Hippurate
 Citrate
 Creatine
 Trigonelline
 Alanine

Formate
 Lactate
 Glycine

Synth 10 Org
 Synth 4 Org
 Real
 4 Org
 10 Org



- Creatinine and hippurate contribute to the COD equally

- Organic composition has changed completely

$$COD_{theo} \neq COD_{meas}$$



- No complete identification of the COD_{meas}

- Real urine contains more propionate and acetate after 43d

Time [d]

COD: chemical oxygen demand

Time [d]

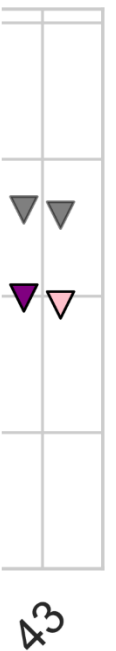
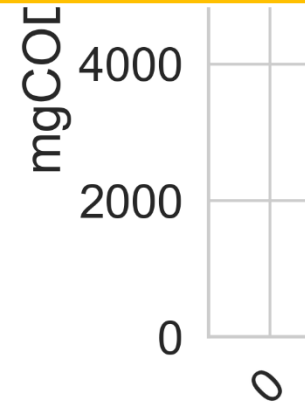
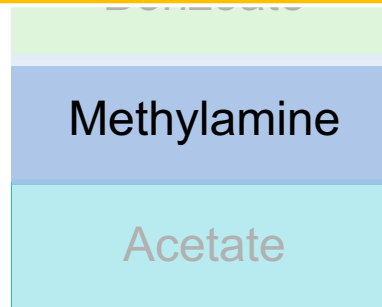
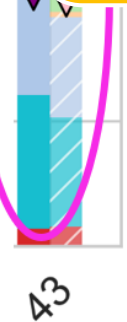
Measured COD is not equal to theoretical COD

Li, et al. (1) report $\frac{COD_{meas}}{COD_{theo}} = 13 \%$

$$COD_{meas} \neq COD_{theo}$$

$$COD_{theo} \neq COD_{meas}$$

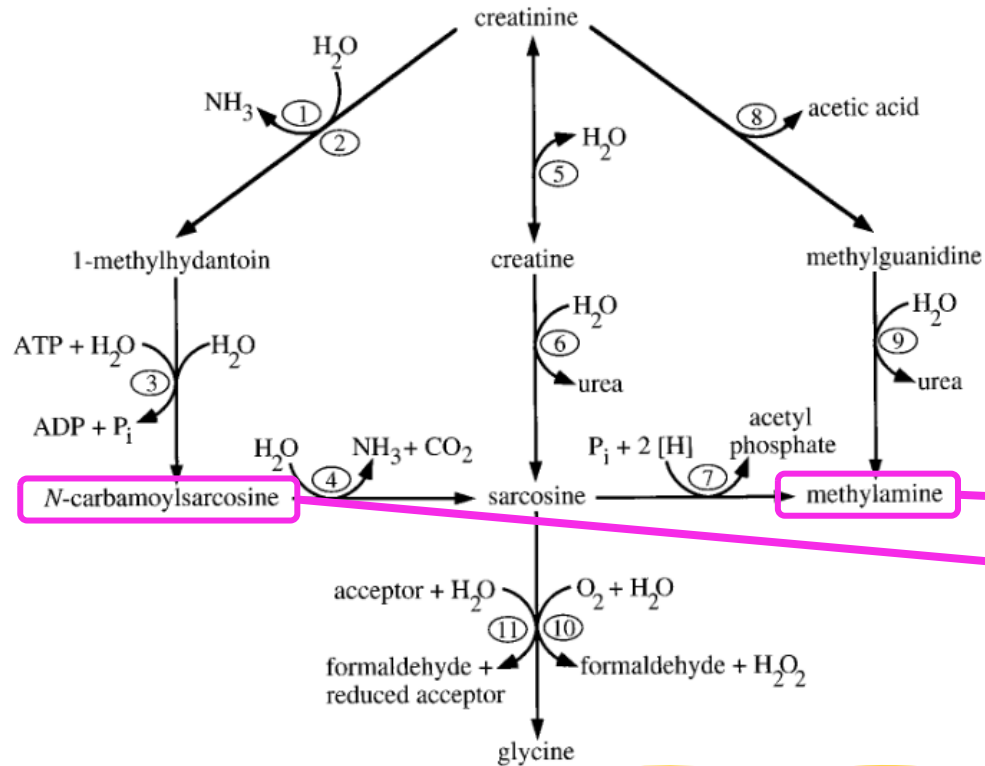
1. COD decrease leads to false conclusions
2. The measured COD does not accurately represent the theoretical COD in a solution
3. COD decreased could be observed in both real and synthetic solutions



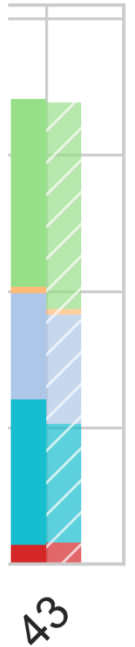
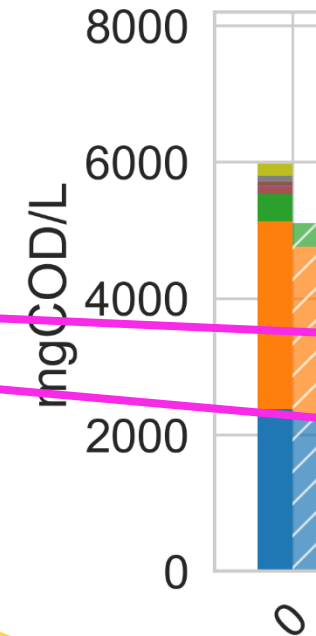
$$COD_{recalculated} = COD_{meas} + (1 - 0.17)COD_{nmr, methylamine}$$

Time [d]

Creatinine does not only follow one pathway



- | | | | |
|----------------|----------------------|-------------------------|----------------|
| ■ Creatinine | ■ Formate | ■ N-Methylcarbamic acid | ▼ Synth 10 Org |
| ■ Hippurate | ■ Lactate | ■ Acetate | ▼ Synth 4 Org |
| ■ Citrate | ■ Glycine | ■ Methylamine | ▼ Real |
| ■ Creatine | ■ Carbamoylsarcosine | ■ Propionate | ▨ 4 Org |
| ■ Trigonelline | ■ Sarcosine | ■ Benzoate | □ 10 Org |
| ■ Alanine | ■ Methylhydantoin | | |



Time [d]

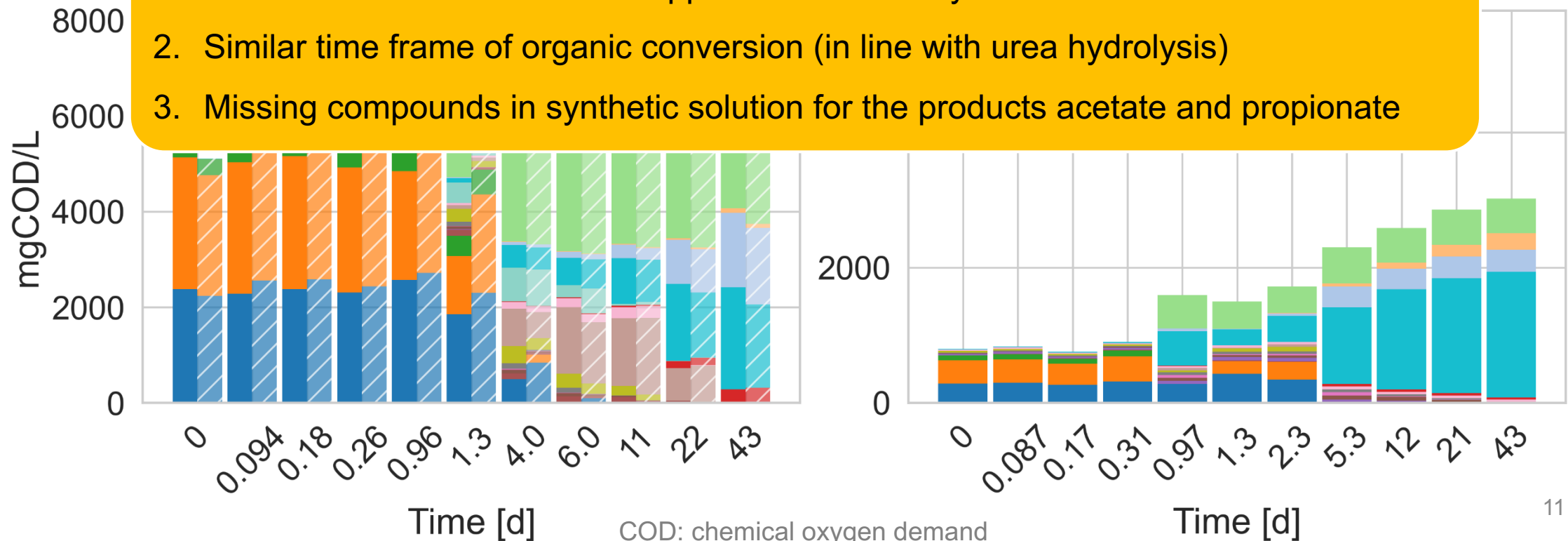
Synthetic solutions can be helpful
to simplify biological processes

COD: chemical oxygen demand

Shining a light on the conversion pathways



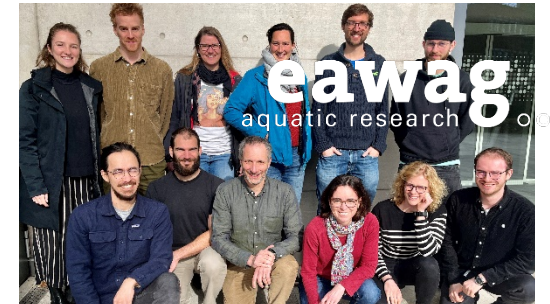
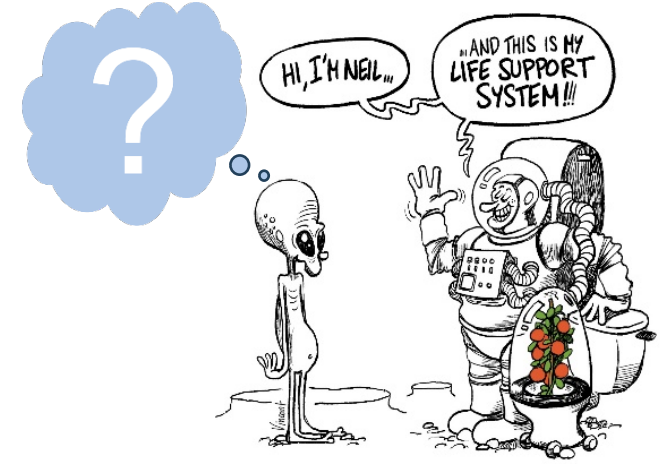
1. Conversion of creatinine and hippurate similar in synthetic and real solutions
2. Similar time frame of organic conversion (in line with urea hydrolysis)
3. Missing compounds in synthetic solution for the products acetate and propionate



Final Destination – Synthetic Urine?



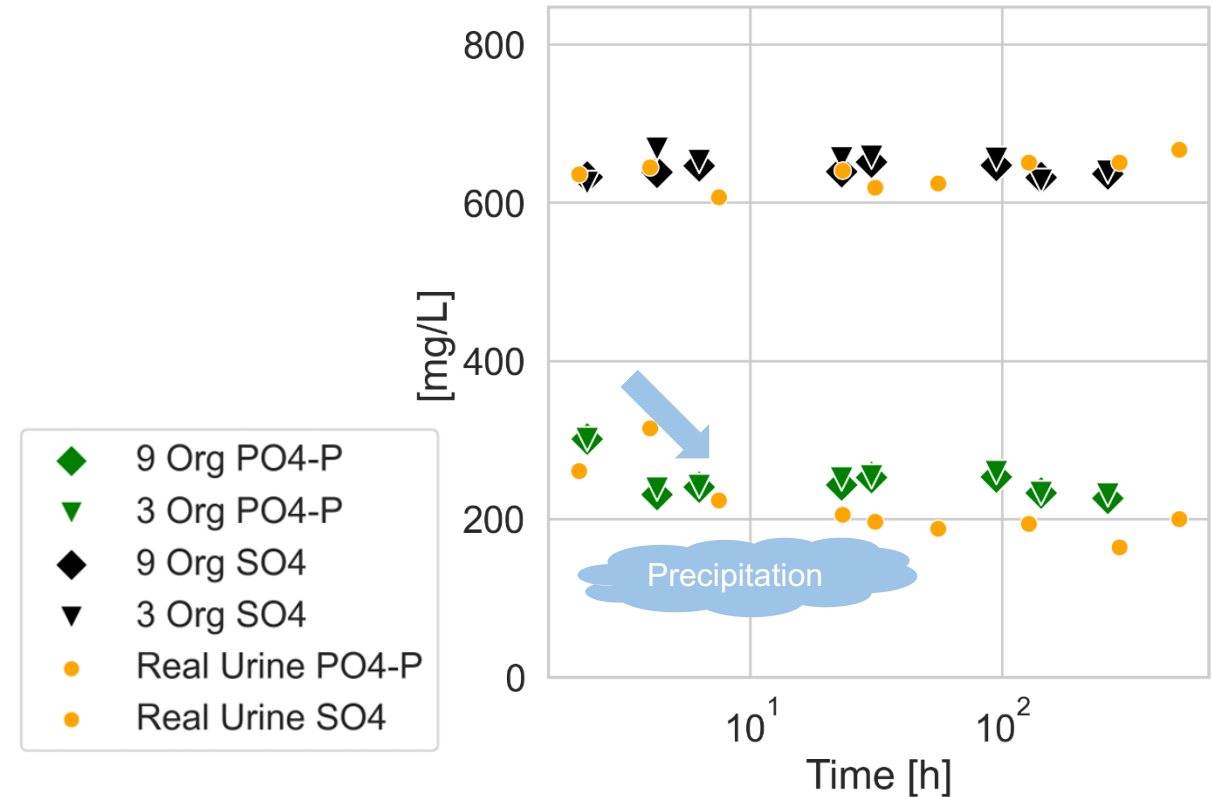
Are the synthetic solutions **representative** of real urine



Synthetic Solutions

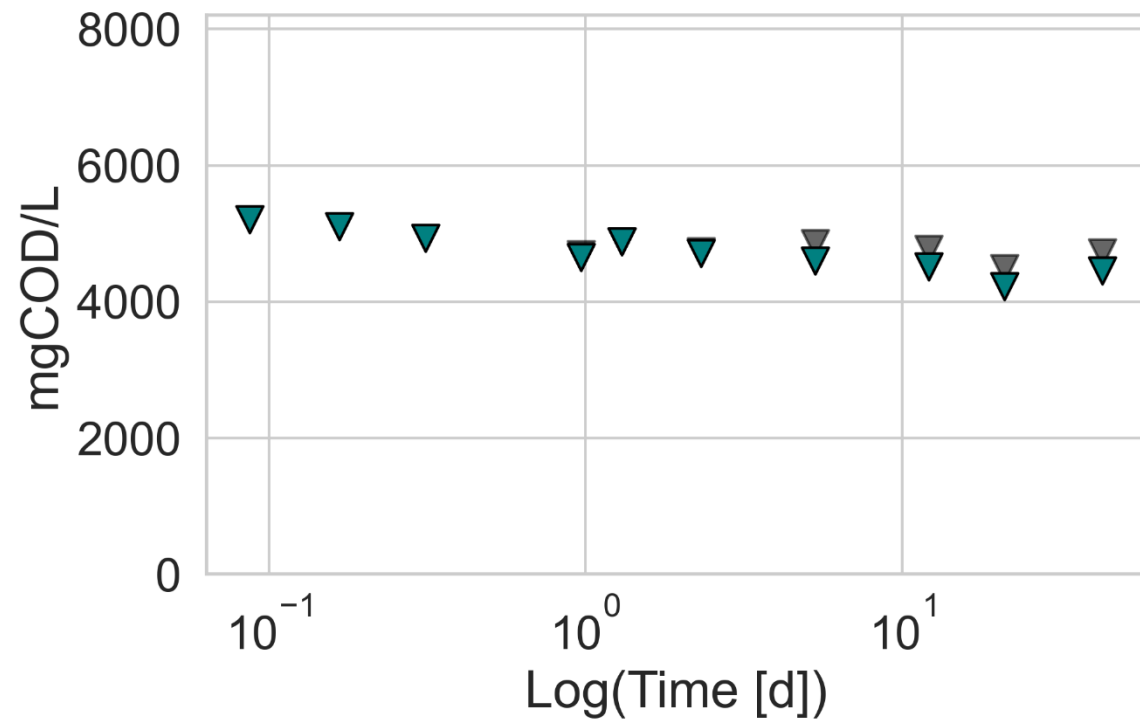
Chemicals		24-hour urine	24-hour urine	Daytime urine
		10 organics	4 organics	10 organics
		[g/L]	[g/L]	[g/L]
NaCl		1.94	1.94	1.89
MgSO ₄ *5H ₂ O		0.697	0.697	0.665
KH ₂ PO ₄		1.44	1.44	0.964
KCl		0.701	0.701	1.74
NH ₄ Cl		0.299	0.299	0.238
CaCl ₂ *2H ₂ O		0.198	0.198	0.145
NaOH *		0.92	0.922	0.857
K ₂ SO ₄		0.709	0.709	0.384
CH ₄ N ₂ O	Urea	8.54	8.54	7.19
C ₄ H ₇ N ₃ O	Creatinine	2.5	2.69	2.13
C ₆ H ₈ O ₇	Citric acid	0.87	0.937	0.906
C ₉ H ₉ NO ₃	Hippurate	1.56	1.68	1.26
CH ₂ O ₂ *	formic acid	0.0347		0.035
C ₇ H ₇ NO ₂ HCl *	Trigonelline Hydrochloride	0.01		0.0078
C ₂ H ₅ NO ₂	Glycine	0.241		0.242
C ₄ H ₉ N ₃ O ₂	Creatine	0.107		0.0818
C ₃ H ₆ O ₃ *	Lactate	0.0869		0.137
C ₃ H ₇ NO ₂	Alanine	0.0507		0.0475

Phosphate Precipitation and Other Compounds



☑ 25% P precipitation

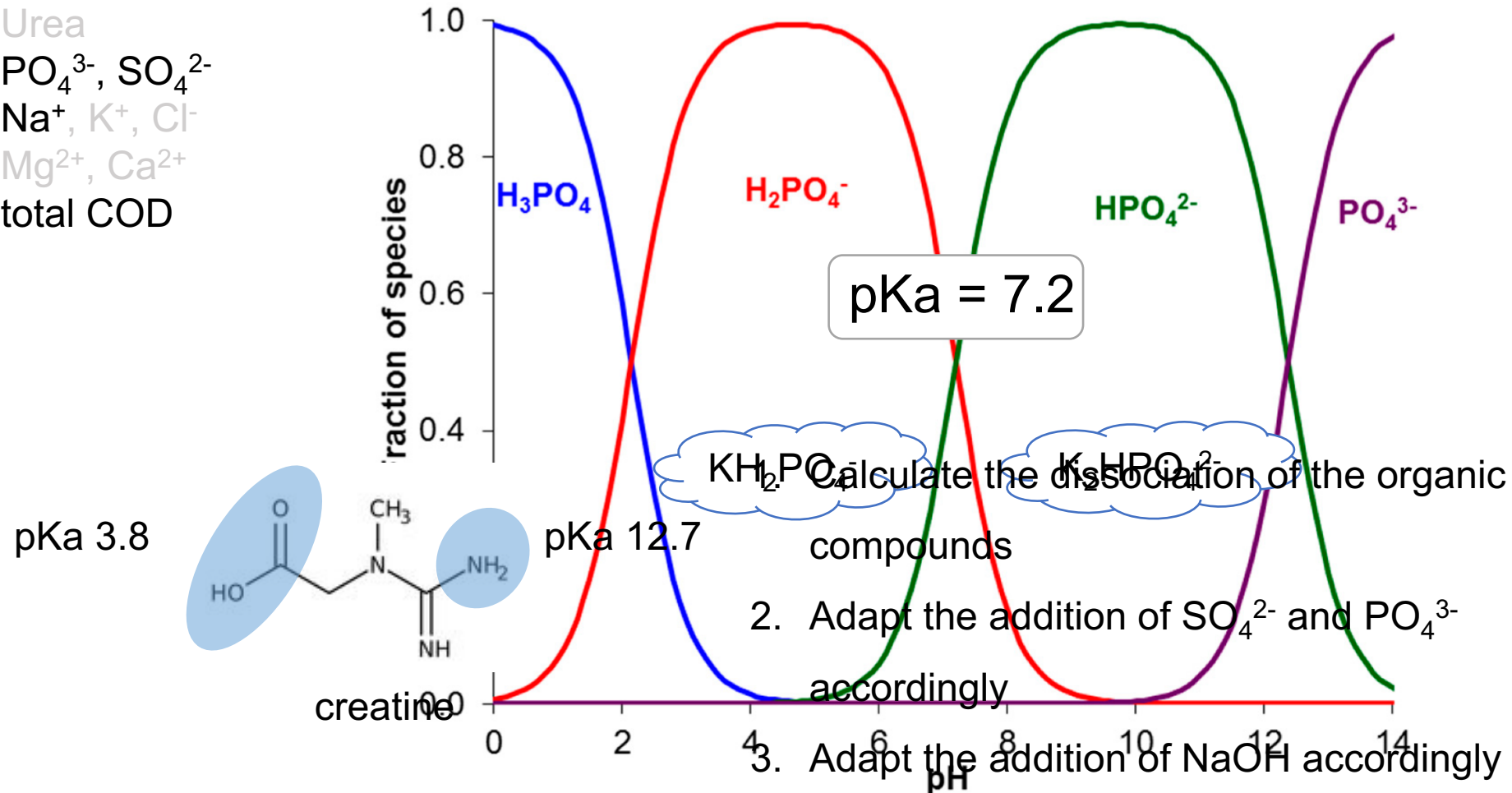
COD recalculated for real urine



$$\text{Alkalinity} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] - [\text{H}^+]$$

«fixed» parameters

- Urea
- PO_4^{3-} , SO_4^{2-}
- Na^+ , K^+ , Cl^-
- Mg^{2+} , Ca^{2+}
- total COD



pH 6.2
COD/N 1.1

Carbon during Anaerobic Storage as COD

- Creatinine
- Hippurate
- Citrate
- Creatine
- Trigonelline
- Alanine

1. Organic conversion similar, whether 10 or 4 organics are added
2. Organic conversion for the most abundant compounds in urine similar
3. Some organic compounds not represented in synthetic solutions

