

# Sustainable hydrolysates-based basal medium (HBBM) and its application in circular cell culture

For The Unjournal · Pivotal Questions Initiative Cultivated Meat Workshop  
8.04.2026



# Basal media: What's the problem with DMEM/F12?

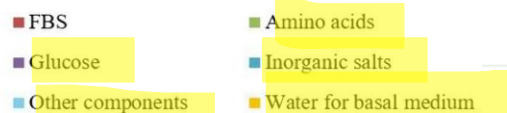
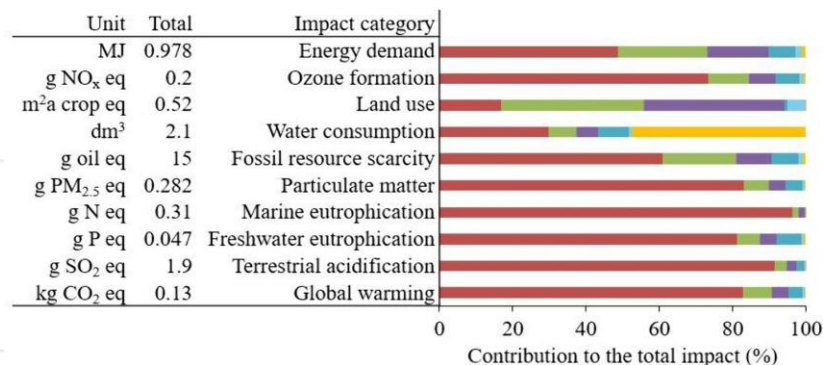


♥ DMEM/F12 (or other similar chemically fully defined basal media) is standardly used in CM/CSF areas\*

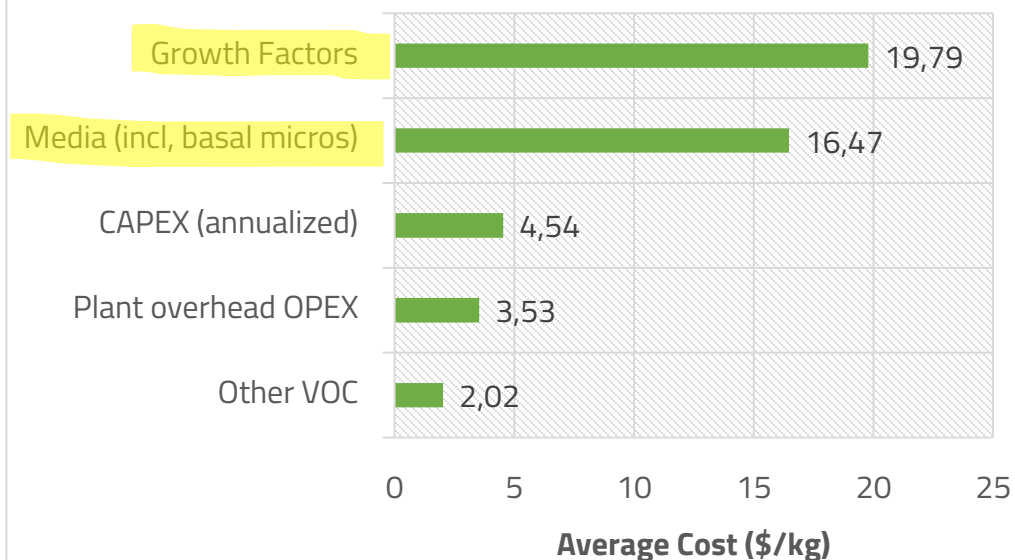
⚠ But it has quite a significant environmental impact

💰 It is also quite pricy

## Environmental impact results of 1 L 10% FBS medium preparation



## Cost Breakdown by Component (Total: \$46/kg)\*\*



\*Leber et al. 2025 preprint: <http://dx.doi.org/10.2139/ssrn.5310265>

\*\*Figure 1 from Wali, M.E., Karinen, H., Rønning, S.B. et al. Int J Life Cycle Assess 29, 2077–2093 (2024). <https://doi.org/10.1007/s11367-024-02350-6>

\*\*\*Courtesy of Unjournal project: [https://unjournal.github.io/cm\\_pq\\_modeling/](https://unjournal.github.io/cm_pq_modeling/)

# Basal media: Can hydrolysates be the alternative?



- ✔ Yeast, microalgae, and plant hydrolysates have been explored as potential substitutes for FBS\*
- ✘ This approach was not successful with CM/CSF-relevant cell lines so far\*
- ⚠ Microalgal hydrolysates were shown to partially substitute DMEM in circular cell culture (CCC) system\*\*

SO.....

- ❓ Can they be applied as a full basal medium substitution?
- 🕒 Microalgae tend to grow slow – can hydrolysates from faster growing species substitute basal medium?
- ♻️ Can food-grade agri-waste be used for such hydrolysates?

\*Leber et al. 2025 preprint: <http://dx.doi.org/10.2139/ssrn.5310265>)

\*\*Haraguchi, Y., Okamoto, Y. & Shimizu, T. A circular cell culture system using microalgae and mammalian myoblasts for the production of sustainable cultured meat. Arch Microbiol 204, 615 (2022).

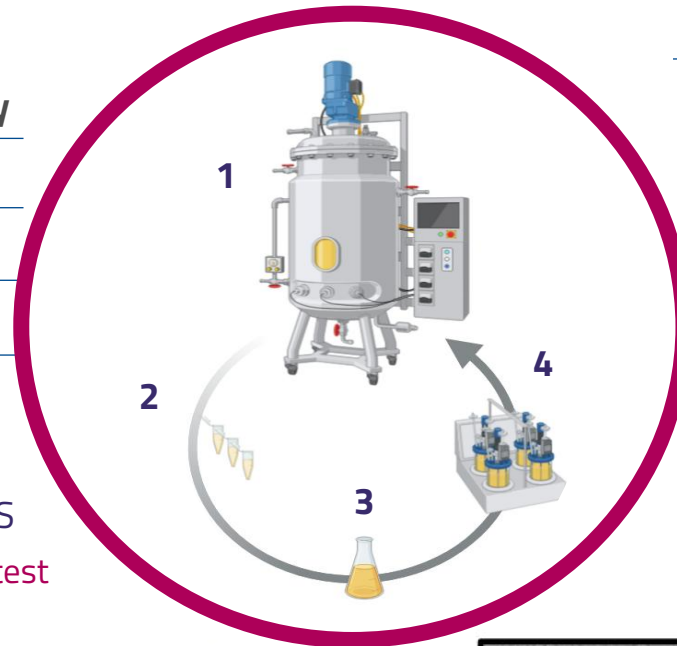
<https://doi.org/10.1007/s00203-022-03234-9>

# Results: Production of food-GMP grade plant protein hydrolysates from **common** food plants

## DEVELOPED & PRODUCED PROTEIN HYDROLYSATES, FOOD-GMP GRADE\*

	Type	Possible Annual Production Capacity
01.	Wheat Hydrolysate	100'000 kg
02.	Rice Hydrolysate	100'000 kg
03.	Soy Hydrolysate	100'000 kg

\* Application / Use: Cell culture media component & food ingredient

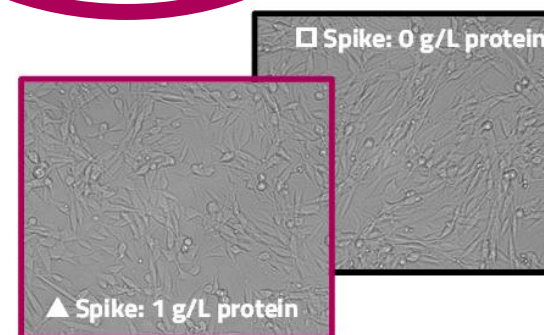
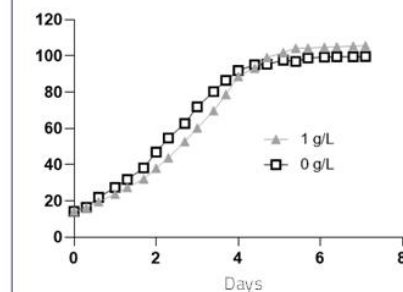
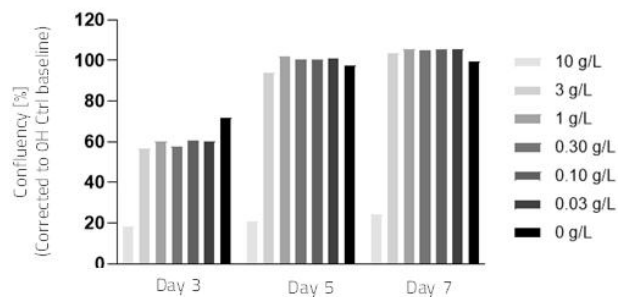


## STRATEGY: NATURTLE'S PROPRIETARY PROCESS DEVELOPMENT

1. Identify suitable GMP-certified commercial production-plant
2. Down-scale process flow
3. Adapt process to crop hydrolyzation assuring tight compatibility with commercial plant work-flow
4. Upscale to commercial scale without any need for process adjustment or capex

## HYDROLYSATE PERFORMANCE, PRIMARY BOVINE CELLS

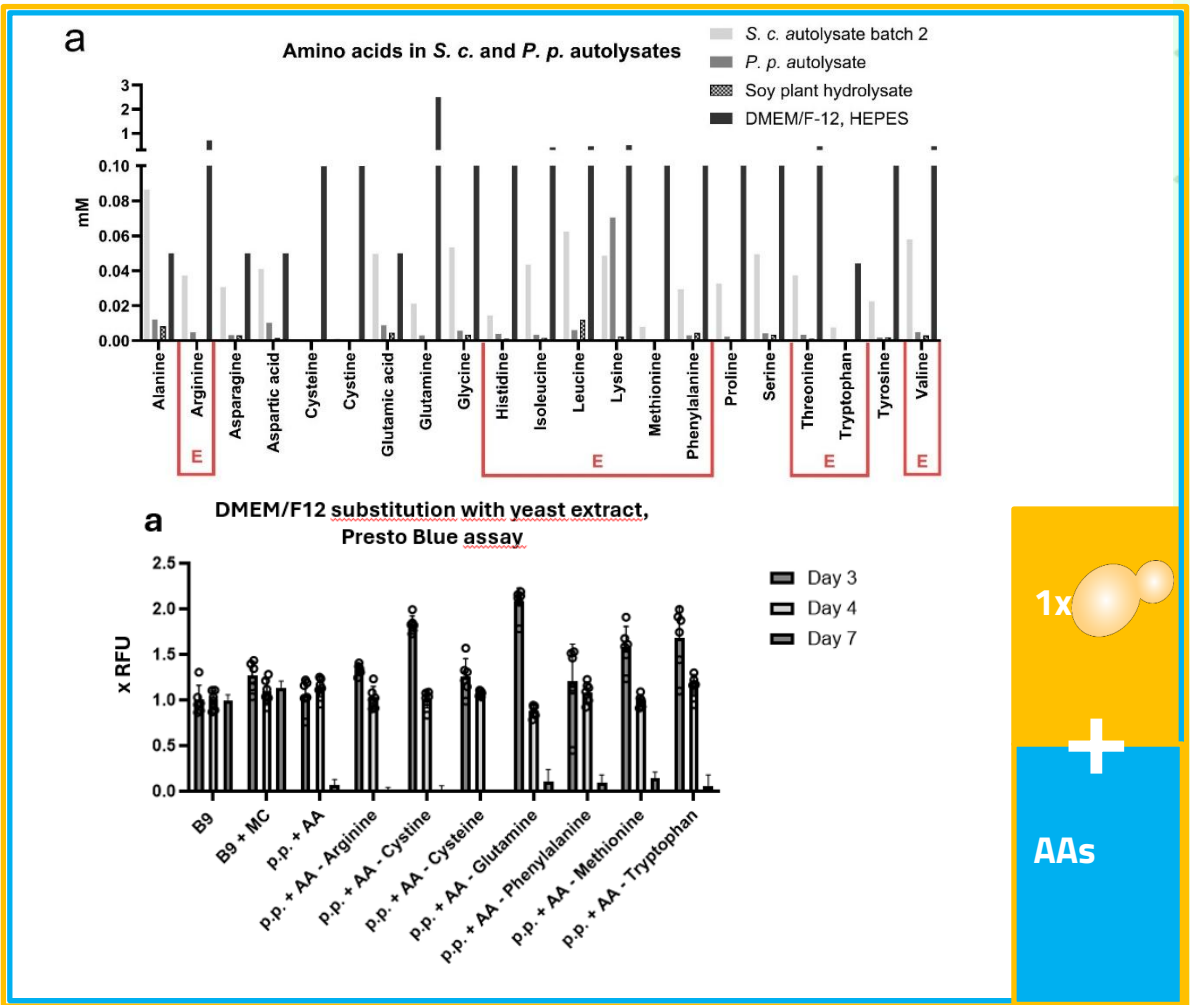
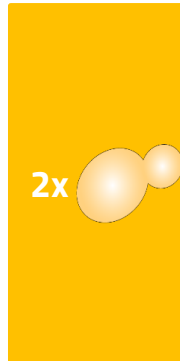
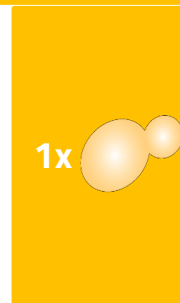
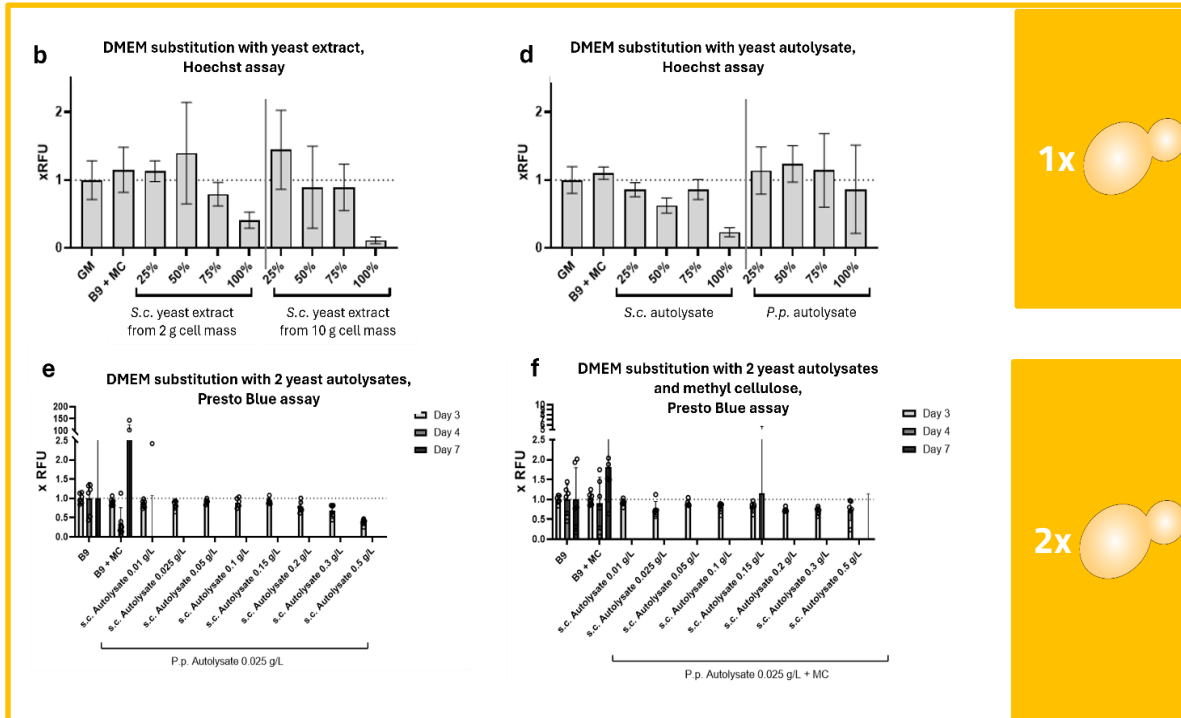
Example Shown: *Wheat Hydrolysate, media spike-in toxicity test*








## CONCLUSION

- A commercial-scale-ready process for the production of three food plant hydrolysates
- Toxicity tests show concentration dependent growth and growth-advantages for hydrolysates in standard cell culture media
- No morphological differences are observed between samples and control at suitable concentrations

# Results: Full substitution of DMEM/F12 solely with yeast hydrolysates-based medium?

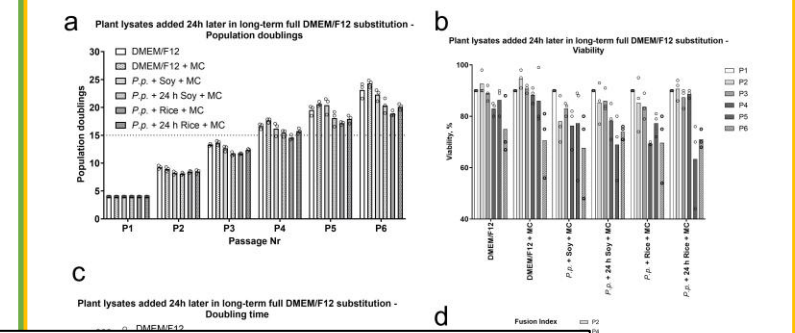
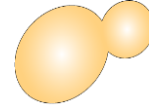
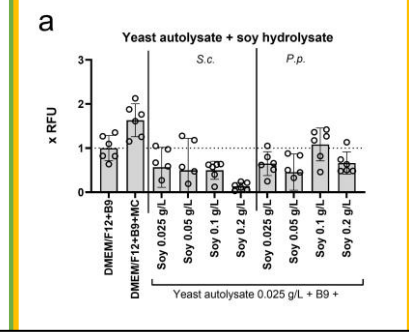
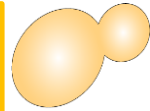
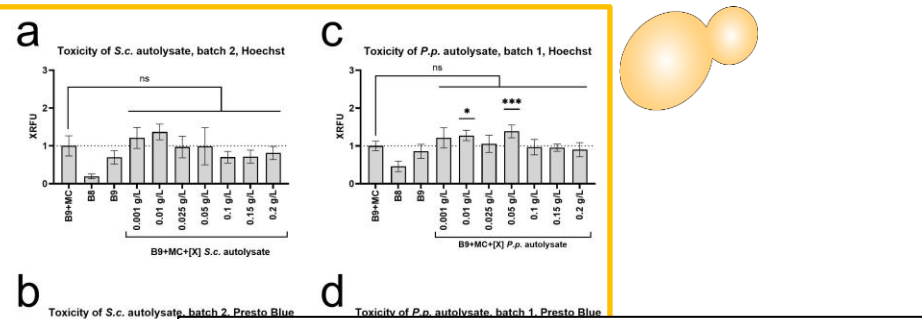




➤ HBBM (  +  ) -> 

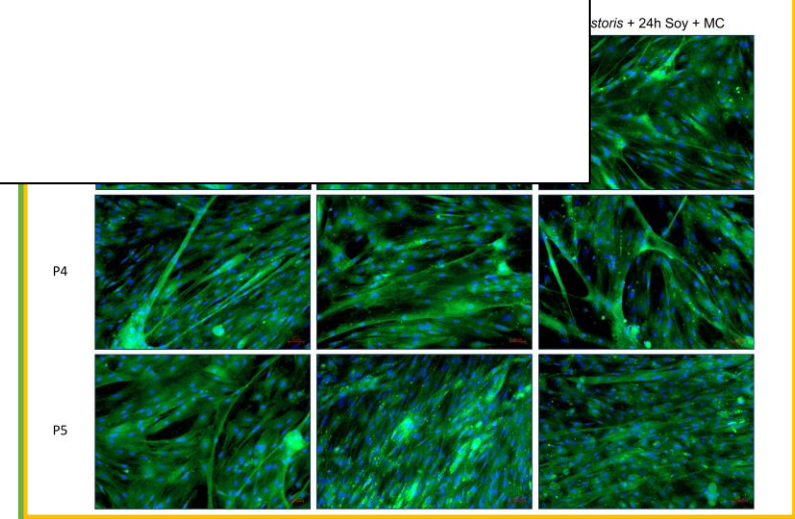
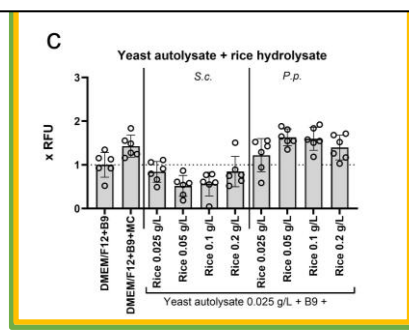
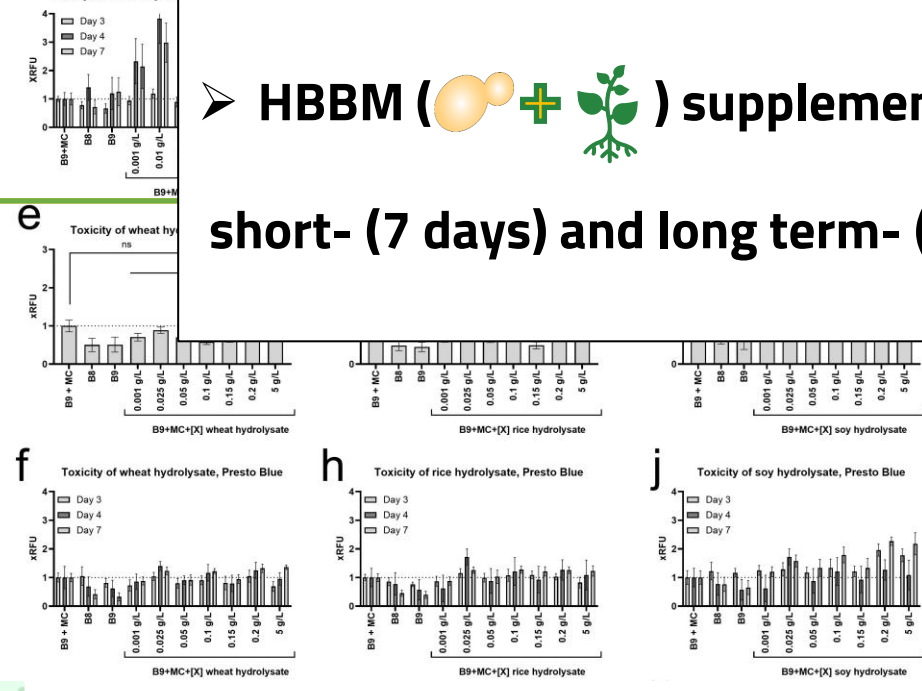
➤ HBBM (  + essential/missing AA ) -> 

Substitution of DMEM/F12 short term with yeast hydrolysates supplemented with essential/missing AAs

# Results: Full substitution of DMEM/F12 with yeast and plant hydrolysates-based medium (HBBM)



➤ **HBBM (  +  ) supplemented with dextrose can fully substitute DMEM/F12 in short- (7 days) and long term- (6 passages) cultivations**

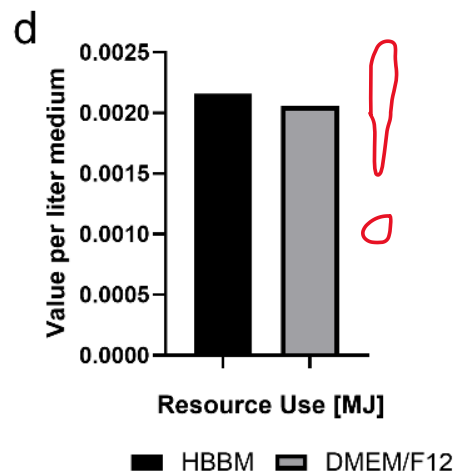
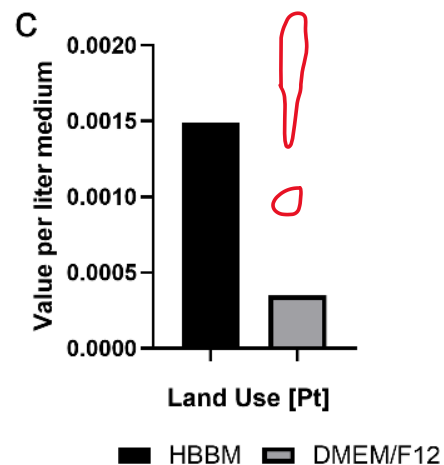
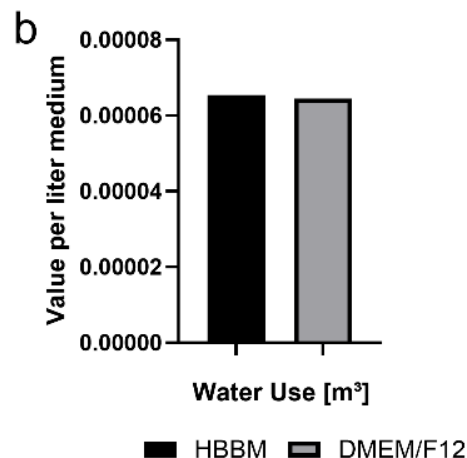
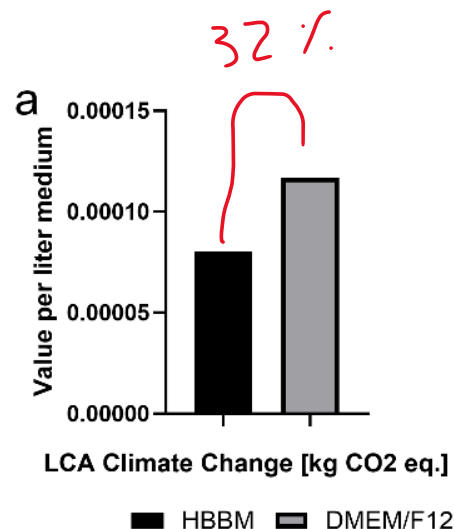


**Short term toxicity of hydrolysates**

**Short term full substitution of DMEM/F12 with hydrolysates**

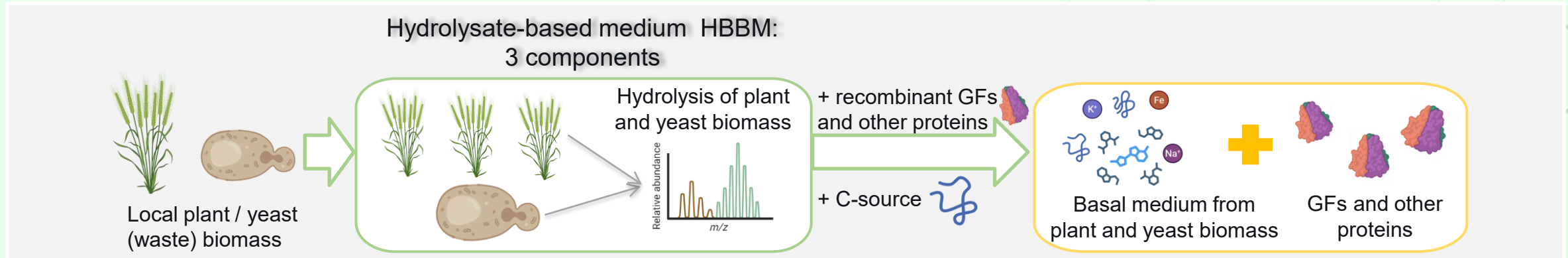
**Long term validation of hydrolysates-based basal medium**

# Results: Life cycle assessment of the AAs and vitamins in HBBM and DMEM/F12 medium



- **HBBM reduces climate-related impacts**
- **but introducing trade-offs in land and resource use**
- **while water use stays largely the same**

# Conclusion

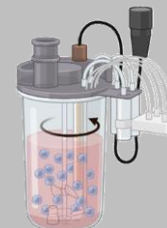


Chemically defined medium DMEM/F12: approx. 60 components

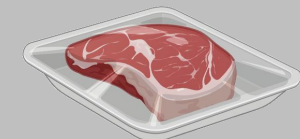
DMEM/F12 basal medium #D0547		Amino Acids		Vitamins	
Inorganic Salts	g/L	g/L	g/L	g/L	g/L
Ammonium Molybdate • 4H2O	0,00000618	L-Alanine	0,00445	D-Biotin	0,0000035
Ammonium Metavandate	0,00000058	L-Arginine • HCl	0,1475	Choline Chloride	0,00898
Calcium Chloride	0,1166	L-Asparagine • H2O	0,0075	Folic Acid	0,00265
Cupric Sulfate • 5H2O	0,0000013	L-Aspartic Acid	0,00665	myo-Inositol	0,0126
Ferric Nitrate • 9H2O	0,00005	L-Cystine • 2HCl	0,03129	Niacinamide	0,00202
Ferrous Sulfate • 7H2O	0,000417	L-Cysteine • HCl • H2O	0,01756	D-Pantothenic Acid (hemicalcium)	0,00224
Manganese Sulfate	1,51E-07	L-Glutamic Acid	0,00735	Pyridoxal • HCl	0,002
Magnesium Chloride • 6H2O	0,0612	L-Glutamine	0,365	Pyridoxine • HCl	0,002031
Magnesium Sulfate (anhydrous)	0,04884	Glycine	0,01875	Riboflavin	0,000219
Nickel Chloride	0,00000012	L-Histidine • HCl • H2O	0,03148	Thiamine • HCl	0,00217
Potassium Chloride	0,3118	L-Isoleucine	0,05447	Vitamin B12	0,00068
Sodium Bicarbonate	—	L-Leucine	0,05905	<b>Other</b>	
Sodium Chloride	6,996	L-Lysine • HCl	0,09125	D-Glucose	3,15
Sodium Metasilicate • 9H2O	0,0000142	L-Methionine	0,01724	Hypoxanthine	0,0021
Sodium Selenite	0,00000519	L-Phenylalanine	0,03548	Linoleic Acid	0,000042
Sodium Phosphate Dibasic (anhydrous)	0,07102	L-Proline	0,01725	Phenol Red • Na	0,00863
Sodium Phosphate Monobasic (anhydrous)	0,0543	L-Serine	0,02625	Putrescine • HCl	0,000081
Stannous Chloride • 2H2O	0,00000011	L-Threonine	0,05345	Pyruvic Acid • Na	0,11
Zinc Sulfate • 7H2O	0,000432	L-Tryptophan	0,00902	DL-Thioctic Acid	0,000105
		L-Tyrosine • 2Na • 2H2O	0,05579	Thymidine	0,000365
		L-Valine	0,05285		

## CONCLUSION

- Substitution for DMEM/F12 is possible
- For up to 5 passages;
- Lower component concentrations than in DMEM/F12;
- LCA suggest improved sustainability
- Rough price estimate: 0.07 €/L HBBM vs 2.5 €/L DMEM/F12

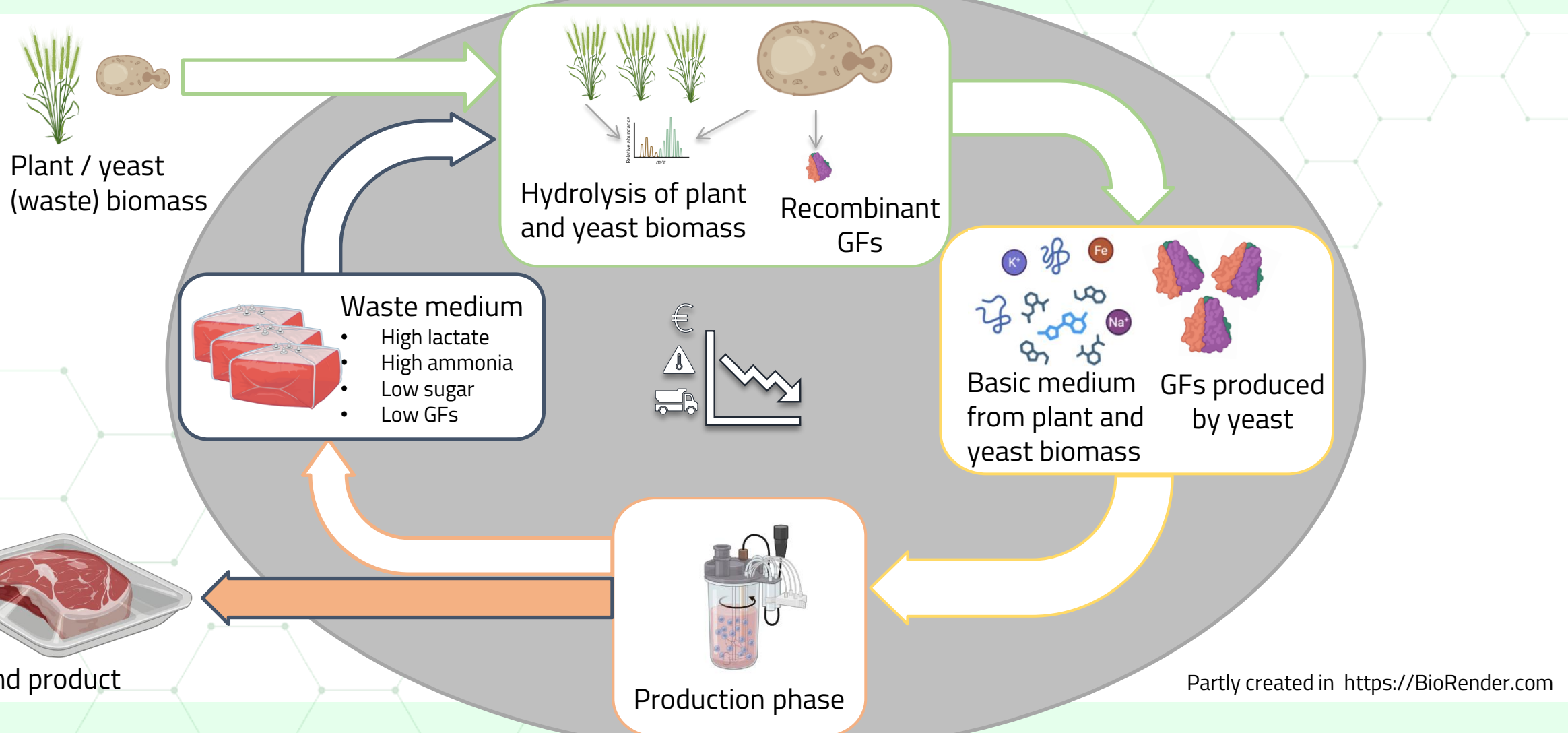


DMEM/F12 → HBBM



Partly created in <https://BioRender.com>

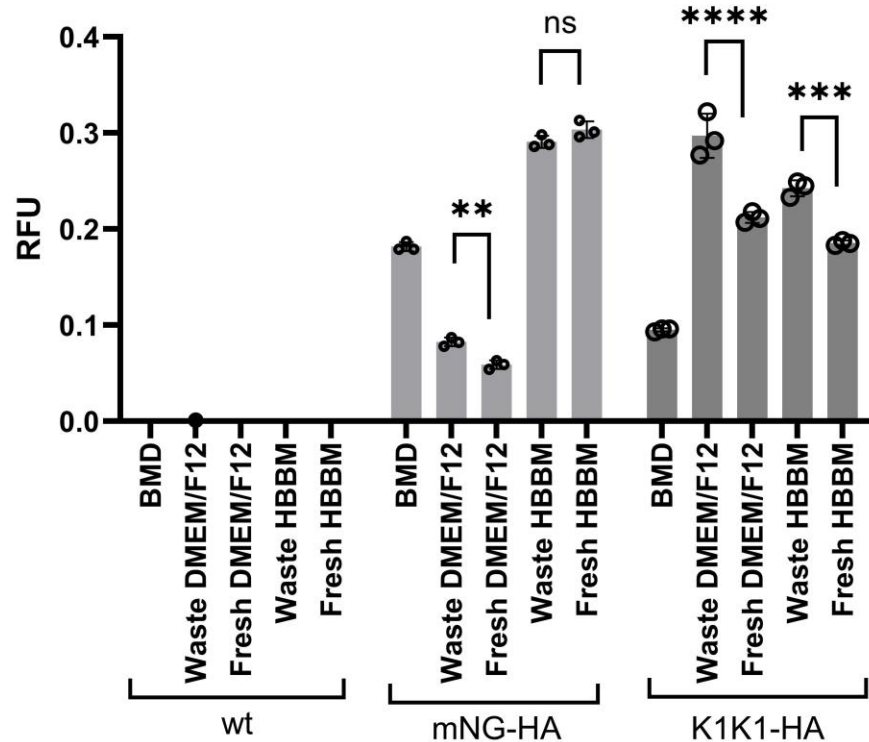
# Concept: CCC system based on yeast and plant lysates



Partly created in <https://BioRender.com>

# Results: Waste medium is suited for GF production by yeasts

**Waste cell culture media for recombinant protein production**

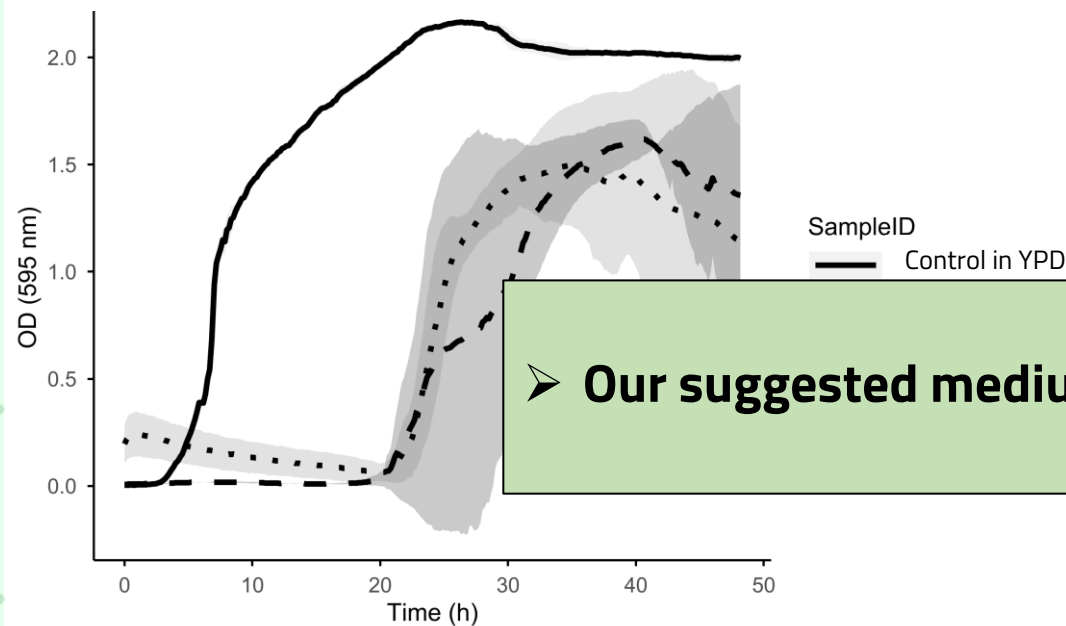


Medium type	BMD 2%	Waste HBBM with 2% dextrose	Fresh HBBM with 2% dextrose
Productivity	100%	255% - 443%	195% - 337%
Improvement	-	In average 100% higher in waste medium	

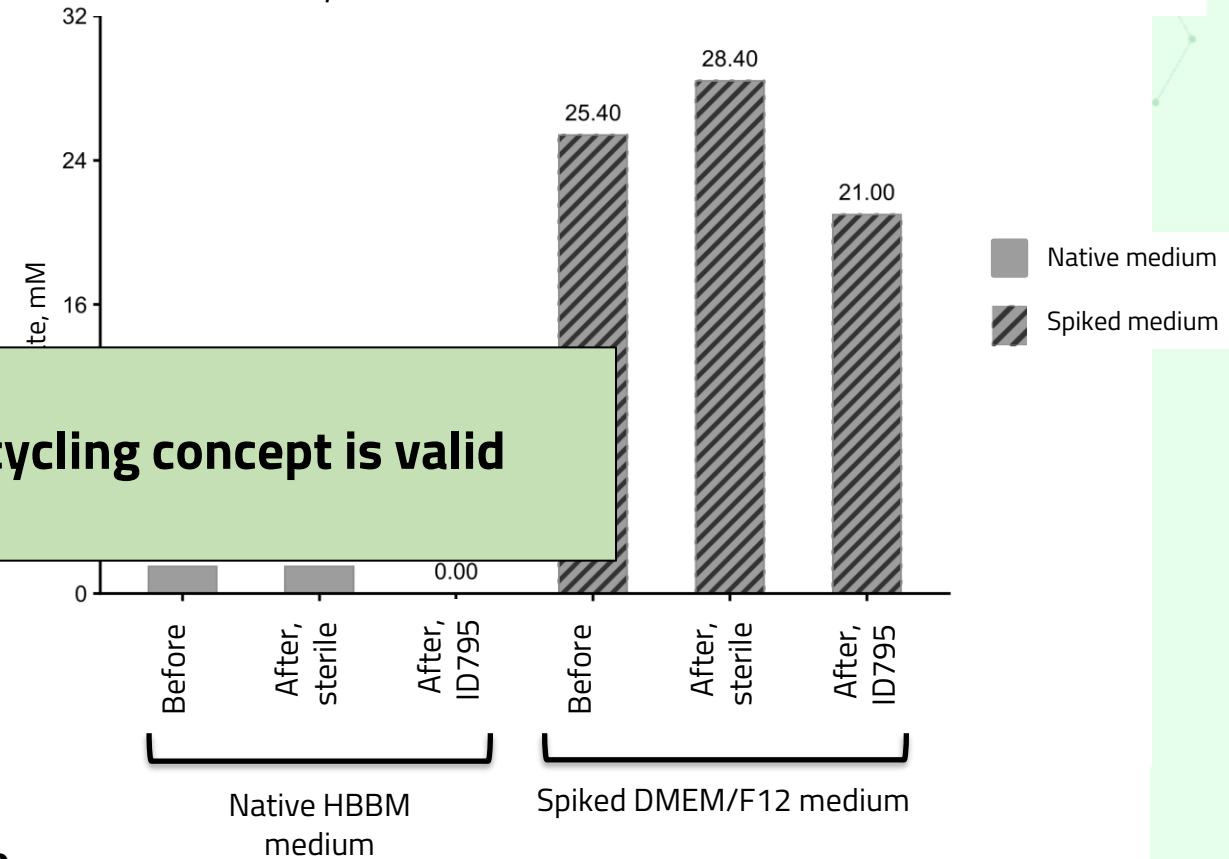
- **Growth factor expression in HBBM is significantly higher than in the standard yeast minimal expression BMD medium**
- **Productivity in waste HBBM is significantly higher than in fresh HBBM.**

# Results: Waste medium valorization

Growth of *Pseudomonas putida* (ID795) in waste DMEM/F12



*Pseudomonas putida* (ID795) consumes lactate in waste DMEM/F12



➤ Our suggested medium recycling concept is valid

- *Pseudomonas putida* strain can consume both high and low concentrations of lactate
- Co-cultivation with *P. pastoris* or cloning lactate pathway into *P. pastoris* are open for a full recycling of cell culture medium



# Our team



# Thank you!



**Group leader:** Assoc.Prof. Harald Pichler

**Project lead:** Dr. Aleksandra Fuchs

**Project staff:** B.Sc. Lisa Schenzle, M.Sc. Ella Siegl, B.Sc. Rita Beiral, M.Sc. Beate Berchtold, Dr. Regina Leber

**Collaborators:**

NaTurtle

Dr. Jürg Stebler

MedUni Graz

Assoc.Prof. Beate Rinner,  
Dr. Stefan Wernitznig

EurA AG, Erfurt

M.Sc. Shashank Goyal

VetMedUni Vienna

Assoc.Prof. Evelyne Selberherr,  
Dr. Dzieciol Monika

BOKU Vienna

Prof. Johannes Buyel

# Consortium Partners

