HELMHOLTZ
Centre for Environmental Research

Yeast-based production of carboxylic acids from renewable resources and wastes

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4. DBFZ-Bioraffinerietag
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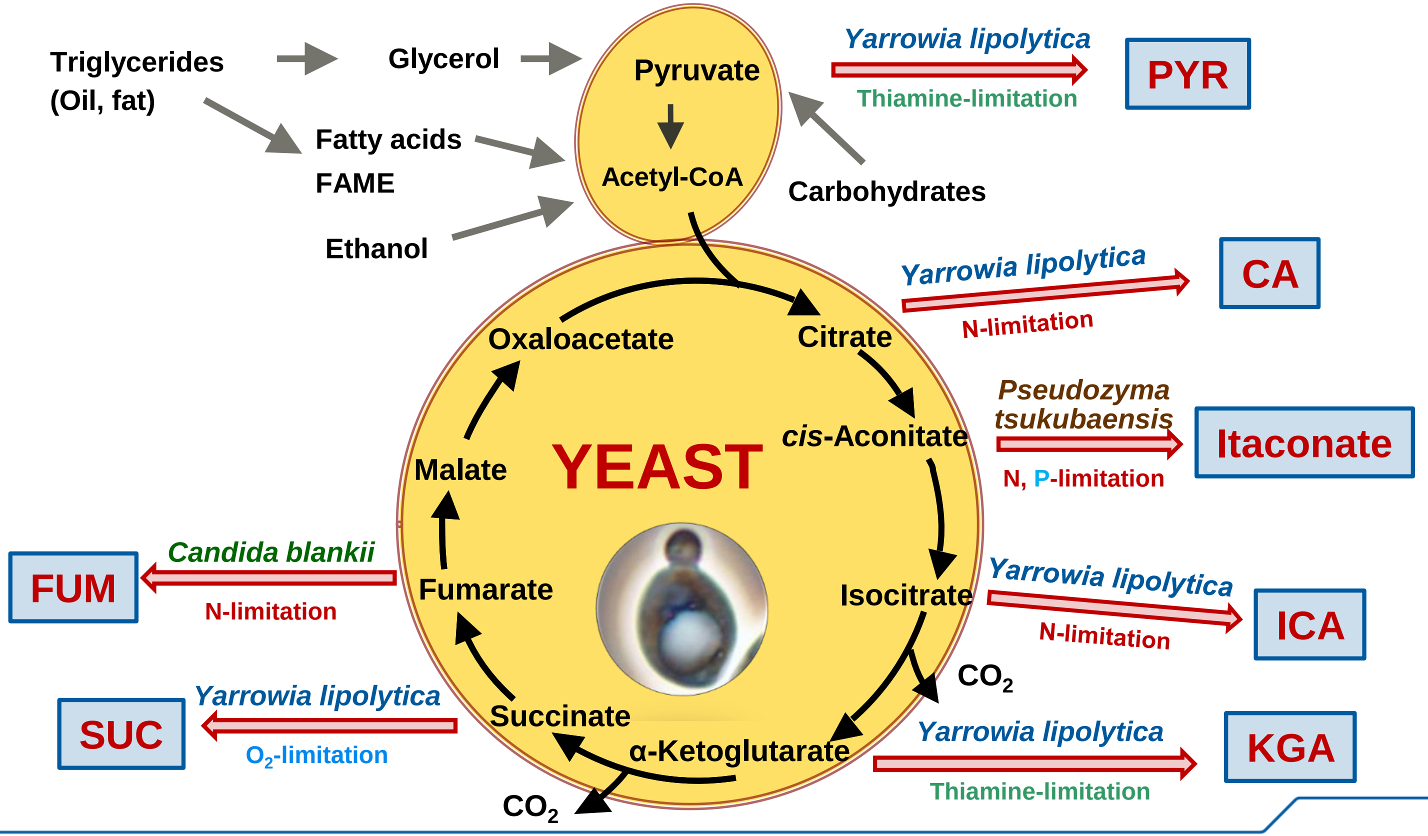
Yeast based bio-refinery - overproduction of Citrate cycle intermediates



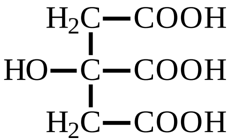
Results of our yeast-based bio-processes with renewable resources

Carboxylic acid / Yeast strain	GMO / wt	Substrate	Concentr. [g l^{-1}]	Selectivity [%]	Productivity [g l^{-1} h^{-1}]	References
Citric acid						
Yarrowia lipolytica H222	wt	**glucose**	**120-140**	**90-92**	0.7-1.2	Moeller et al. (2011, 2012, 2013)
Y. lipolytica H222-S4 (p67ICL1)	GMO	**sucrose**	**140**	**95-96**	0.8	Förster, Aurich et al. (2007) Appl Microbiol Biotechnol 75
Yarrowia lipolytica H181	wt	**sunflower oil**	**165-205**	**95**	1.70	Aurich et al. (2003) Biotechnol Adv 21
		raw glycerol	**190**	**95**	1.54	
Fumaric acid						
Candida blankii IFO1973	wt	**glucose**	**49**	**85**	0.14	WO 2013/120924
Isocitric acid						
Yarrowia lipolytica EH59	wt	**sunflower oil**	**93**	**53**	0.56	Herretsch, Aurich et al. (2008) Angew Chem Int Ed 47
Y. lipolytica H222-S4(p67ACO1)	GMO	**sunflower oil**	**68**	**76**	0.47	Aurich (2012) Subcell Biochem 64 Holz et al. (2009) Appl Microbiol Biotechnol 81
Itaconic acid						
Pseudozyma tsukubaensis CBS422.96	Wt	**glucose** **glycerol**	**66-75** **42**	**87-90** **90**	0.19 0.13	DE 102008011854 DE 102008011854
α-Ketoglutaric acid						
Yarrowia lipolytica H355	wt	**rapeseed oil**	**115**	**96**	0.45	Aurich et al. (2012) Subcell Biochem 64
Pyruvic acid						
Yarrowia lipolytica H355	wt	**raw glycerol**	**64**	**80**	0.90	
Succinic acid						
Yarrowia lipolytica H222-AZ9	GMO	**raw glycerol**	**91**	**93.5**	0.33	DE 102011056297 A1; Jost (2015) Appl Micro Biotech 99

GMO=genetically modified organism, wt= wild-type strain

Why is citric acid (CA) a target for bio-economy?

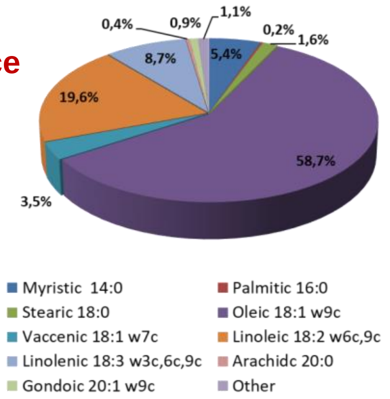
- **Market size:** about **2,000,000 t/a** (2019), produced by fungi *Aspergillus niger*
- **Main consumers**: Western Europe, America
- **Applications** (Citric Acid Anhydrous & Citric Acid Monohydrate):
  - Food and Beverages
  - Detergents and Cleaners (decalcifier, acidification, metal complexing agent)
  - Pharmaceuticals and Personal Care (e.g. blood stabilizer)



- Use of **sugars** as carbon source → **food** vs. **fuel** controversy
- Centralized world scale production → **local** consumption

Our alternative: Non-sterile CA bio-process with *Yarrowia lipolytica* using wastes and wastewater (ww)

Waste frying oil (WFO) as Carbon rich source



Wastewater from food processing

Results of non-sterile CAprocess at conventional bio-reactors

Wastewater + WFO	Time (h)	Citrate (g/L)	Producti-vity (g/L*h)
Oil & Fat separator discharge	95-165	128-145	0.8 - 1.2
Kitchen cleaning discharge	190	182	0.95
Urban WW	190	134	0.7
Tap water	166-214	166-192	0.9-1.15

- **Patent granted:**
  Aurich et al. (2023) Method for producing carboxylic acids under unsterile conditions; **US11597952, EP3642347**

Challenge: From high-tech to low-tech - Designing a downgraded bioreactor for decentralized operation

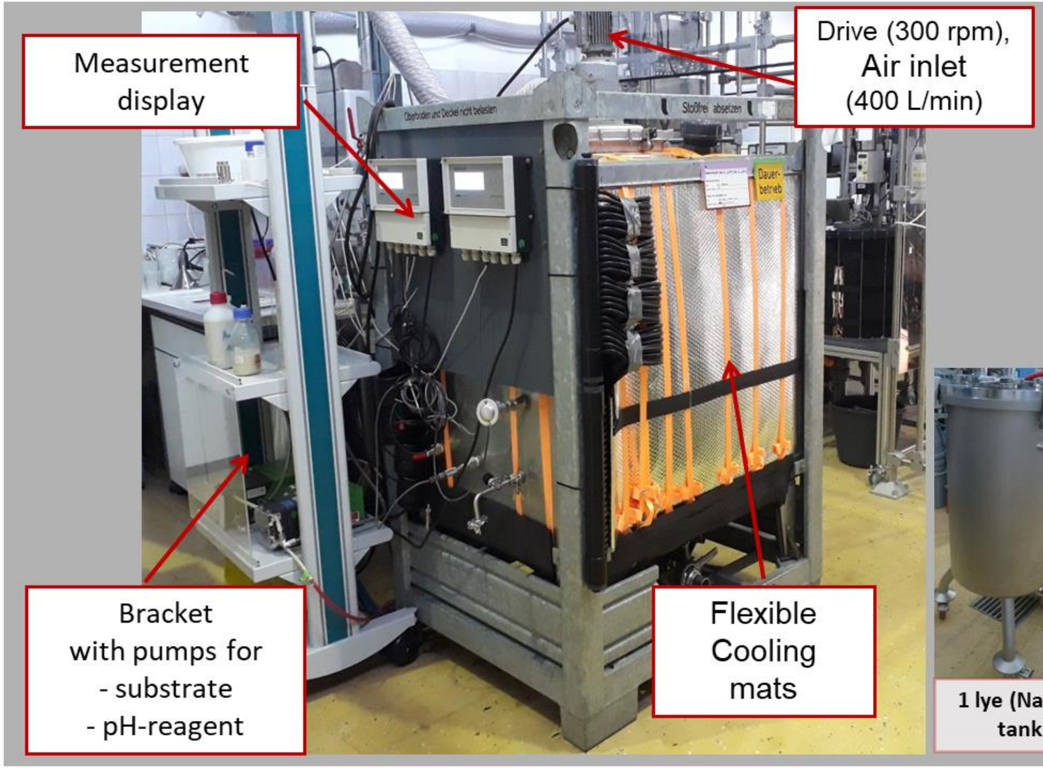
Conventional sterile Stirred tank bioreactors

- Efficient O_2, heat transfer system
- Designed for sterile operation
- Control and analysis of many parameters
  → T, pH, pO_2 , OUR, CPR
- High invest costs: >**400,000 €** for 1 m³

Downgraded non-sterile bioreactor

- Based on **standard equipment** for operation of wastewater treatment (e.g**. IBC** container)
- Modular for **decentralized** operation
- Simple robust control system
- Low invest costs: ~**35,000 €** for 1m³

Final design of 1m³ IBC container based bioreactor for decentralized yeast based Citric acid production



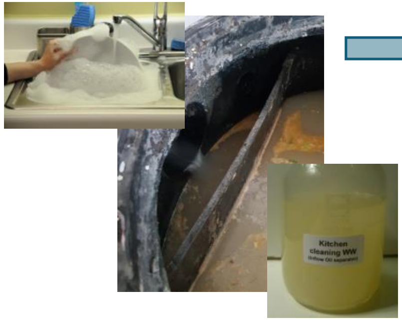
Key figures 1 m³ IBC reactor

- Working volume: 0.6-0.8 m^3
- Cooling area: ~2.7 m^2
- Cooler: 5 kW
- Measurement: T; pO2, pH
- Automatic control: pH
- Control: T

Exemplary decentralized concept & solution: Canteen & Catering UFZ

Feedstocks

Kitchen cleaning WW

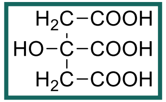


Waste frying oil

- **Yeast *Yarrowia lipolytica***
- **Non-sterile operation**
- **Aerobic conditions, pH 5**

Citric acid, solution

CA, cryst.



Filtration

Yeast biomass

Biogas

Exemplary concept – Canteen UFZ

- Kitchen cleaning discharge
- 600-700 lunch meals daily

Biogenic waste:

- 50-60 L WFO/week

Process duration:

- 2 weeks

Citrate: 120 kg/m³

Bioprocess mode:

- Two-stage decoupled Growth and Production phase
- Precultures in conventional bioreactor
- High cell density process for yeast biomass

What are we looking for ?

University, non-university and industrial R&D partners for

- Further development / optimization of downgraded bioreactor systems
- Development / optimization of downstream processing for carboxylic acids
- Opening up new fields of application for yeasts / carboxylic acids

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Thank you for your attention !