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HAN BioCentre

Biobased products by microbial fermentation

Example: production of microbial oil

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CBBE Docentendag

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HAN University

HAN University of Applied Sciences

Faculty of Technical Studies

Institute of Applied Sciences

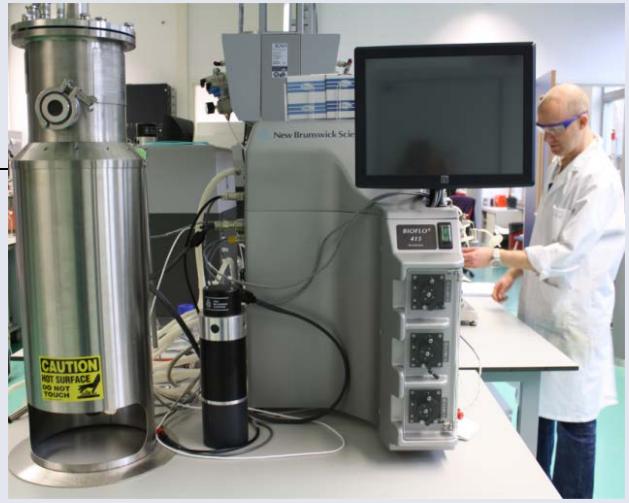
IJ5Lab

MLO

HLO

Master
MMLS

HAN BioCentre:
HAN Centre of expertise
in biotechnology & analysis



Industry ⟷ Research Biotechnology & analysis ⟷ Education Biotechnology & analysis

Staff BioCentre

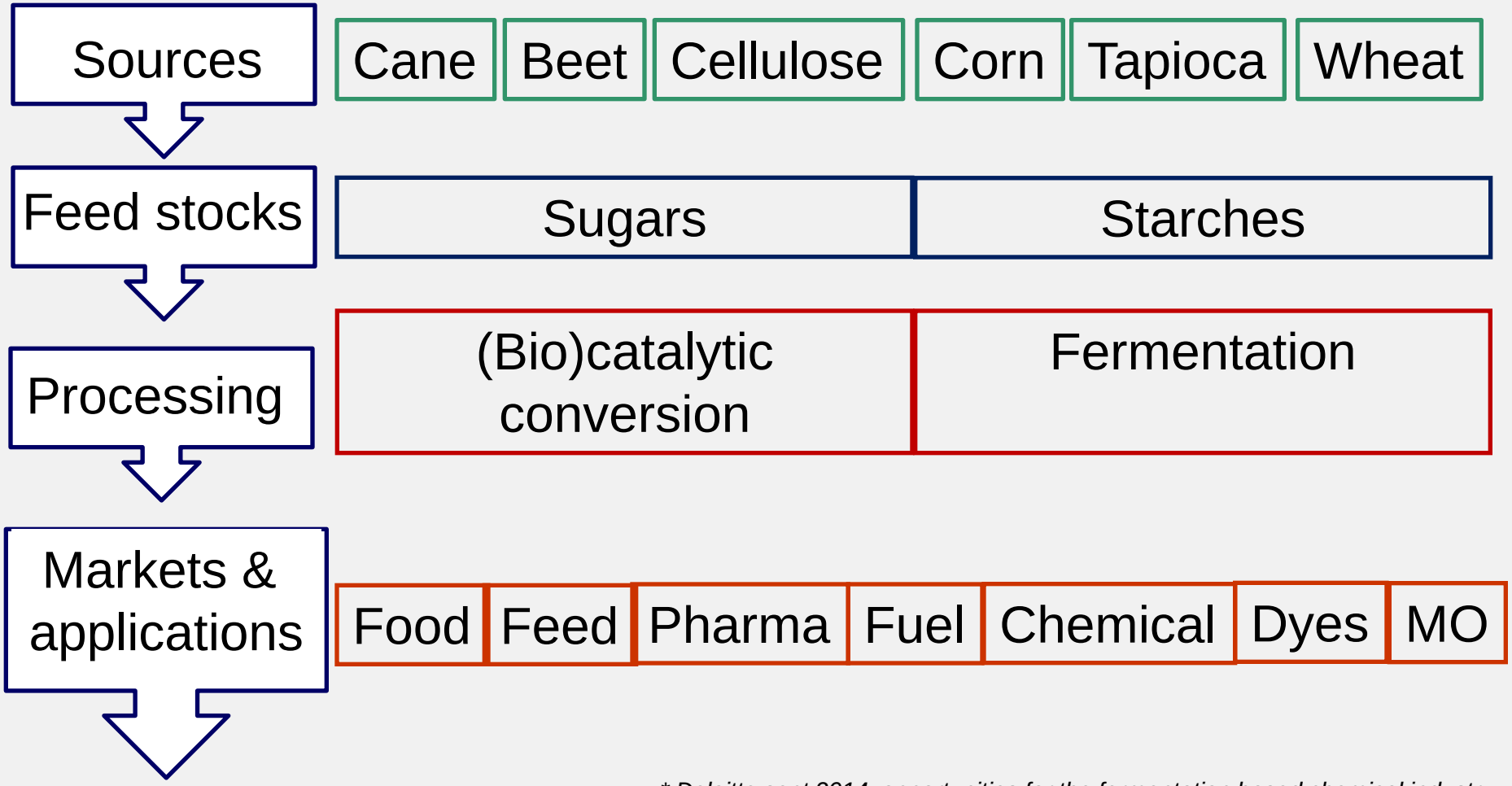
1 lector/manager
5 project leaders/researchers
3-5 technicians
1 PhD student
in addition 5-10 students

Life Sciences education

Master programme
Bachelor programme

Tailor made course:
Fermentation technology
Introduction LC-MS
Applied Microbial Genomics

Fermentation to biobased products



** Deloitte sept 2014, opportunities for the fermentation based chemical industry*

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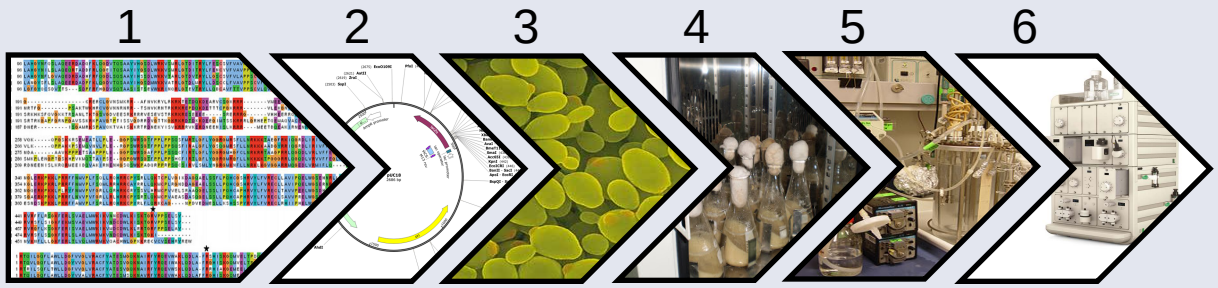
Key biobased products using microbial fermentation

Product	Market size* (mln ton)	Examples	Main production organisms	Theoretical yield* (ton product / ton glucose)
Antibiotics	0.2	Penicillin, tetracyclin	*Penicillium*, *Streptomyces*	1.0
Industrial enzymes, pharm proteins	0.1	Protease, lipase, amylase, cellulase, laccases	*Bacillus*, *Aspergillus,* hetero-logous expression in *E. coli, Saccharomyces, P. pastoris*	1.0
Food & feed additives	7.3	Vitamins, amino acids	*Corynebacterium* (lysin), *Propionibacterium* (B_{12})	0.92-0.96
Chemicals: org acids, polymers	3.1	Lactic acid, succinic acid, PHA, alcohols, fatty acids/oil	lactic acid bacteria, *Bacillus*, *Pseudomonas*	0.93-1.05
Fuels	99.9	Bioethanol, biogas, biodiesel	*Saccharomyces* & other yeasts	0.27-0.51

** Deloitte sept 2014, opportunities for the fermentation based chemical industry*

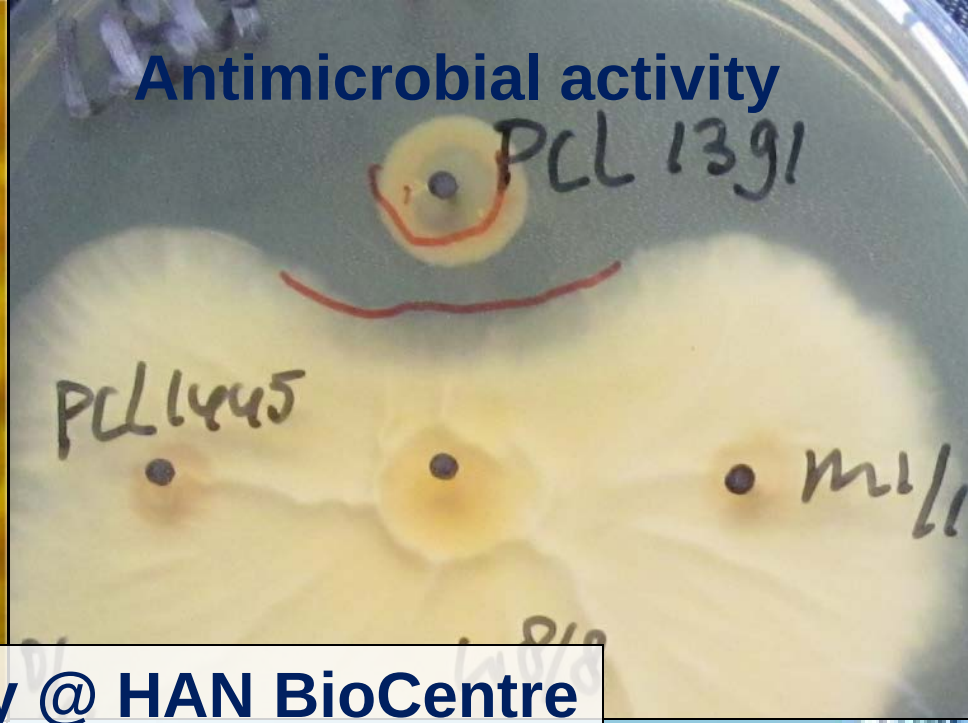
Enzyme pipeline



1 = Gene selection	Bioinformatics
2 = Vector integration	Molecular biology
3 = Expression system	Molecular biology
4 = Flask culture analysis	Microbiology
5 = Fermentation process	Fermentation technology
6 = DSP	Biochemistry

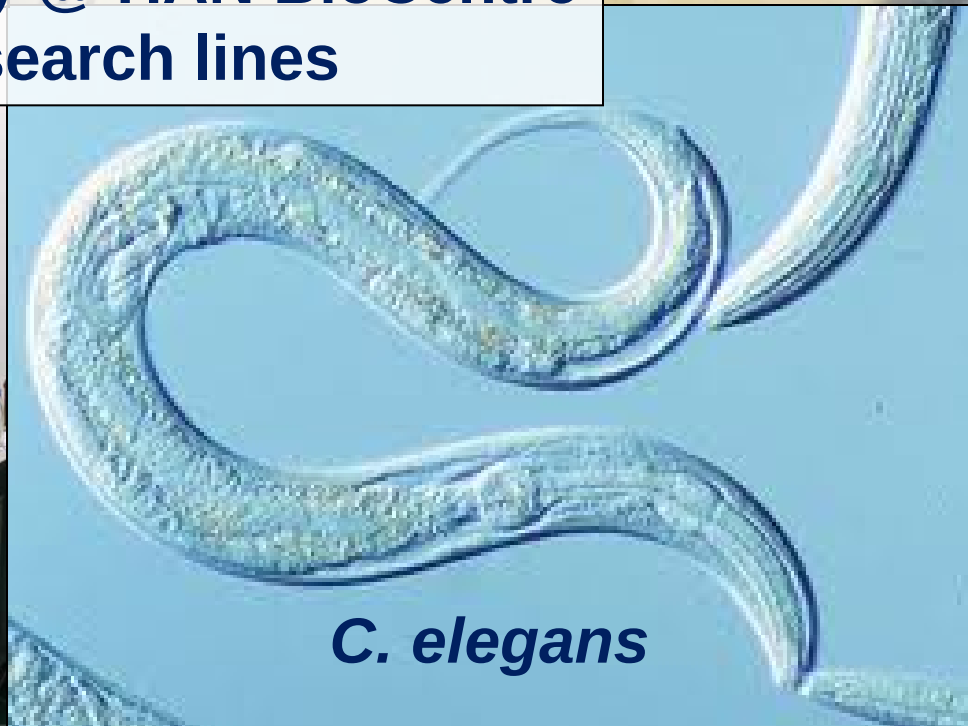
Oil from Yeast

Antimicrobial activity



Biodiscovery @ HAN BioCentre
4 research lines

Enzyme production



C. elegans

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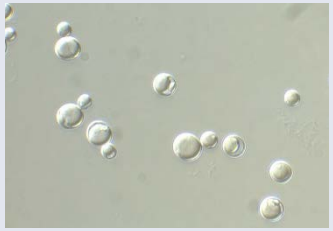
BioCentre fermentation

- Microbial oil



- Enzyme production

- BioCentre labs

Fermentation in education

- minor DAPB
- HLO/MLO students
- master program (metabolic engineering)

- master program (fermentation week)
- bachelor biology
- HLO/MLO students

- bachelor/master: fermentation and DSP theory and practical work

- Fermentation lab for education

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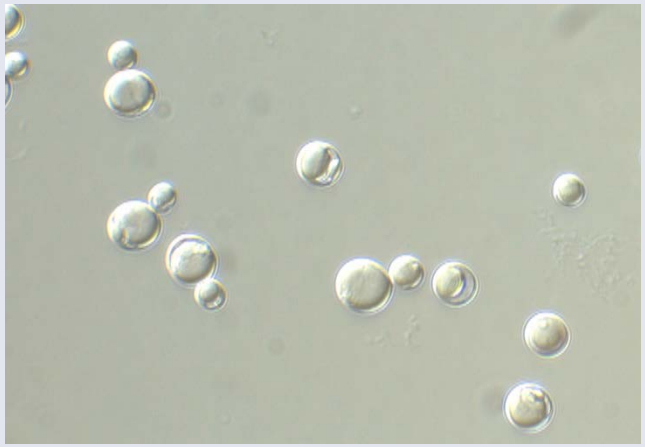

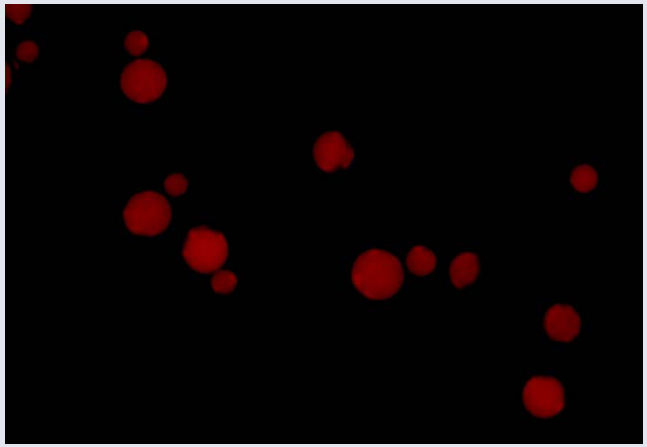

Production of biobased oil by oleaginous yeast

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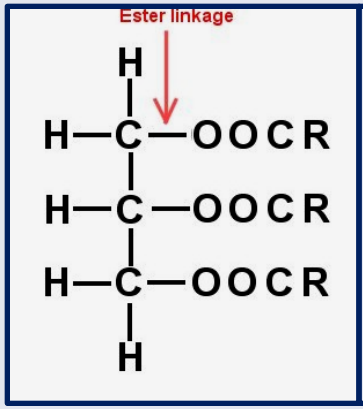
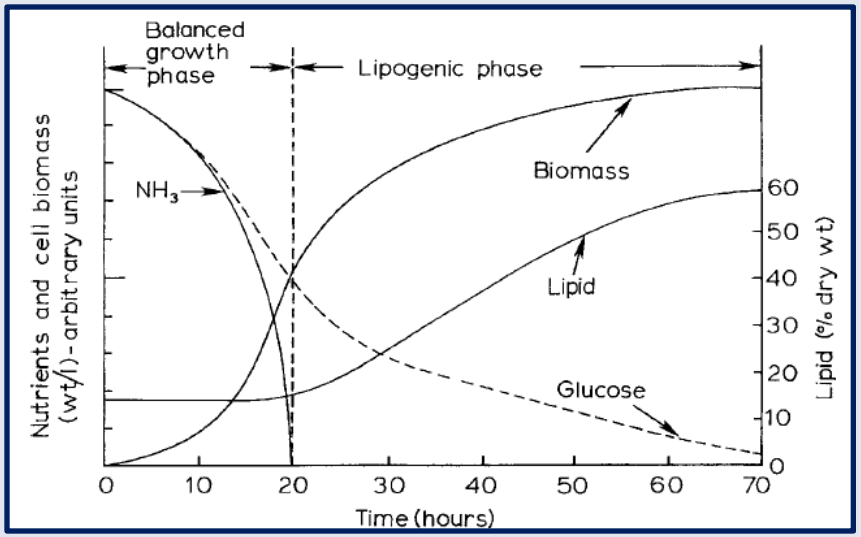
Triacylglycerol (TAG)

- Natural oils consist of TAGs
- TAGs are a natural stock of energy in
  - plants
  - animals
  - microorganism
- Oleaginous species
- Accumulation > 20% of their biomass as oil
- Yeast, fungi, algae



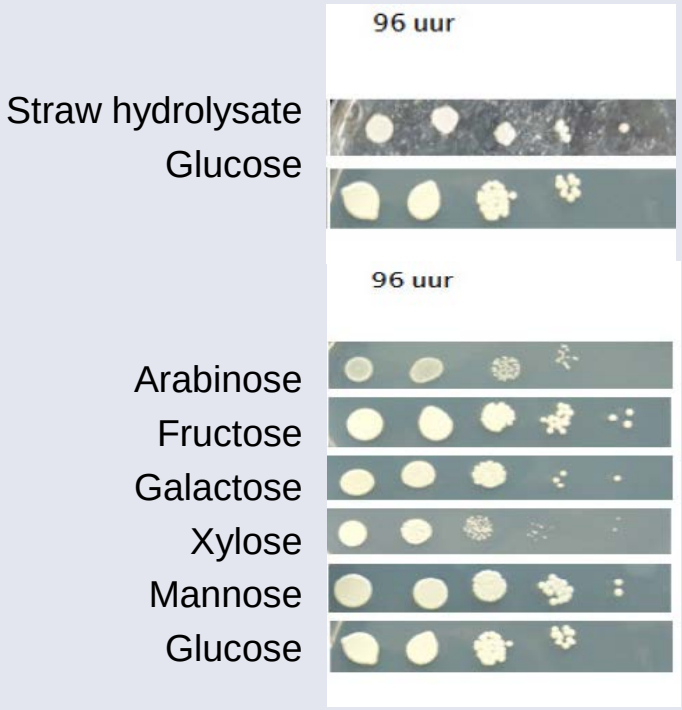
TAG

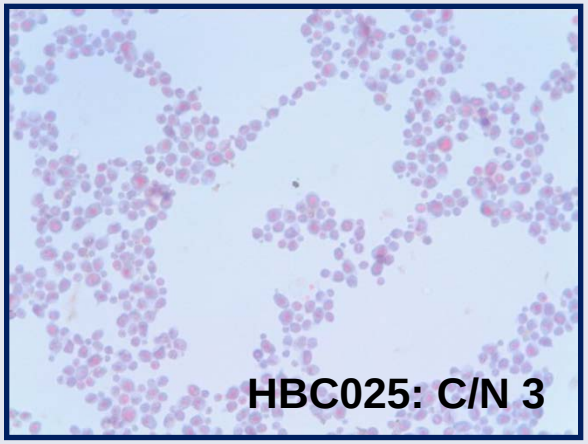
Fatty acids of glycerol consisting mainly of **palmitic, stearic, oleic or linoleic acid**

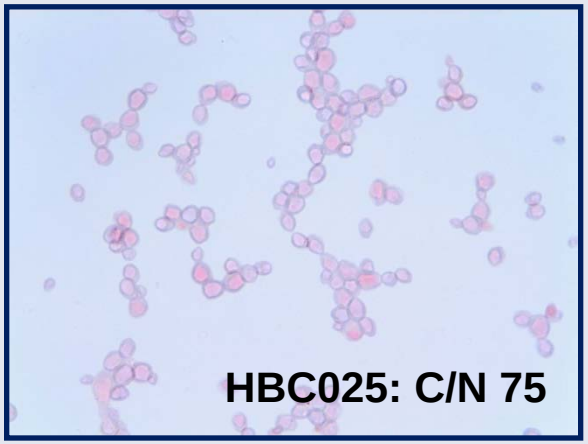


Wynn & Ratlegde 2005

Selection of HBC025







Selection criteria:

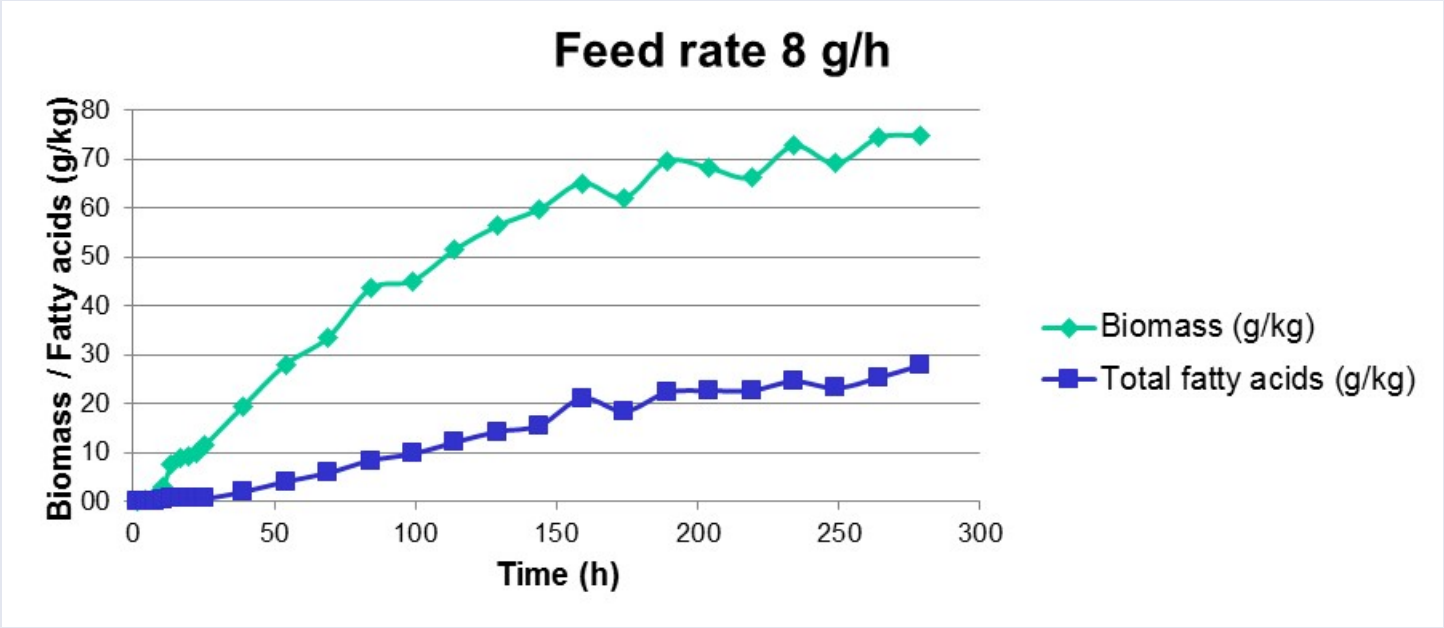
Robust
High biomass
High lipid yield
Growth on various C-sources

Nick van Biezen et al. in preparation

Average composition of natural oils (%)						Other	Costs & applications
	C16:0	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**other**	**Costs & applications**
	Palmitic	**Stearic**	**Oleic**	**Linoleic**	**Linolenic**		
Sunflower	8	3	20	68	1		1,3-2,0 €/kg, paint, alkyd resins
Soybean	12	4	25	52	6		1,5-1,6 €/kg
Palm oil	43	4	37	9	0,5		
Olive oil	13	3	69	12	1		4,9-5,4 €/kg
Linseed oil	6	4	18	17	53		Flooring, road construction
Rapeseed oil	4	1,5	17	13	9	C 20:1 C 22:1	1,7-1,8 €/kg, road construction
Fatty acid profile HBC025 (%)							
Batch 30g Glucose	14	2,5	58	16	6,6	C16:1	
Fed-batch 150 g Glucose	15	1,7	69	4,5	1,0	C16:1	

Costs taken from Huang et al. 2013 Biotechnology advances 31: 129

Fed-batch process (glucose feed / increase in C/N)



- increasing feed rate does not give more biomass and fatty acids

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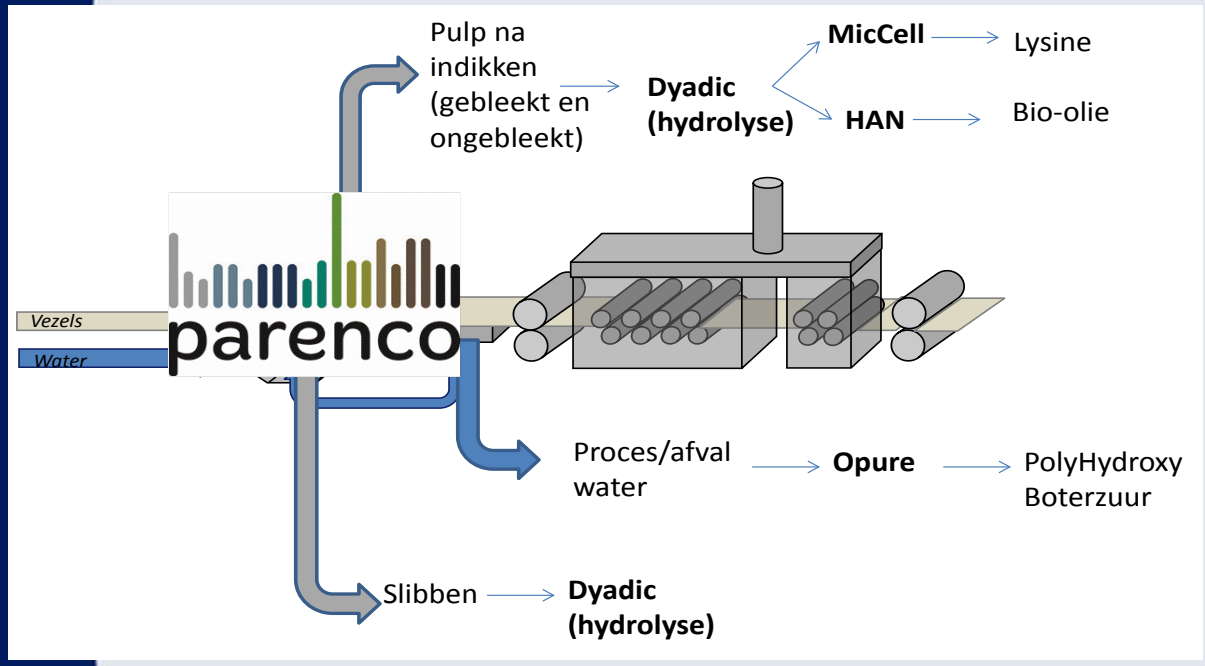
Biobased oil: Parenco project



provincie GELDERLAND

Composition paper waste

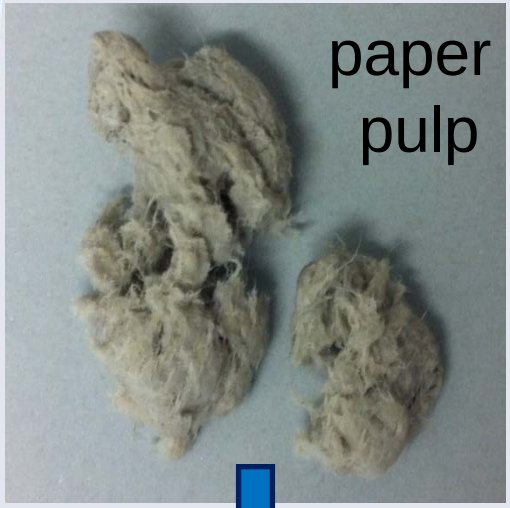
Table 1
Composition of waste papers (all results are presented as percentages of oven dry weight and brackets show SE of triplicate samples).

	Newspaper	Office paper	Magazine	Cardboard
Glucan	47.20 (0.05)	58.64 (0.05)	35.91 (0.08)	52.61 (0.06)
Xylan	7.11 (0.02)	14.65 (0.08)	4.72 (0.06)	8.23 (0.06)
Galactan	1.90 (0.02)	0.00[a]	1.98 (0.02)	1.89 (0.01)
Mannan	7.31 (0.01)	0.00	5.67 (0.04)	5.07 (0.01)
Arabinan	1.86 (0.07)	0.00	1.82 (0.03)	1.55 (0.03)
Total carbohydrates	65.38	73.29	50.10	69.35
ASL	1.06 (0.01)	1.41 (0.01)	0.98 (0.03)	1.59 (0.02)
AIL	17.08 (0.43)	4.68 (0.29)	13.85 (0.29)	14.18 (0.19)
Total lignin	18.14	6.09	14.83	15.77
Extractives	3.93 (0.05)	1.97 (0.05)	3.45 (0.06)	2.55 (0.02)
$CaCO_3$	2.13 (0.04)	8.12 (0.02)	2.63 (0.04)	4.20 (0.01)
Ash	10.51 (0.11)	7.97 (0.08)	30.14 (0.16)	9.89 (0.07)
Mass closure[b]	100.09	97.44	101.15	101.76

[a] Zero was reported here since trace amount was found under detection limit of HPLC.

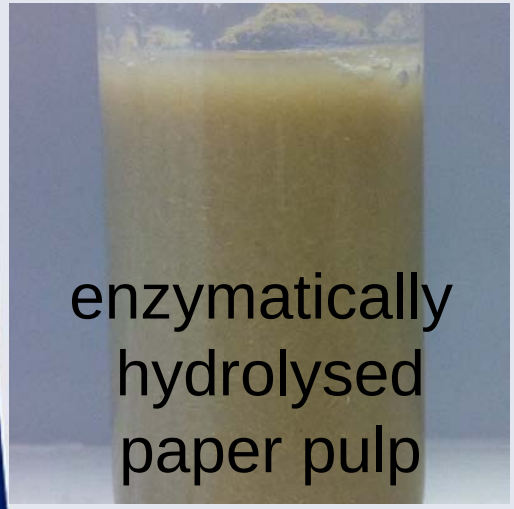
[b] A mass closure exceeding 100% is due to the intrinsic variation of component means.

Wang et al. 2012 Applied Energy 99: 23



SSF

SHF



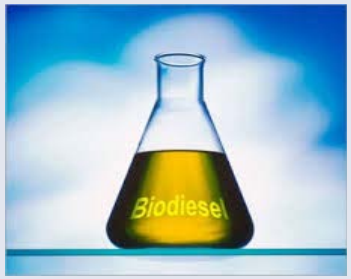
Fermentation

Results

- HBC025 can grow and produce microbial oil on pre-hydrolysed paper pulp (no toxic effect)
- Depending on enzyme dosage medium additions are needed
- Economic feasibility promising when also components from yeast biomass are marketed (biorefinery)

- Applications in non-food sector:

Alkyd resins/paint, cosmetics, biosurfactants, road construction, flooring systems



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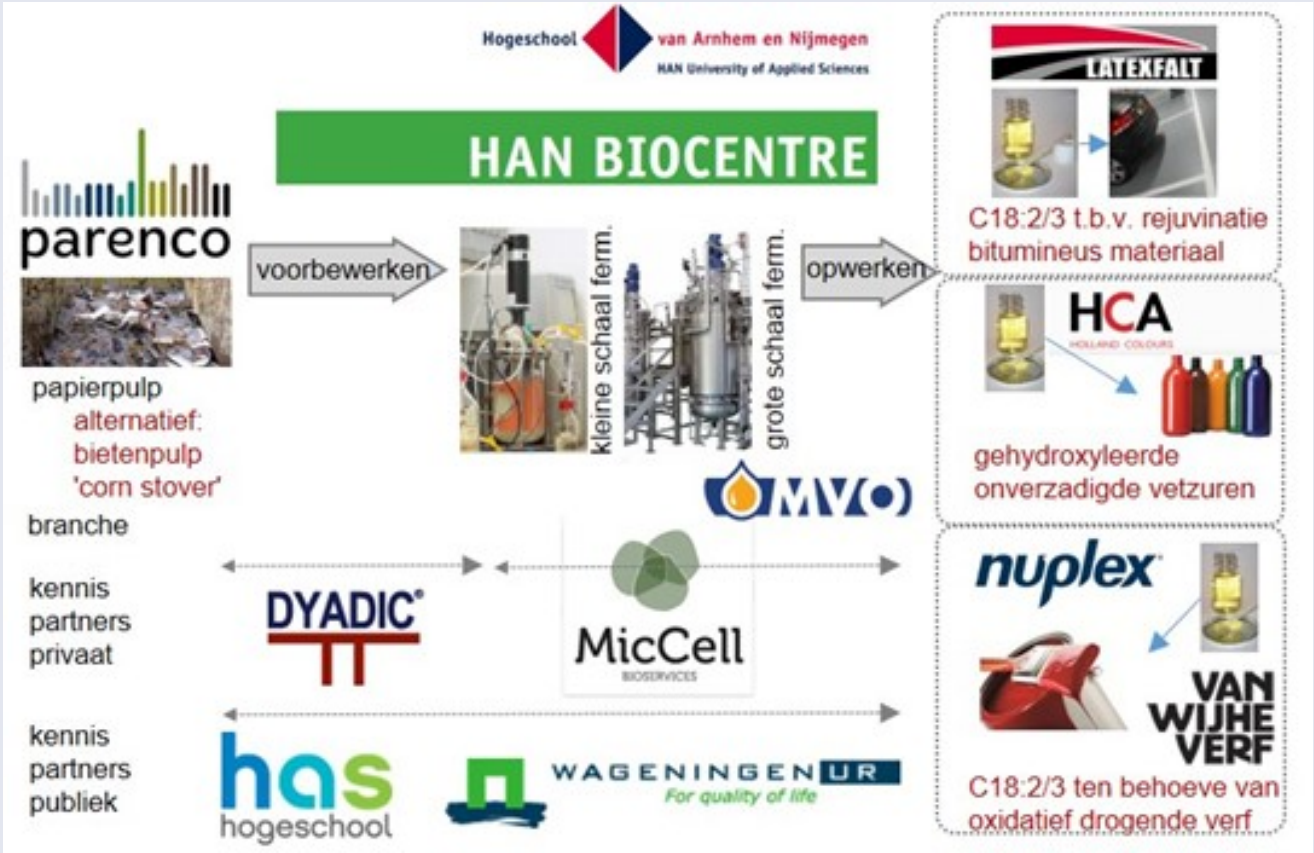
RAAK-PRO started: ‘Microbiële olie op maat’



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Oil project in education

- **Minor students / students biology-chemistry-bioinformatics**
  - strain selection & conditions for oil production
  - process with alternative C-sources ao. (waste) glycerol
  - high C18:2 producers
  - genome sequence and annotation HBC025, bio-informatic tools
  - DSP: oil extraction and decolouring of oil
  - deskstudy biomass sources
  - transformation of HBC025

- **MMLS students**
  - metabolic engineering assignment

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Acknowlegdements

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Questions ??