

A biorefinery for the conversion of glycerol to value added products

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Dept. Systems Biology, DTU



BioGasol ApS, Denmark



A&A Biotechnology, Poland



MEROCO, Slovakia



The Institute for Energy and Environmental Research, Germany



ProChimia Surfaces, Poland

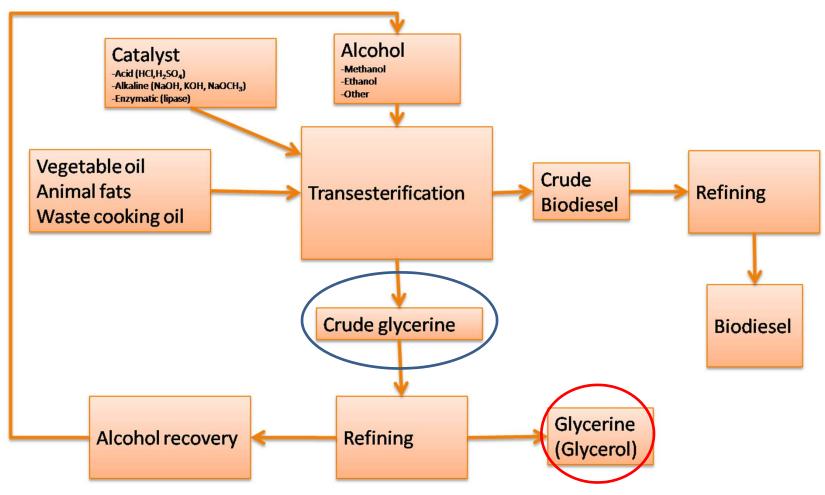
Project period: March 2008 – February 2012







# Biodiesel production process

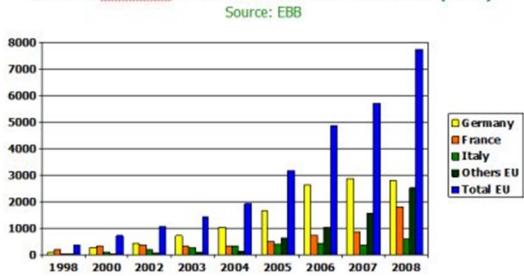




#### Biofuel production in Europe



#### EU and Member States' Biodiesel Production ('000 t)



Over 7.7 million tons of biodiesel were produced in the European Union in 2008, representing an increase of 35.7% over the previous year.

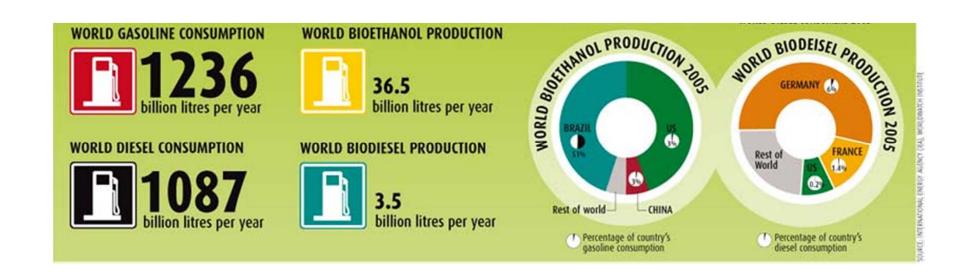
Biodiesel accounted for over 80 % of the total biofuels production.









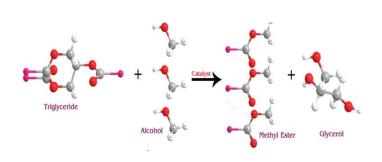




#### Biofuels: a historical perspective









1900
Diesel engine
run on peanut
oil at the
World's Fair in
Paris

1937
Chavanne granted
patent for "Procedure for
the transformation of
vegetable oils for their
use as fuels"

Petroleum replacement fuel

1977
Brazilian scientist
Expedito Parente
filed patent for same
process

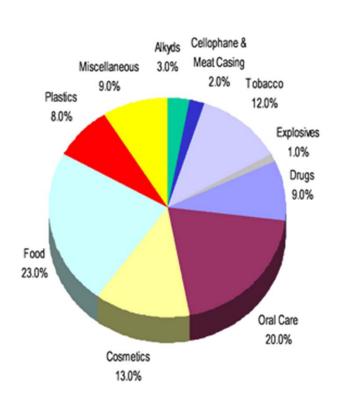
**Biofuels** 

Center for Microbial Biotechnology Department of Systems Biology









Over 2000 established uses for glycerol in the drug, food, beverage, chemicals and synthetic materials industries.

Disposal of surplus glycerol is by incineration.









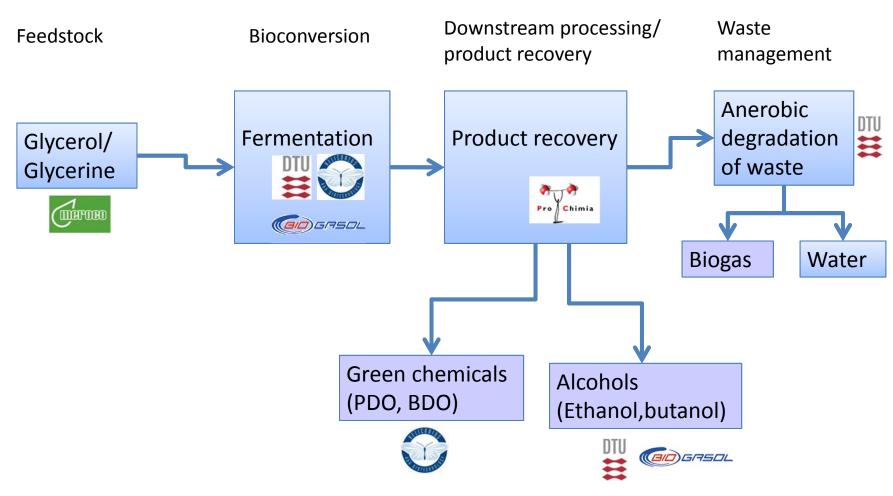
- Targeted to development of novel technologies based on biological conversion of glycerol
- Aimed at producing new and known advanced liquid biofuels, bioenergy and biochemicals
- Integrated biorefinery concept
- Improve economics of the biodiesel biorefinery by enhancing energy conversion efficiency





#### Integrated GLYFINERY concept



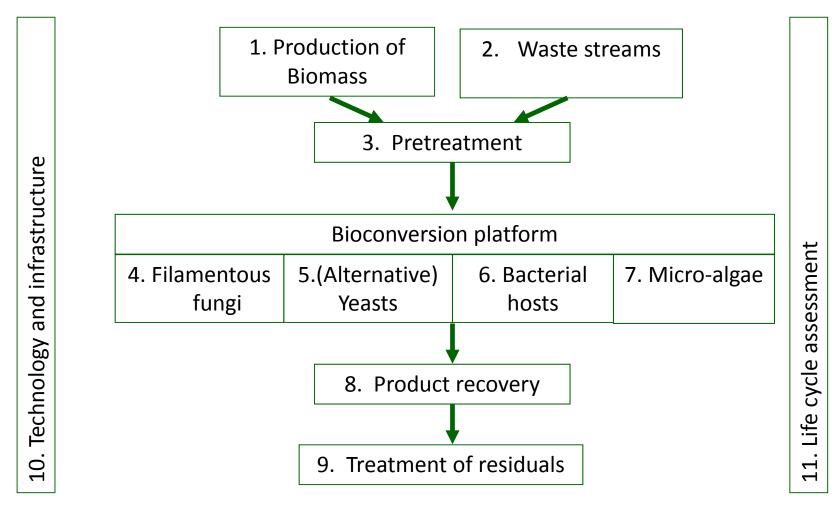








# Biorefinery concept



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Green chemicals

1,3 – propanediol, produced by *Clostridia* (A&A Biotechnology, Poland)

Alcohols

Ethanol and butanol produced by yeasts and anaerobic bacteria (BioGasol and DTU, Denmark)

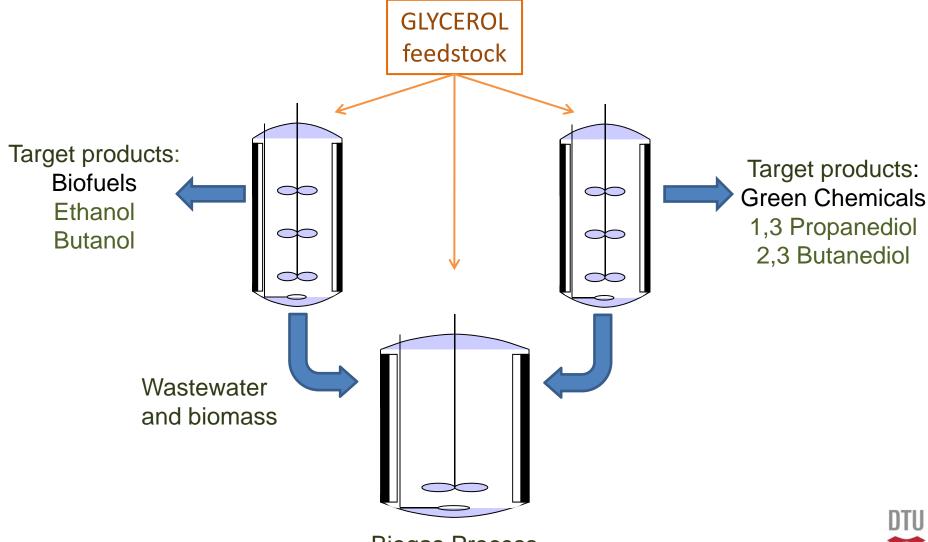
Biogas
 Biomethane (DTU/SLU)

Process choice and design guided by Life Cycle Assessment (economic, environmental and technical (IFEU, Germany) Product recovery solutions (ProChimia Surfaces, Poland)



## Target products





**Biogas Process** Target product: Biomethane



#### Production of biofuels

 Ethanol production in the non-conventional yeast Pachysolen tannophilus (DTU)

Butanol production by Clostridia (BioGasol)









#### The Company

- Developes technologies for cellulosic ethanol production
  - High performance continuous pretreatment process & equipment
  - C5-sugar fermentation with high efficiency
  - Integrated process concept
- Unique combination of skills: combined biotechnology & engineering
  - 30 employes
  - 10 patent families



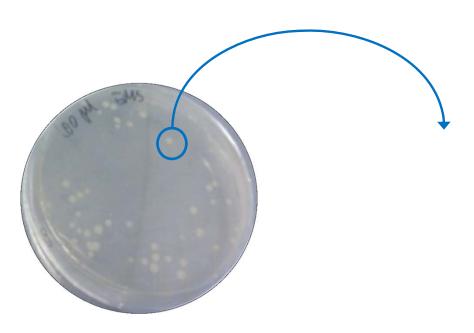
### Comparison of biofuels

Name	Chemical formula	Chemical weight [g/mol]	Boiling point [°C]	Air-fuel ratio [kg/kg]	Energy density [MJ/I]	Specific energy [MJ/kg air]	Enthalpy of vaporization	Solubility % w/w at 20°C	RON (octane number)
Methanol	СН₃ОН	32.04	65	6.5	16	3.1	1.2	Completely	104
Ethanol	CH <sub>3</sub> CH <sub>2</sub> (OH)	46.1	78	9.0	19.6	3.0	0.92	Completely	129
Butanol	C <sub>2</sub> H <sub>5</sub> CH <sub>2</sub> CH <sub>2</sub> (OH)	74.12	117	11.2	29.2	3.2	0.43	8	96
Gasoline				14.6	32	2.9	0.36	insoluble	91-99

(Lide 2005; Varde et al. 2007).







Mutant strain having increased tolerance towards the crude glycerol.

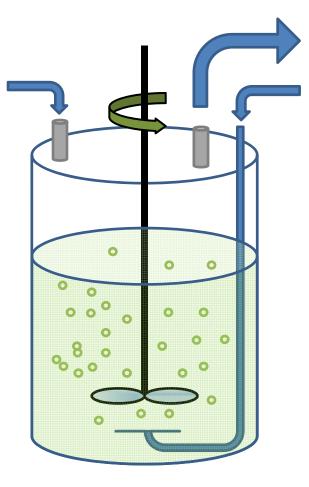
Producing almost equal amounts of 1,3-propanediol and butanol

# Two products are produced simultaneously



Butanol is removed *in situ* 1,3-Propanediol is accumulated in the fermentation broth

Fresh media with crude glycerol



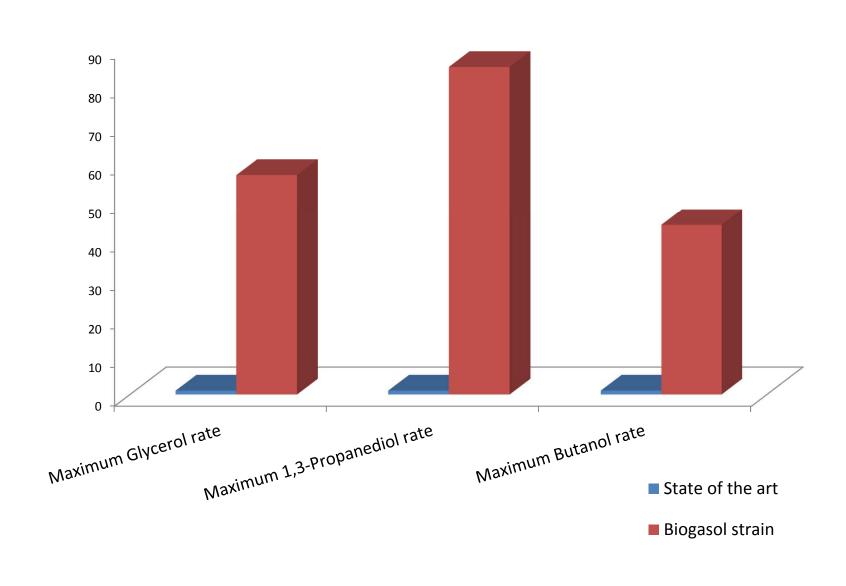
Butanol<sup>(gas)</sup>

Gas-flow

The less toxic 1,3propanediol is accumulated

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#### Relative increase in rates



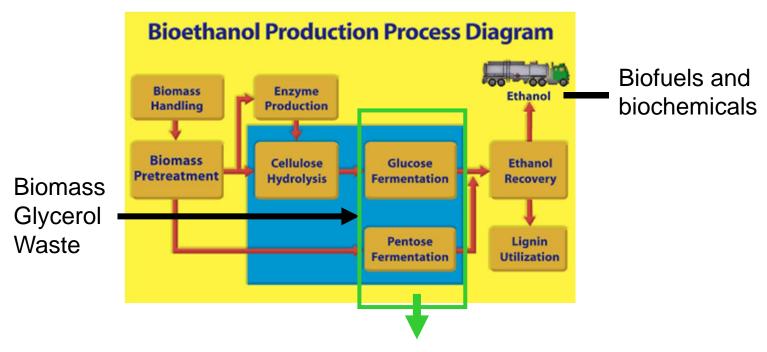




- Screening of potential cell factories for conversion of glycerol
- Quantitative microbial physiology
- Fermentation optimisation



#### Systems Biotechnology in Biorefineries Research



Development of microbial production platforms by:

- Strain evolution, selection and screening
- Optimise stress resistance
- Metabolic engineering and synthetic biology
- Modelling and Systems Biology
- Fermentation optimisation



#### Screening of non-conventional yeasts

#### Pacchysolen tannophilus

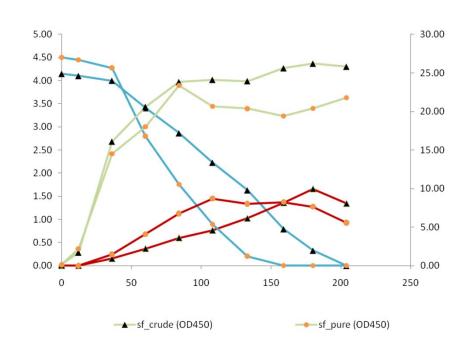
- Known producer of ethanol from D-xylose
- Previously shown to ferment glycerol (Maleszka, 1982)
- No growth anaerobically





## Supply of glycerol



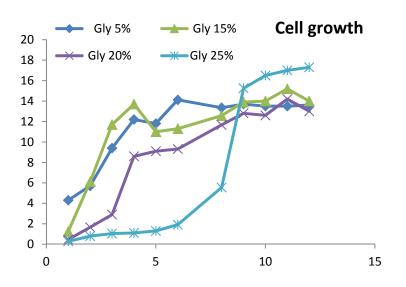


- Biodiesel industry typically uses variety of oil blends
- Meroco rape seed oil biodiesel
- Blends with used cooking oil
- *P . tannophilus* not affected by variations in glycerol batches from Meroco

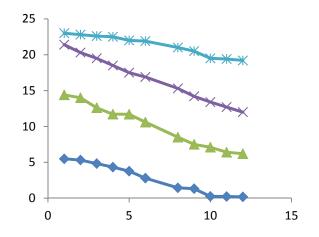




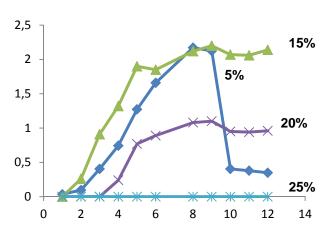
#### P. tannophilus growth on glycerol







#### **Ethanol production**

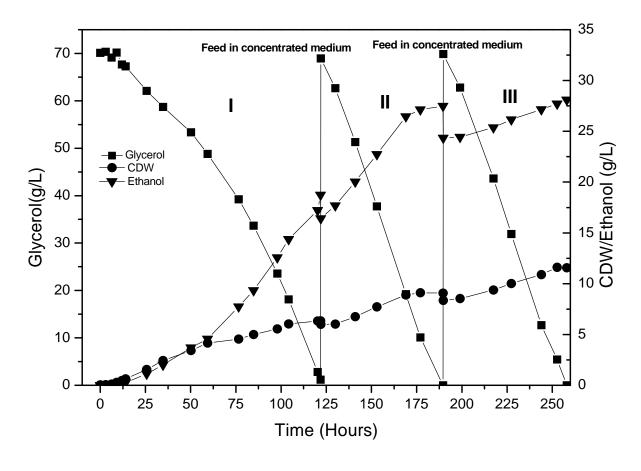


• *P. tannophilus* is capable of growing on 25% glycerol in shake flasks but no ethanol produced at this concentration.

Liu, Jensen and Workman (2011) Bioresource Technology. In press



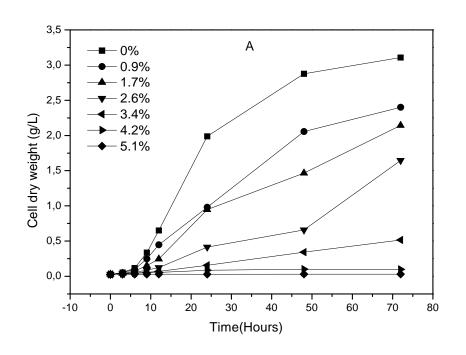




Highest ethanol production obtained for a yeast species growing on glycerol







Further steps to increase ethanol tolerance by adaptive evolution

Liu, Jensen and Workman (2011) Bioresource Technology. In press

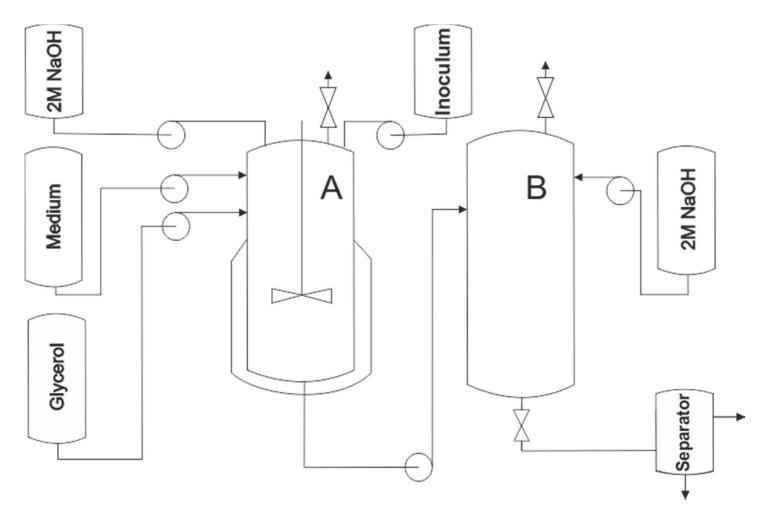




# Production of green chemicals



Proposed model of fermentation of crude glycerol and production of butanol and 1,3-PDO



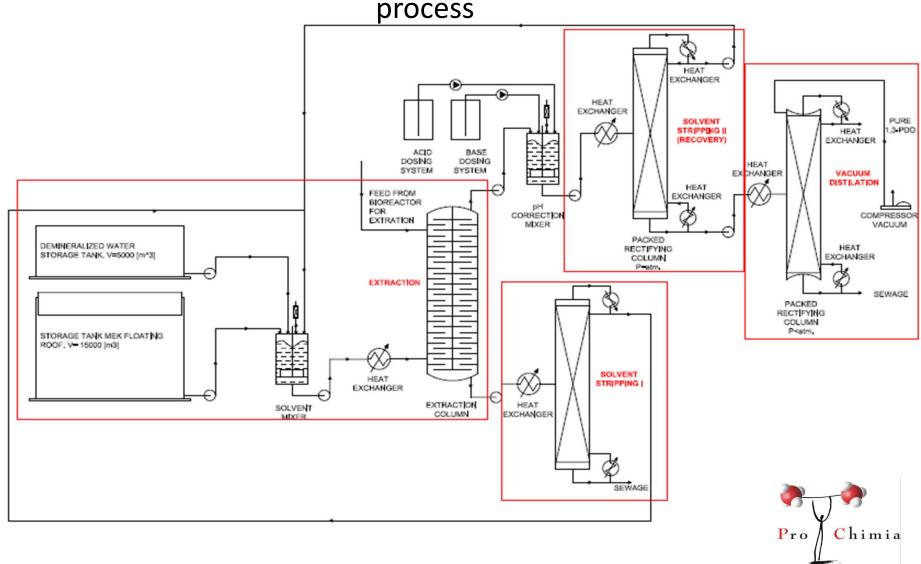
#### Results



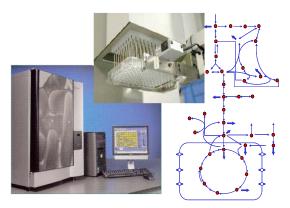
- The best 1,3-PDO productivity is maintaned at 15g/l of glycerol
- Application of 2-stage fermenter system allows for complete removal of glycerol, which is anecessary parameter for 1,3-PDO extraction procedure (Prochimia)
- The highest obtained concentration of 1,3-PDO was 30,2 g/l
- The total 1,3-PDO production efficiency was 0.56 g PDO/1g glycerol
- The glycerol feeding was 1,31 g/h/l

# Technological scheme of 1,3-PDO recovery system The equipment collection and set-up of 1,3-PDO extraction

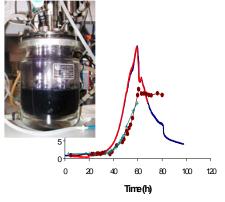




## Timeline to pilot scale GLYFINERY



Isolation, screening and improvement



Process development Product recovery





Process integration: pilot plant

March 2008 March 2010

March 2012

#### Acknowledgements

- Xiaoying Liu (PhD student)
- Philippe Holt (PhD student)









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# Further information Follow our progress at www.glyfinery.net



