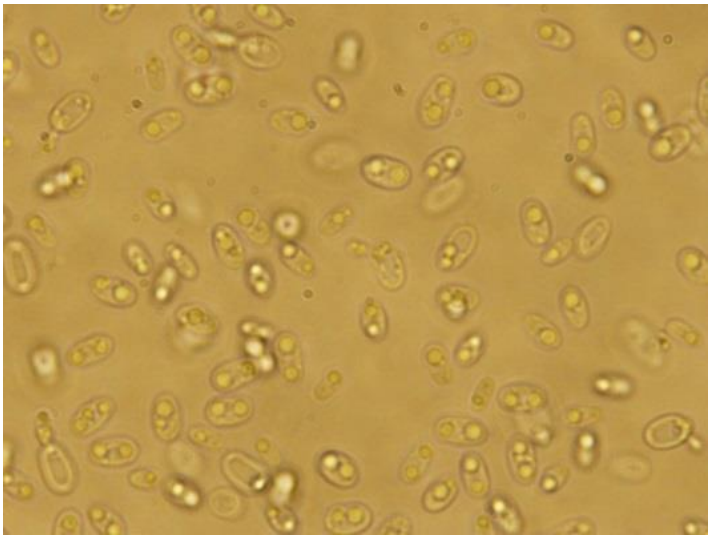

Enhanced microbial lipid production with genetically modified yeast and fungus

Pacific Rim Summit on Industrial Biotechnology and Bioenergy 2014

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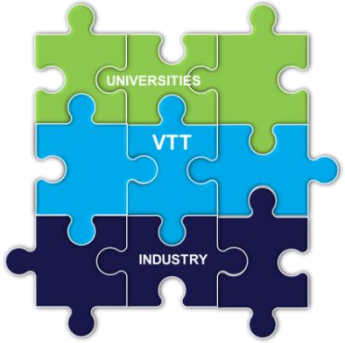
VTT Technical Research Centre of Finland

VTT Technical Research Centre of Finland

- VTT is the biggest multi-technological applied research organisation in Northern Europe
- Cross-disciplinary technological and business expertise
- A not-for-profit and impartial research centre

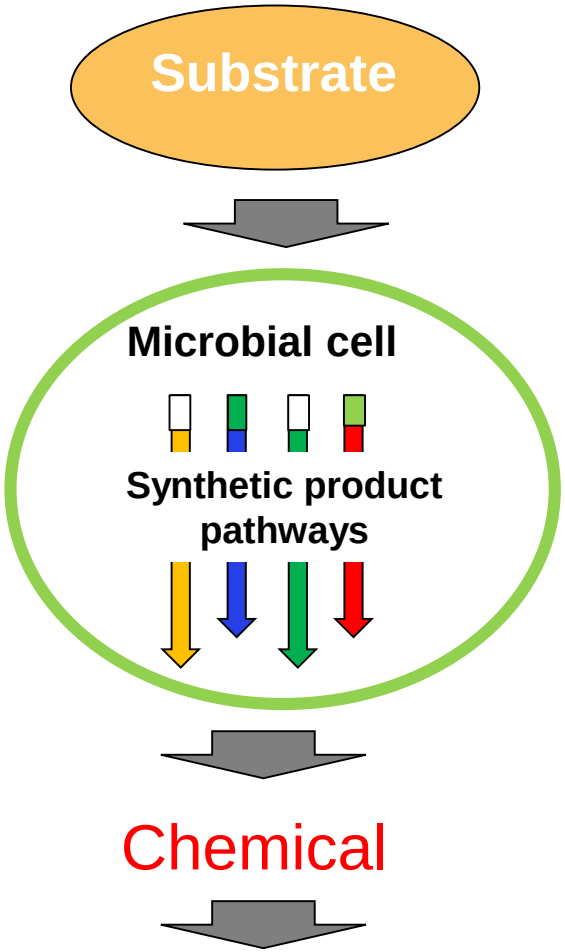


VTT offering covers everything from strain constructions to the end product testing

- Host selection
- Pathway evaluation with metabolic modelling and bioinformatics
- Enzyme discovery, characterization, engineering, synthetic enzymes
- Genetic engineering, synthetic biology
- Systems biology tools for identifying key bottle necks
- Analytics, automation and HTS
- Bioprocess development
- Application testing

Industrial Biotechnology at VTT

- Engineering of broad range of production organisms to the desired product, from PoC to industrial use
- Expertise in non-conventional production organisms: plant, algae, fungi, bacteria

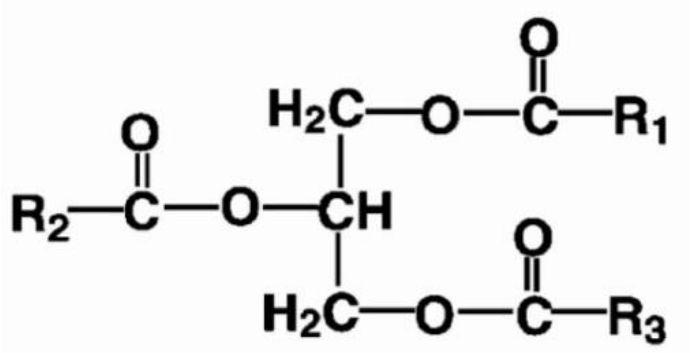


Product portfolio

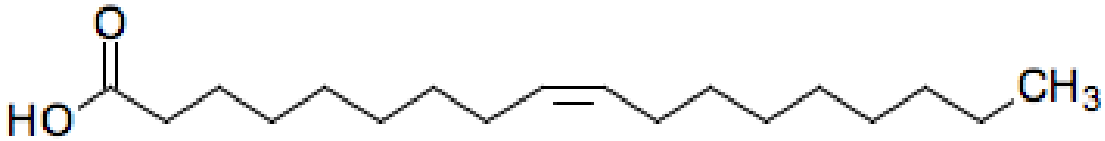
- Various alcohols
- Organic acids; lactic acid, xylonic acid, arabinoic acid, glycolic acid, galactaric acid, galactonic acid, etc.
- Terpenes, volatile hydrocarbons
- Triacylglycerides and other lipid derivatives

Triacylglycerol (TAG) is raw material for biodiesel and renewable diesel

- Vegetable (and other bio-based) oils are mainly triacylglycerols (Oil = lipid = TAG)
- Triacylglycerol (TAG) contains three fatty acid residues esterified with a glycerol molecule



R1, R2, R3 denote aliphatic fatty acid chain (saturated or unsaturated). Usually >15 carbons. E.g. oleic acid.



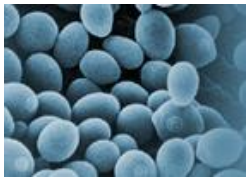
C00712

Oleic acid
(C18:1)

- TAG is used as raw material for biodiesel (transesterified lipids) and renewable diesel (hydrogenated alkanes)
- Composition of vegetable oils is similar to composition of microbial oils => microbial oils can replace vegetable oils

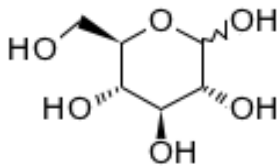
Microbial lipid production

- Some microbes (yeast, filamentous fungi, algae) can produce lipids over 20% of their biomass, i.e. they are **oleaginous** microbes
- The produced lipids are mainly triacylglycerols (TAGs)
- Lipid production requires a lot of carbon and cofactors (especially NADPH). At most **32 g of TAG** can be produced from **100 g glucose** (assuming that all glucose is used for TAG production). **In practise, the max yield has been approx. 22%.**

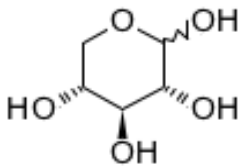
100 g glucose →  → 32 g TAG

Triacylglycerol biosynthesis

Glucose / xylose



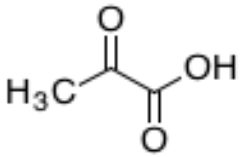
C00031



C00181

Glycolysis

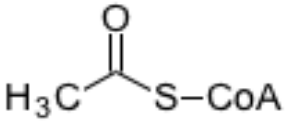
Pyruvate



C00022

Production of cytosolic acetyl-CoA

Acetyl-CoA

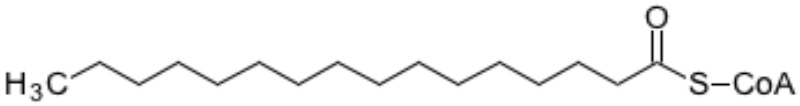


C00024

Synthesis of fatty acids

NADPH

Acyl-ACP/CoA



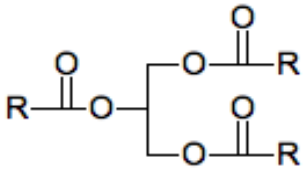
C00154

E.g. palmitoyl-CoA

Synthesis of triacylglycerol

Glycerol +
3x Acyl-CoA

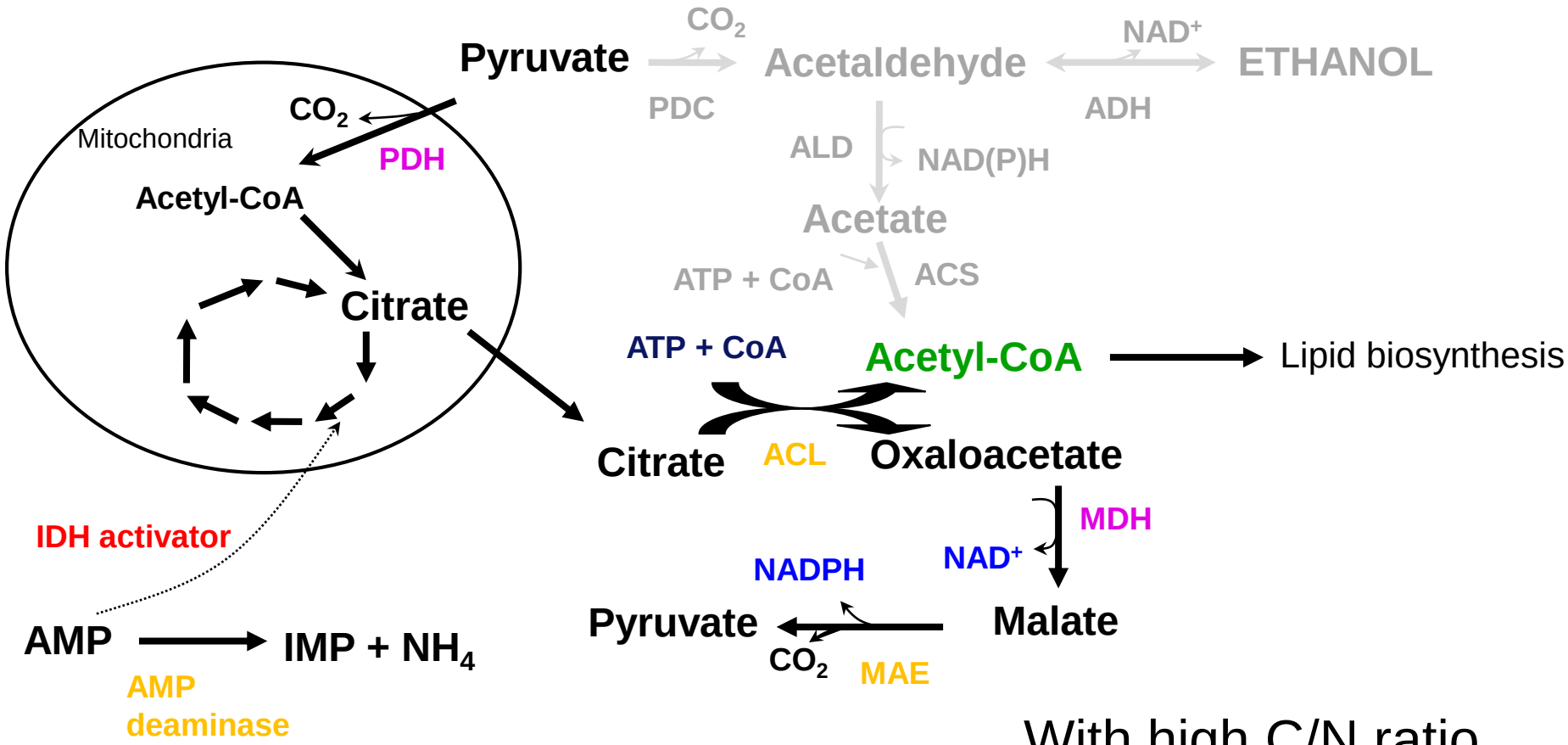
Triacylglycerol



C00422

Production of cytosolic acetyl-CoA differs in oleaginous and non-oleaginous microbes

Cytosolic acetyl-CoA is produced in oleaginous microbes via citrate and in non-oleaginous microbes via acetaldehyde

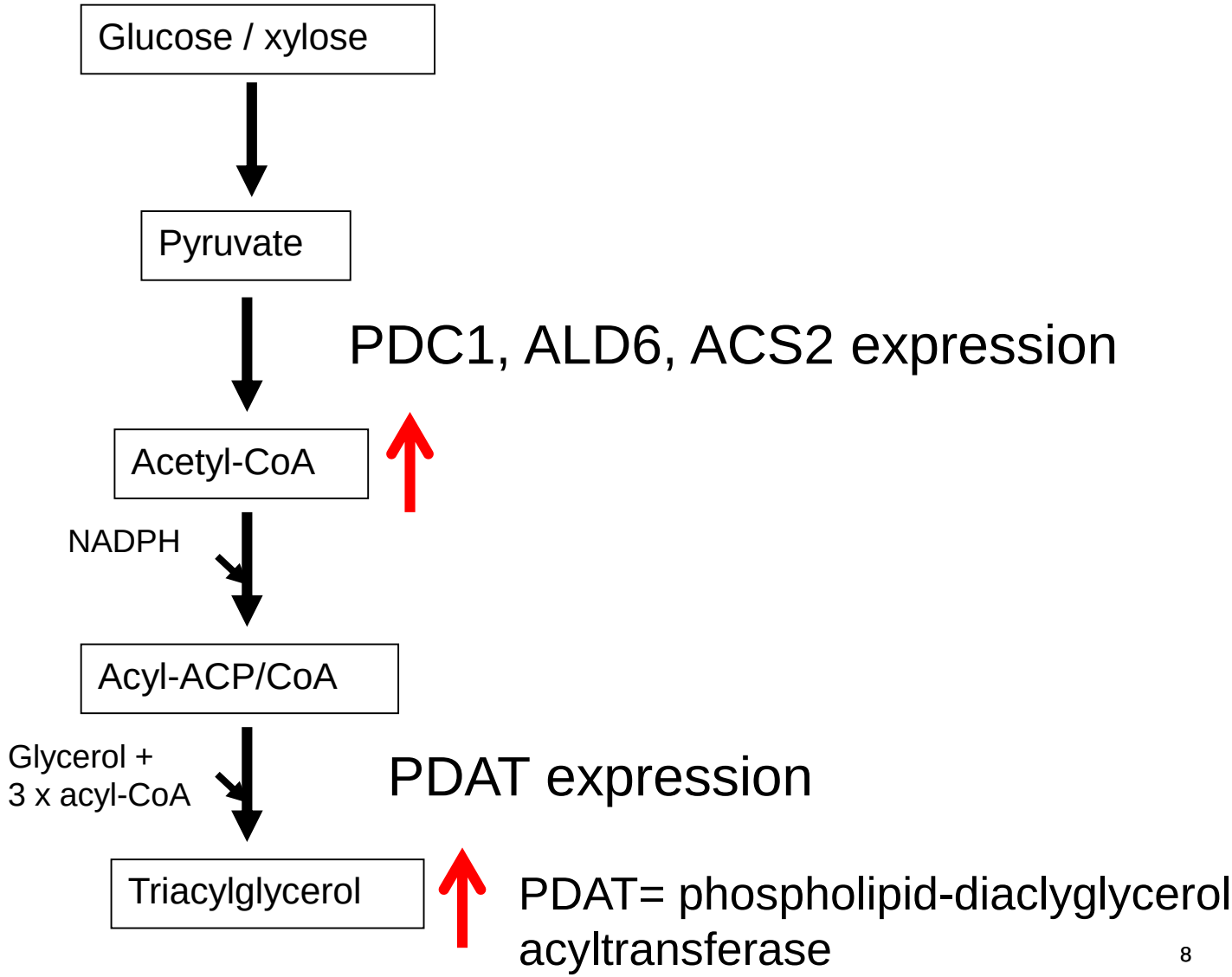


With high C/N ratio high lipid production

Under nitrogen limited conditions decrease of AMP results in decrease of IDH activity → citrate accumulates

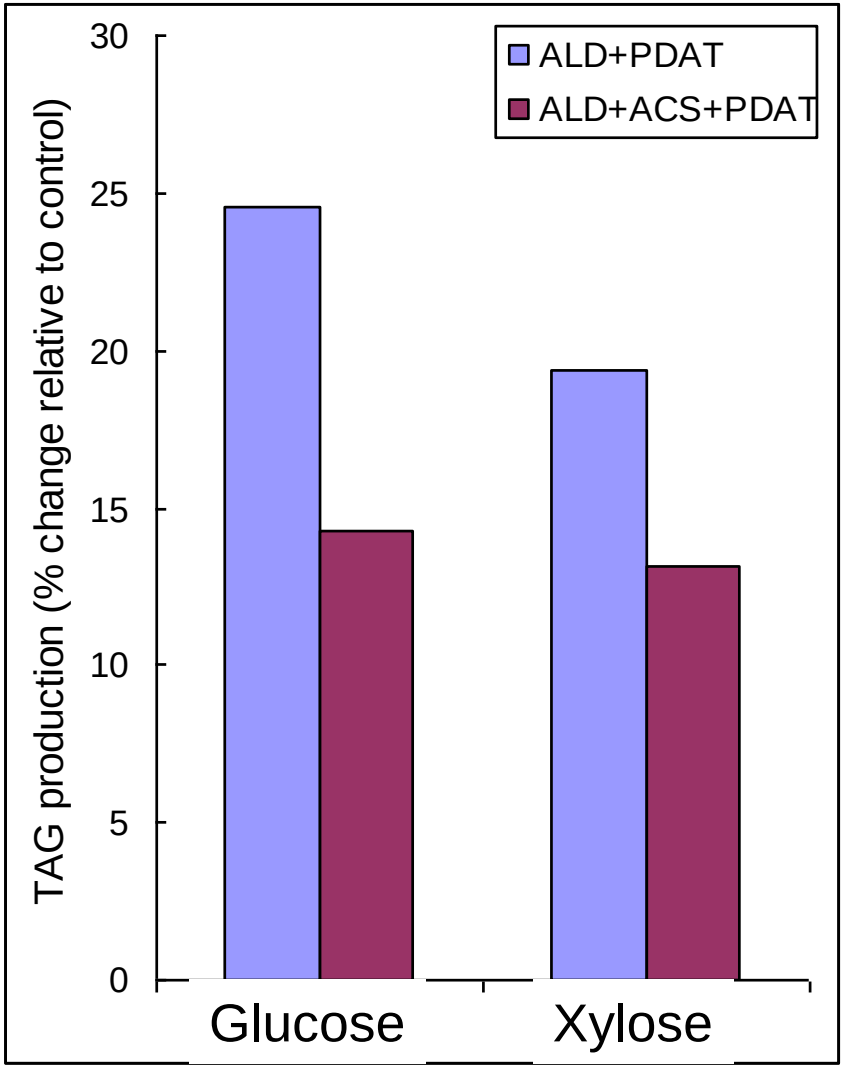
Engineering of microbial strains for oil production

Cryptococcus curvatus (yeast) and *Mucor circinelloides* (fungus) were genetically engineered by over-expressing four genes in different combinations. The codon optimized genes were expressed under endogenous promoters and terminators

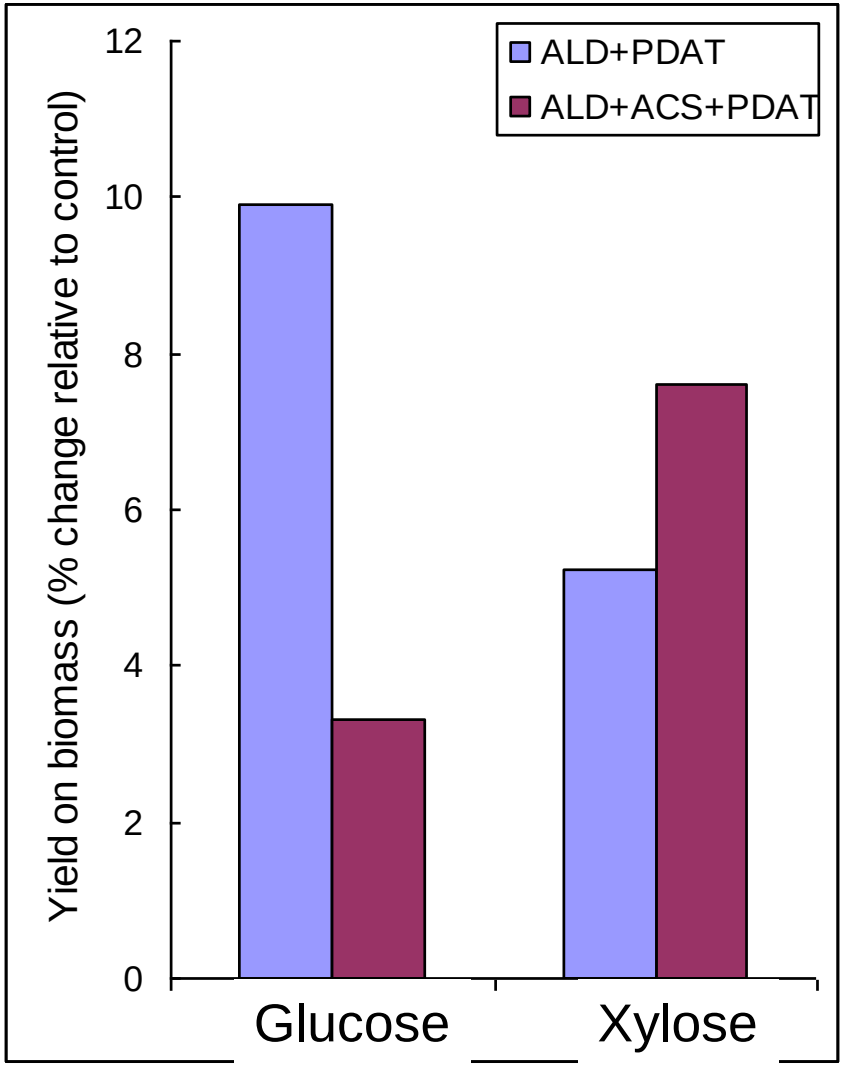


Bioreactor cultivations with *C. curvatus* transformants with C/N ratio of 80

TAG titre (max 21.8 g/l)

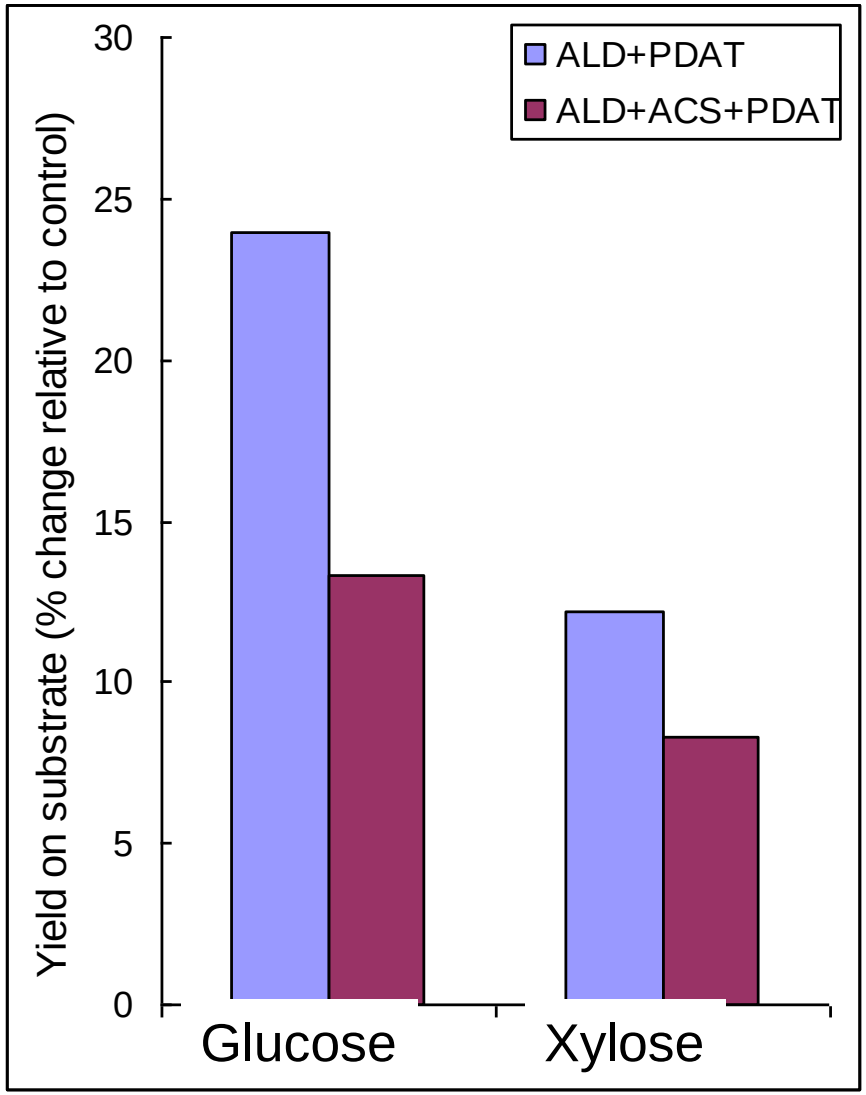


Yield on biomass (max 50.0%)

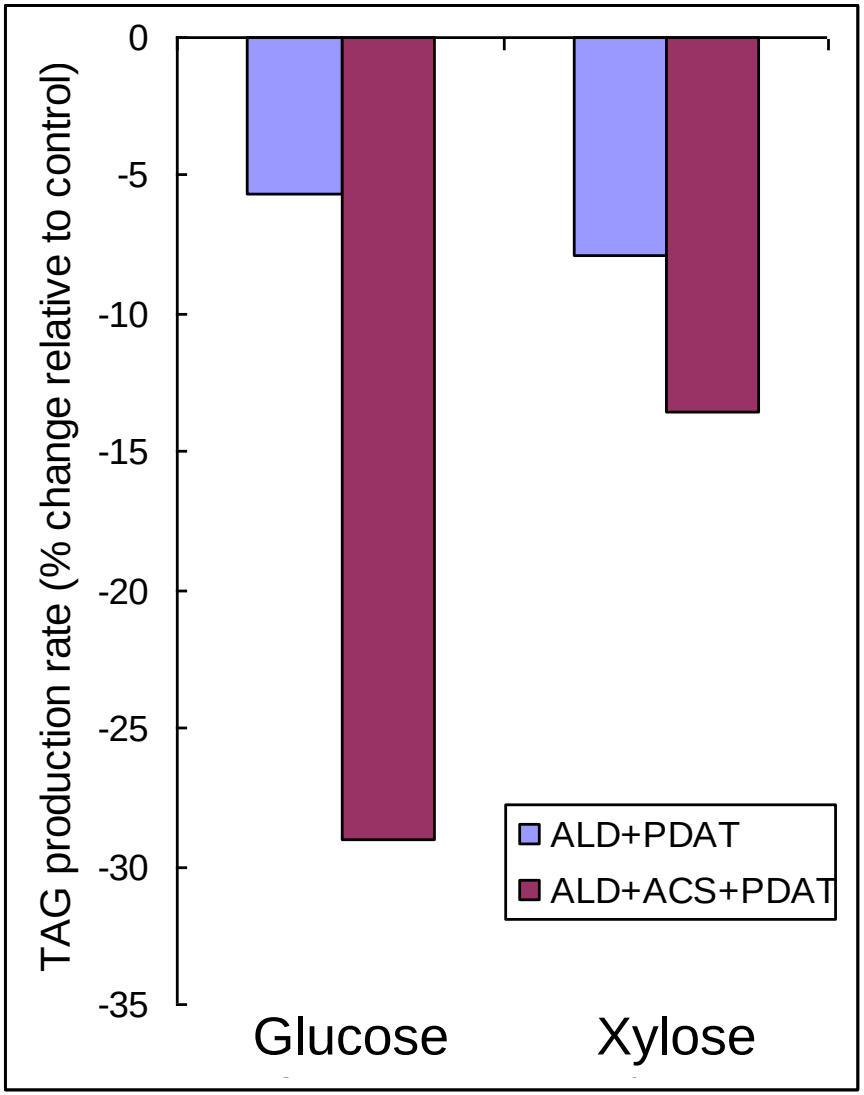


Bioreactor cultivations with *C. curvatus* transformants with C/N ratio of 80

Yield on substrate (max 23.3%)



Production rate (max 0.18 g/l/h)

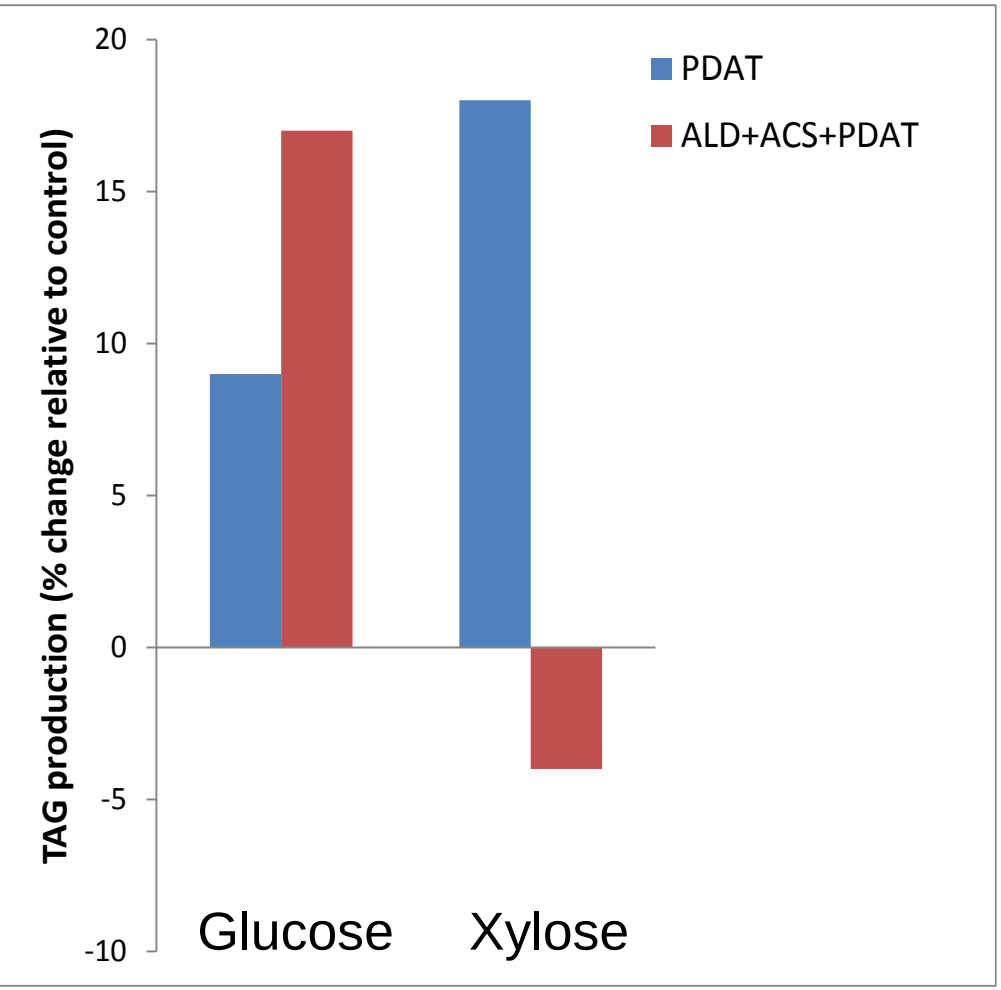


Bioreactor cultivations with *C. curvatus* transformants with C/N ratio of 80

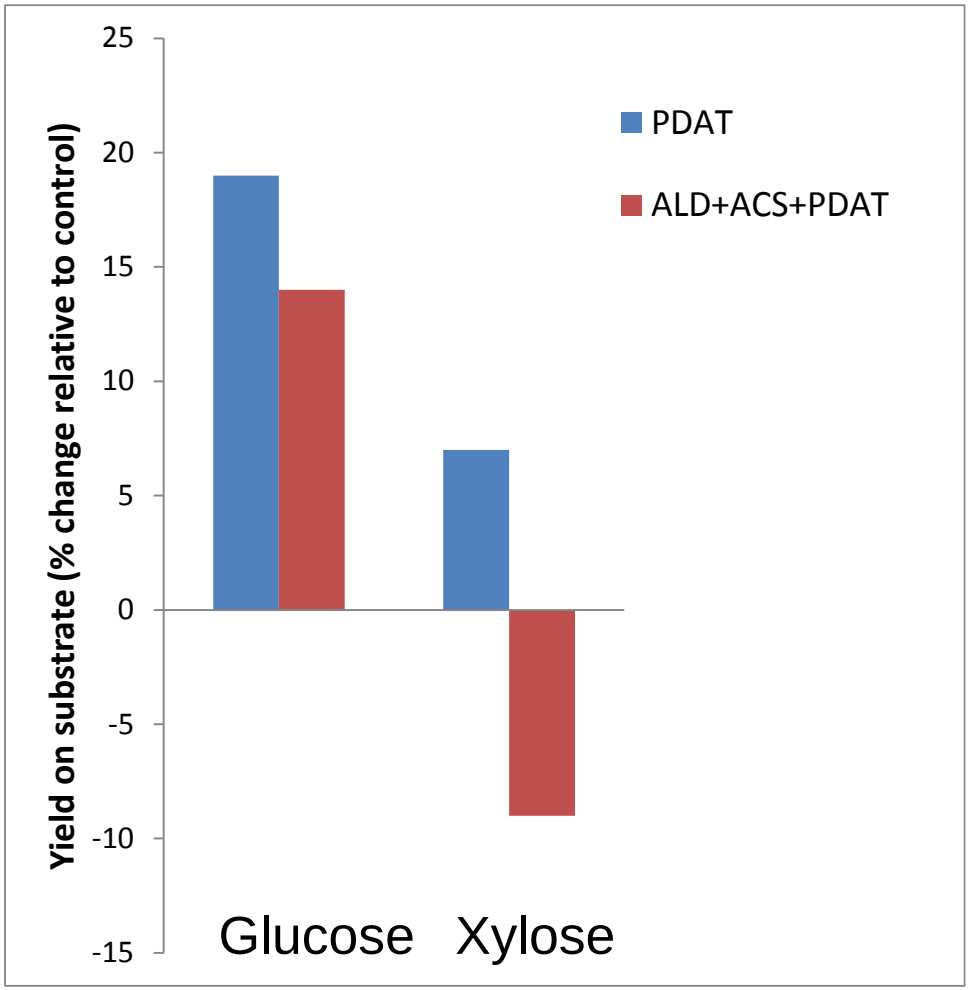
- The ALD+PDAT and the ALD+ACS+PDAT transformants produced more triacylglycerol than wild type *C. curvatus* from glucose or xylose.
- The yields of triacylglycerol on both biomass and substrate were higher in the transformants than in wild type *C. curvatus*, but the rate of triacylglycerol production was lower.
  - Titre and yield on substrate were improved by approximately 25% by addition of the *ALD6* and *PDAT* genes to *C. curvatus*
- The ALD+PDAT transformant was a better triacylglycerol producer than the ALD+ACS+PDAT transformant
- The improvements were generally larger on glucose than on xylose.

Bioreactor cultivations with *M. circinelloides* transformants with C/N ratio of 60

TAG titre (max 11.5 g/L)

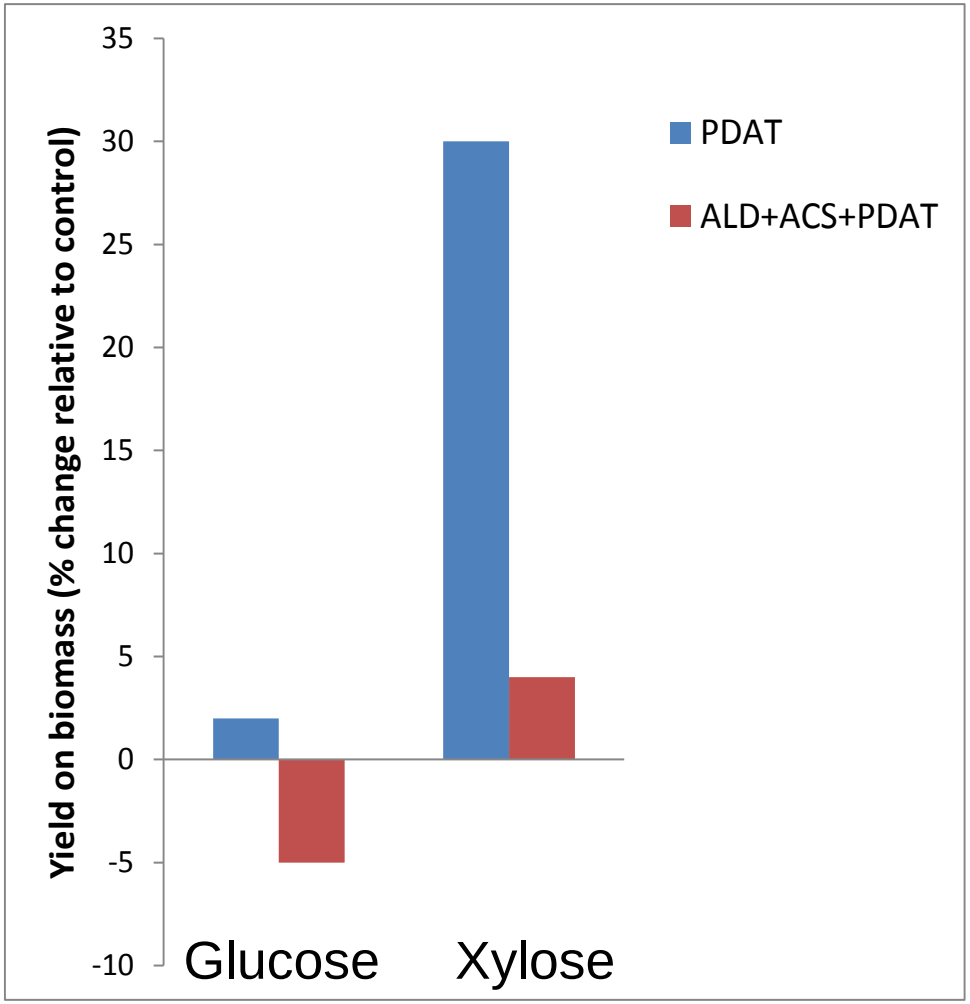


Yield on substrate (max 26.0%)

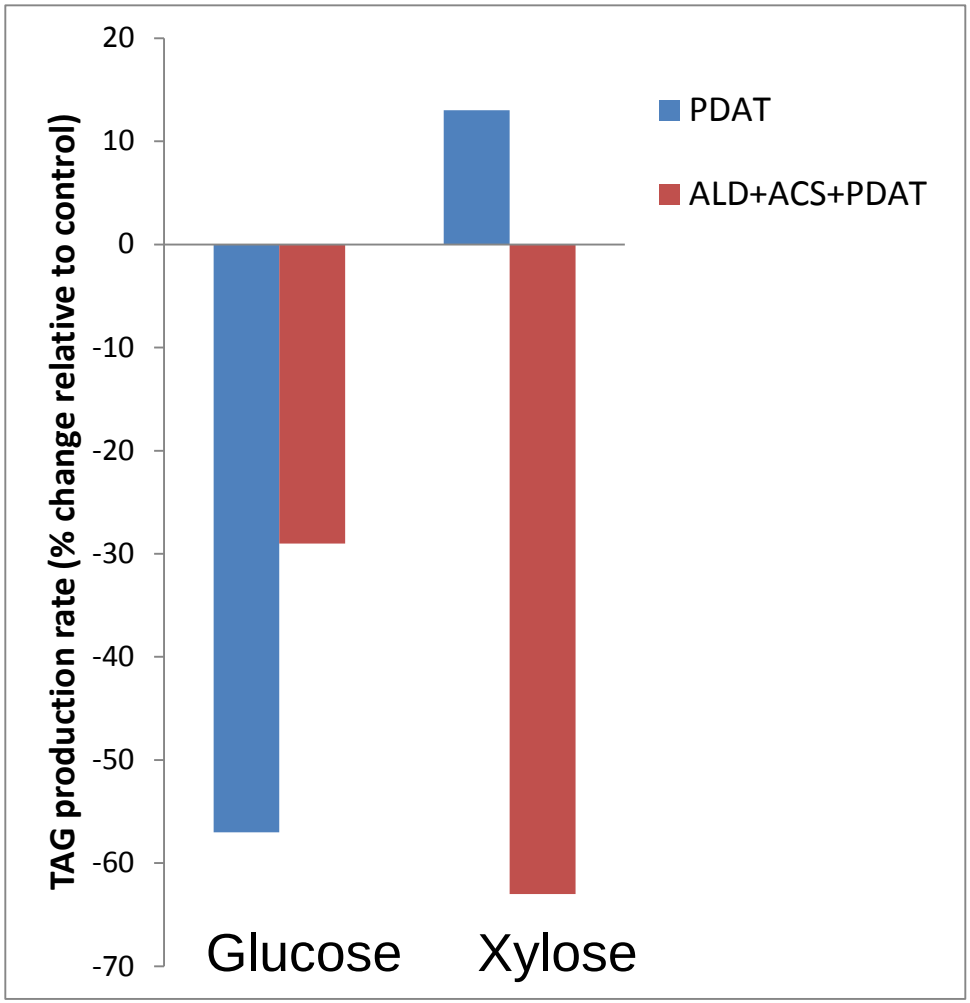


Bioreactor cultivations with *M. circinelloides* transformants with C/N ratio of 60

Yield on biomass (max 62.6%)



Production rate (max 0.10 g/l/h)

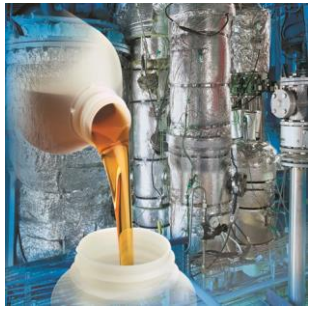


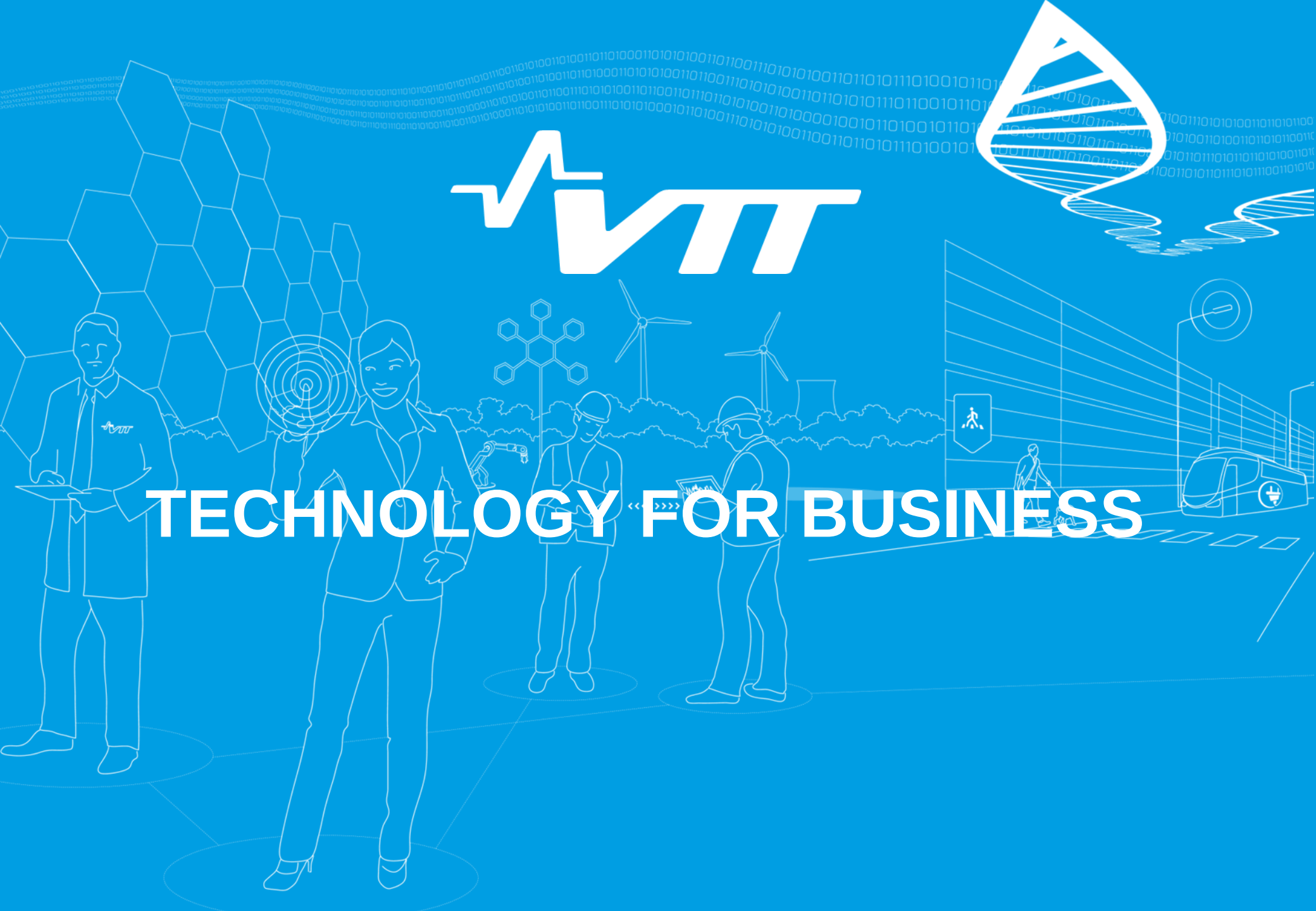
Bioreactor cultivations with *M. circinelloides* transformants with C/N ratio of 60

- The PDAT and the ALD+ACS+PDAT transformants generally produced more triacylglycerol, at higher yields, than wild type *M. circinelloides* from glucose.
- The PDAT transformant also produced more triacylglycerol, at higher yield, than wild type *M. circinelloides* from xylose
- The rate of triacylglycerol production was lower than wild type *M. circinelloides* on glucose, but improved on xylose with the PDAT transformant.

Conclusions

- Targeted genetic modifications to both *C. curvatus* and *M. circinelloides* can improve not only the triacylglycerol content of the cells and the titre of triacylglycerol produced, but also the yield of triacylglycerol from carbohydrate.
- Both *C. curvatus* and *M. circinelloides* use xylose as well as glucose as a substrate for triacylglycerol production
- Non-conventional, oleaginous yeast and fungi can be genetically engineered

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