



Isobutanol to SAF

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Isobutanol yeast development & lab-scale fermentation

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Pilot-scale isobutanol fermentation

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Catalytic conversion of isobutanol to SAF

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Drivers

General

Urgent need for SAF in view of the fight against climate change
Upcoming mandates for SAF in increasing number of countries

Specific for isobutanol

- Four carbon, branched molecule $\leftrightarrow$ ethanol
branched SAF polymers
- Most efficient alcohol to convert into SAF:
easy, cheap conversion
- Isobutanol $\rightarrow$ isobutene $\rightarrow$ isooctane (branched C8 alkane)

Barriers

Replacement of ethanol by isobutanol as fermentation product is cumbersome

- Isobutanol is a natural metabolite of yeast
- Isobutanol biosynthesis pathway is a minor metabolic pathway
- Engineering several overexpressed bacterial genes in yeast is essential
- Unexpected side-products are formed which needs additional genetic engineering
- Major challenge: toxicity of isobutanol >>> toxicity of ethanol (titer $\pm 1.5\%$ versus $\pm 18\%$)
- In-situ product removal is essential

Regulatory barrier in Europe: only 2G isobutanol can be used for SAF

Innovation potentials

Isobutanol production process

Yield

Maximum theoretical yield from glucose: 41% somewhat less than ethanol: 51%

Practical yield: $\pm 70\%$ of max. theoretical yield $\leftrightarrow$ ethanol: $\geq 90\%$

Volumetric productivity

Ethanol: 2.5 – 4.0 g/L.h

Isobutanol: 0.5 – 1.0 g/L.h depending on yeast pitching rate, still acceptable

Titer

Much lower than ethanol: $\pm 1.5\%$ versus $\pm 18\%$ (v/v) ***Main challenge***

Innovation potential

In-situ product removal

→ Isobutanol should be removed continuously from the fermentation medium during its production

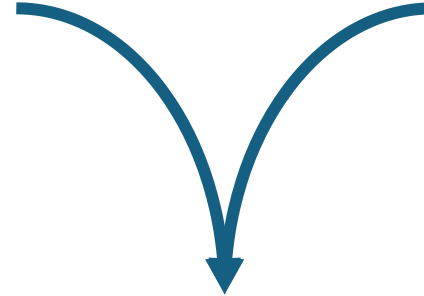
- GEVO: 'GIFT System' (15 years ago)
Distillation at reduced pressure and temperature
Too energy-intensive, not economically viable
- Currently: membrane pervaporation with lipophilic membranes, 20-25% isobutanol, followed by distillation or phase separation
- Membrane pervaporation: already used at commercial scale for separation of butanol and other lipophilic compounds
- Successfully used at pilot scale for isobutanol separation (BBEPP in BioRen project))
- Further developed in Icarus project at LNEG (Lisbon, Portugal)

2G isobutanol production



1G Isobutanol yeast

2G Ethanol yeast



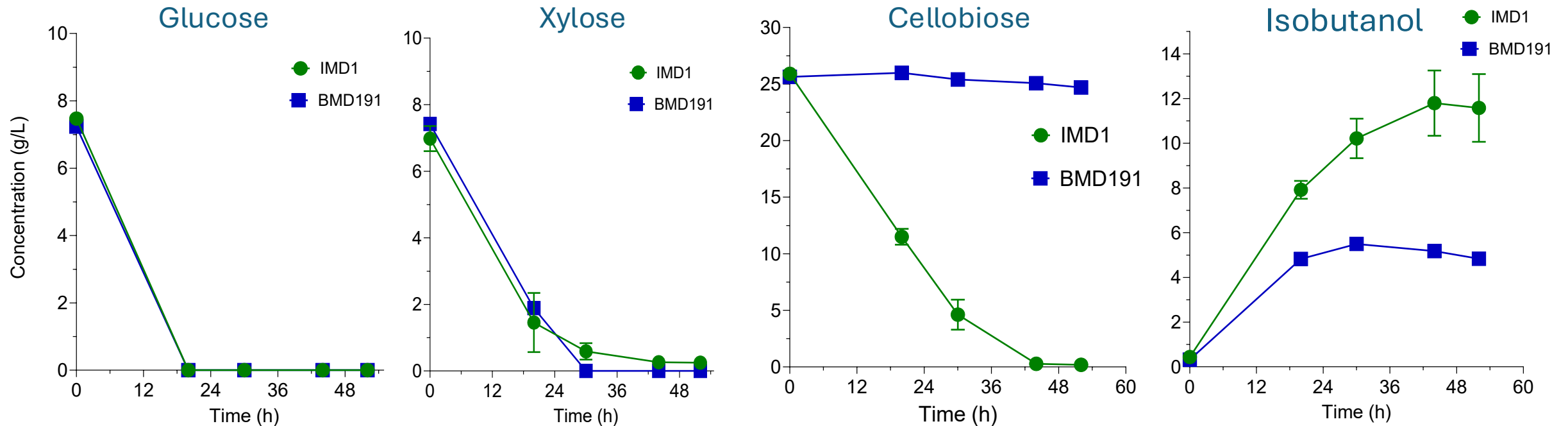
2G Isobutanol yeast
(xylose utilization, high inhibitor tolerance)



Cellulase-secreting 2G Isobutanol yeast

2G isobutanol production with mixture of glucose, xylose and cellobiose

2G Isobutanol yeast with cell-wall attached β -glucosidase for cell surface display (IMD1) versus Parent strain (BMD191)



→ 2G Isobutanol yeast with expression of beta-glucosidase, endoglucanase, cellobiohydrolase I and II available

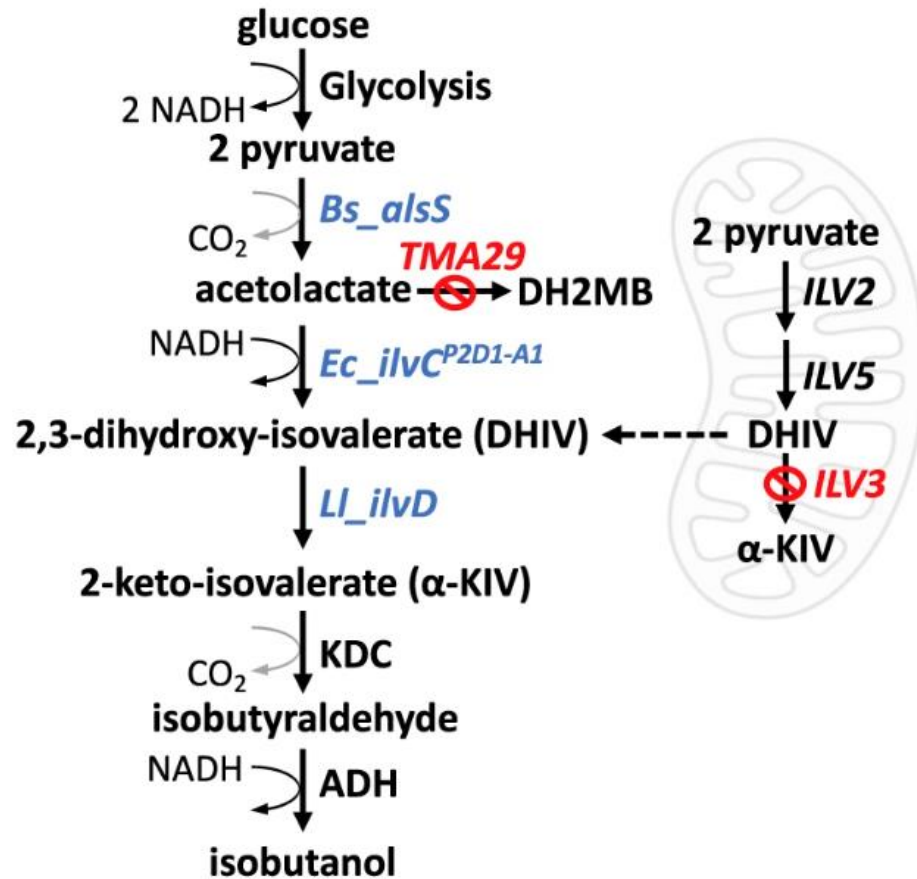
2G isobutanol production with lignocellulose hydrolysates

- Deletion of *TMA29* (*ORA1*), encoding acetolactate reductase that produces the by-product 2,3-dihydroxy-2-methyl butanoate (DH2MB)

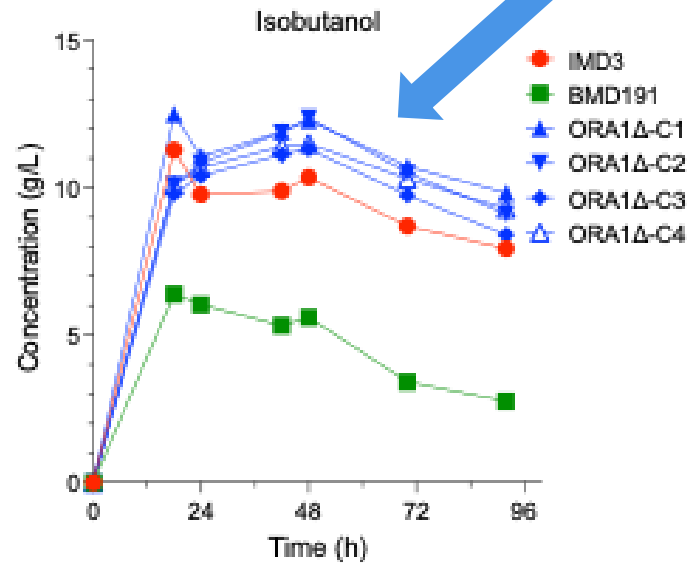
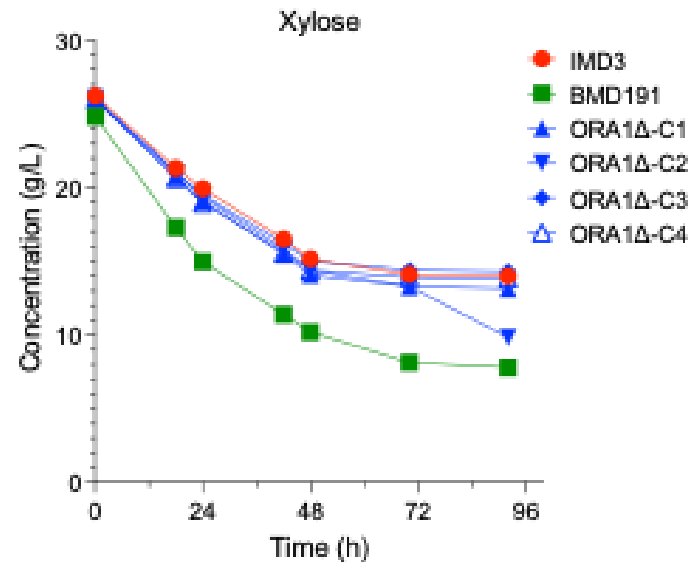
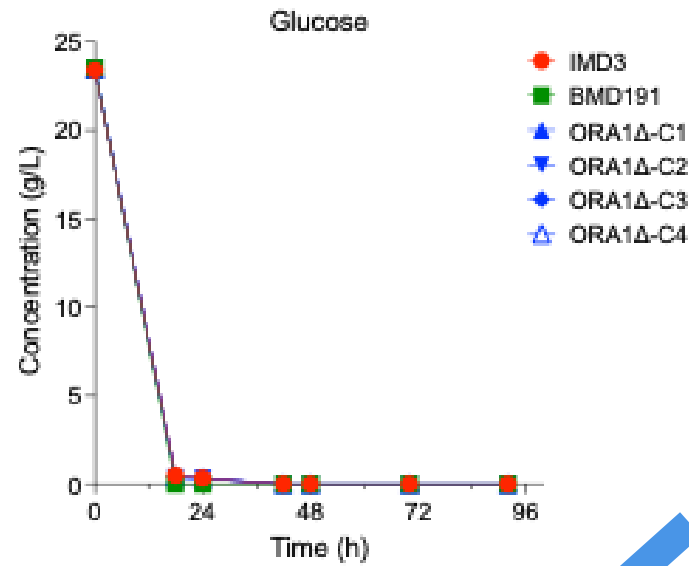
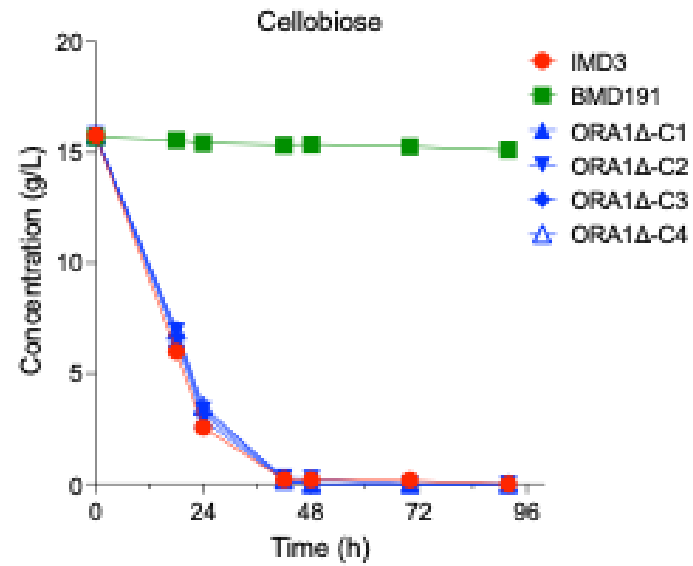
→ acidification of the medium

→ higher toxicity of isobutanol

*Ora1*Δ strain named IMD4



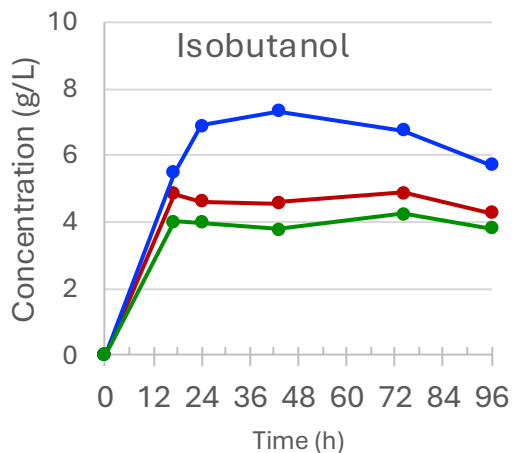
YPDXC: 3% glucose, 3% xylose, 2% cellobiose



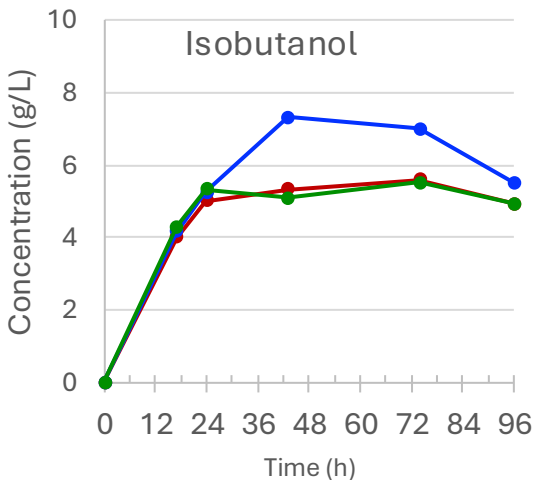
Higher isobutanol by *ORA1* deletion strains (about 13g/L)

2G isobutanol production with lignocellulose hydrolysate

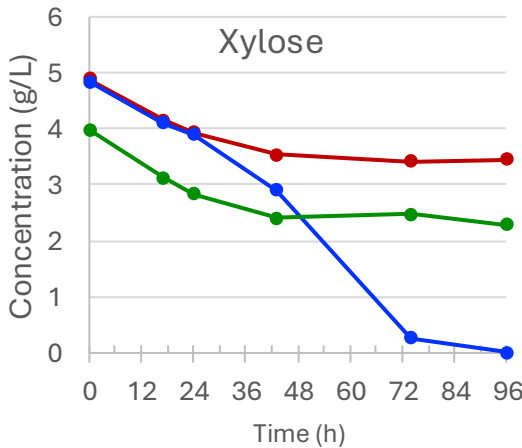
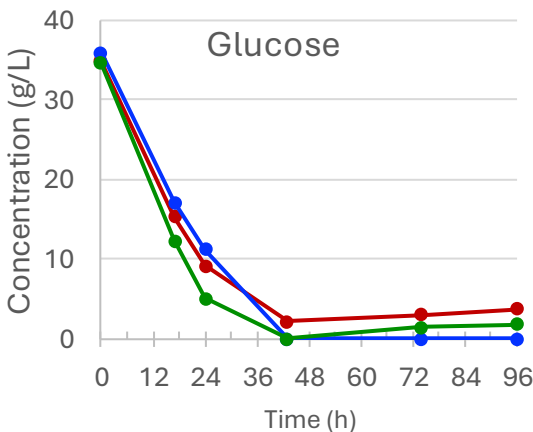
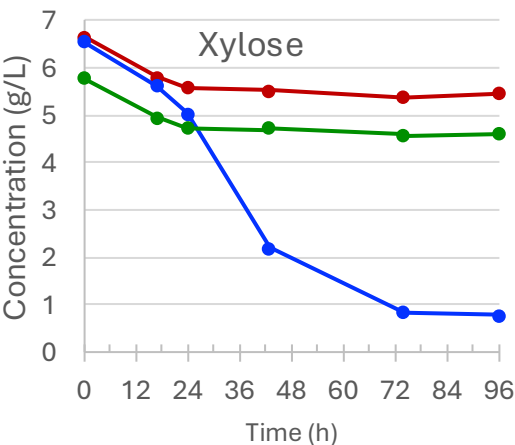
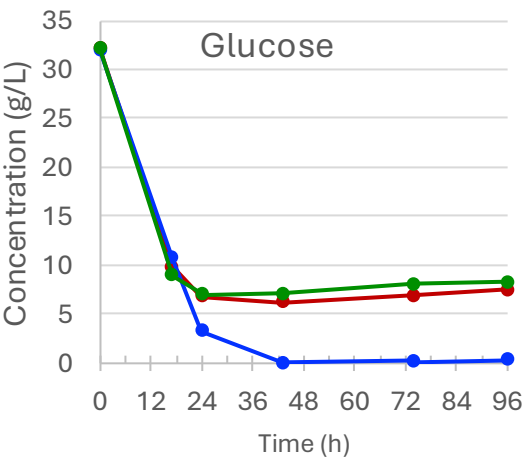
- Strain IMD4 (*ora1Δ*): very good improvement of isobutanol production



Sorghum Pulp



Extract of Sorghum Pulp



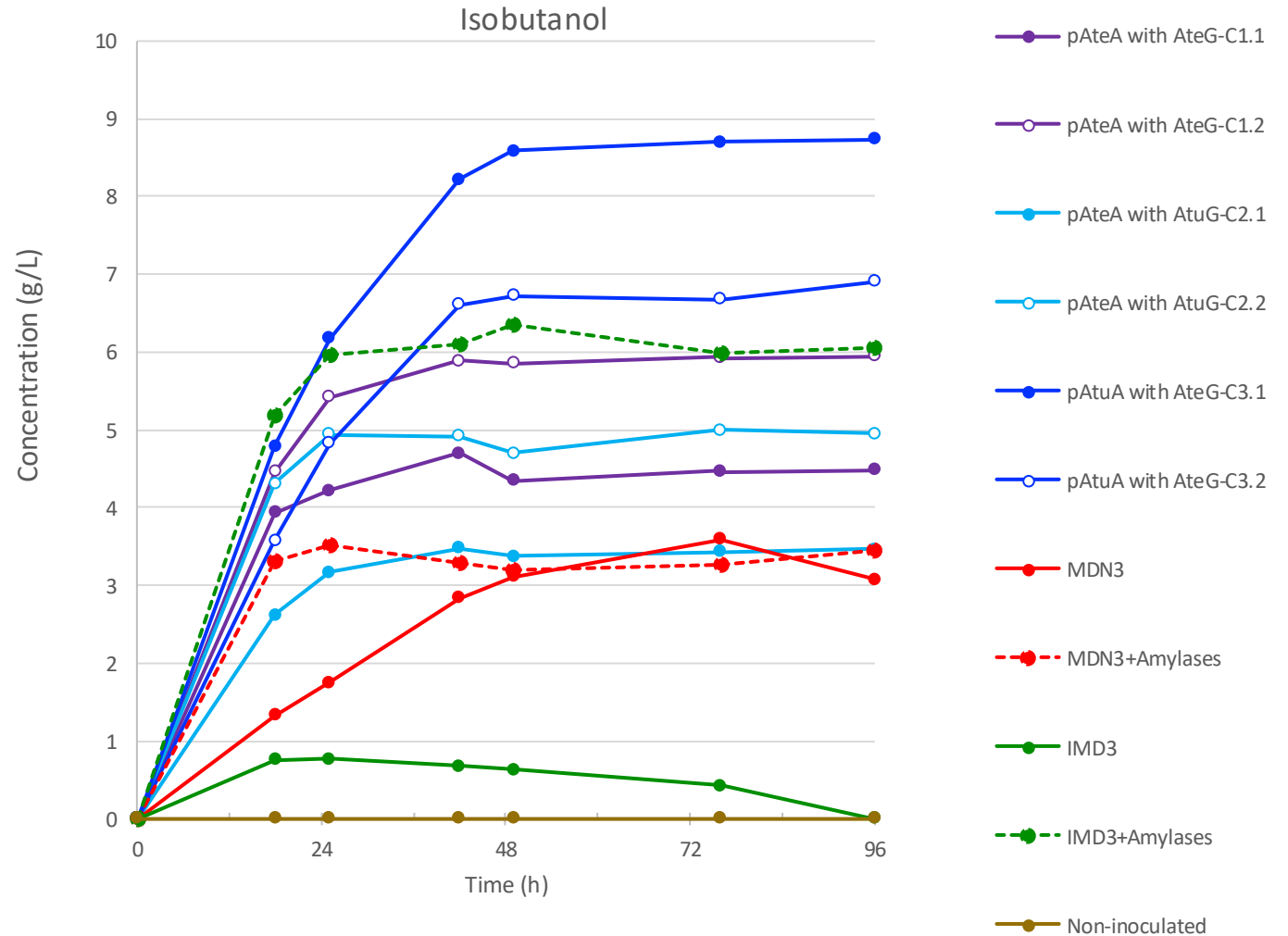
Isobutanol production from corn starch using strains expressing alpha-amylase and glucoamylase in cell surface display

Corn meal

Only hydrothermal treatment:
90°C for 20 min

No addition of commercial
alpha-amylase or glucoamylase

Similar titer as with
commercial alpha-amylase or
glucoamylase but slower rate



Pilot-scale isobutanol fermentation

Isobutanol production in bioreactor: up to 2% $\leftrightarrow$ shake flasks: 1.6%

First results on coupling of isobutanol fermentation with ISPR through membrane pervaporation

Catalytic conversion of isobutanol to SAF

Single catalyst: catalytic dehydration and oligomerization

Patent application submitted

Future plans beyond Icarus

- NovelYeast: new national project granted with VITO for 2G isobutanol production (waste paper/cardboard) with ISPR using membrane pervaporation → pilot scale
- NovelYeast, EHU, LNEG: new collaboration project submitted for isobutanol production and conversion to SAF → pilot scale/demo scale

Many thanks for your attention !



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