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PIMA
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Bioconversion of Lignocellulosic Biomass into Bacterial Bio-Oils

"Trees into Fat"

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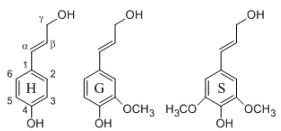
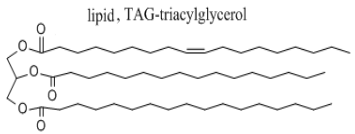
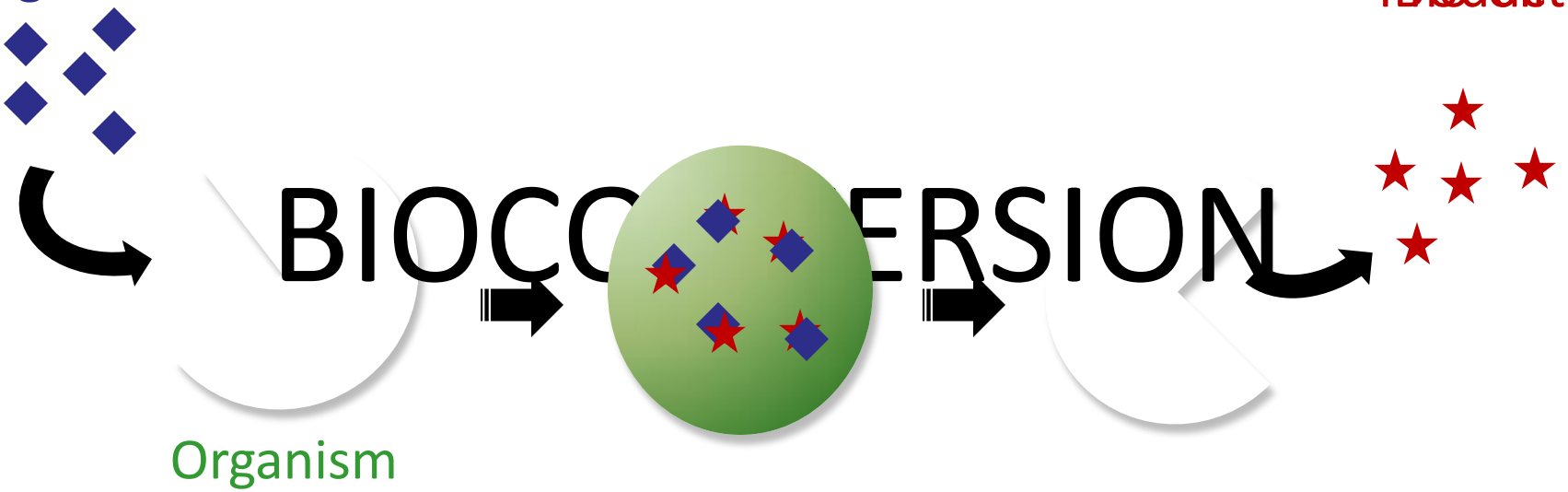


Substrate



Product



Using biological systems to convert abundant starting materials into more valuable compounds

Lignin → Recovered mills → **BIODIESEL**

Potential Domestic Supplemental Fuel Platform

Wells, T. and A.J. Ragauskas, *Biotechnological opportunities with the beta-ketoadipate pathway.* Trends in Biotechnology, 2012. **30**(12): p. 627-637

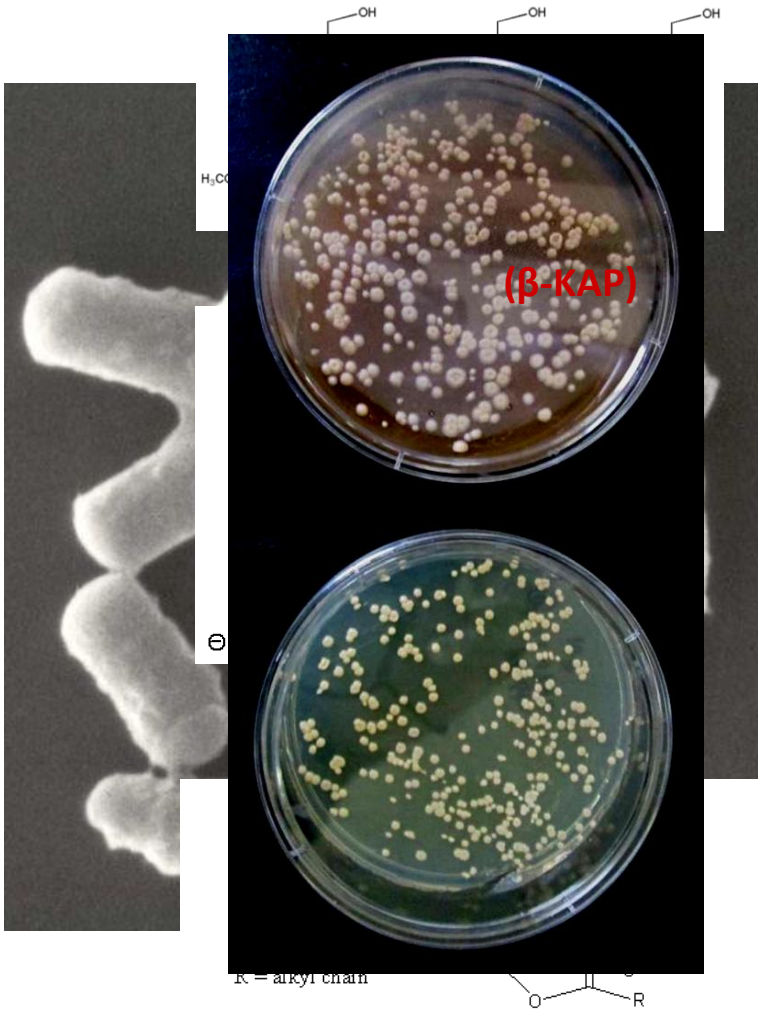
Fundamental Review of "Trees into Fat"

- Background of Bacterial Strains
  - *Rhodoccocus opacus*
- Experimental Work
  - Characterize How Cells Grow
- Results and Discussion
  - Model Compounds Based on Lignin Units
  - Kraft Lignin

Wells, T. and A.J. Ragauskas, *Biotechnological opportunities with the beta-ketoadipate pathway.* Trends in Biotechnology, 2012. **30**(12): p. 627-637

Rhodococcus opacus

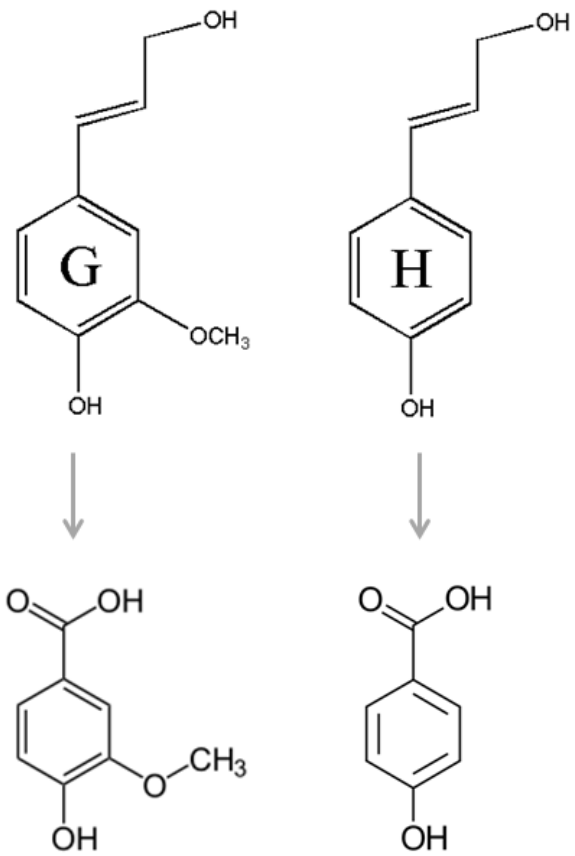
- Soil Bacteria
- Oleaginous
  - >20% of cell dry weight in oil
- Two Strains
  - DSM 1069
  - PD 630
- "High affinity" towards the digestion of lignocellulosic aromatics
  - β-ketoadipate pathway (β-KAP)



Wells, T. and A.J. Ragauskas, *Biotechnological opportunities with the beta-ketoadipate pathway.* Trends in Biotechnology, 2012. **30**(12): p. 627-637

Experimental

- Model Compounds
  - Vanillic acid (G-type)
  - *p*-Hydroxybenzoic acid (PHA/4-HBA) (H-type)

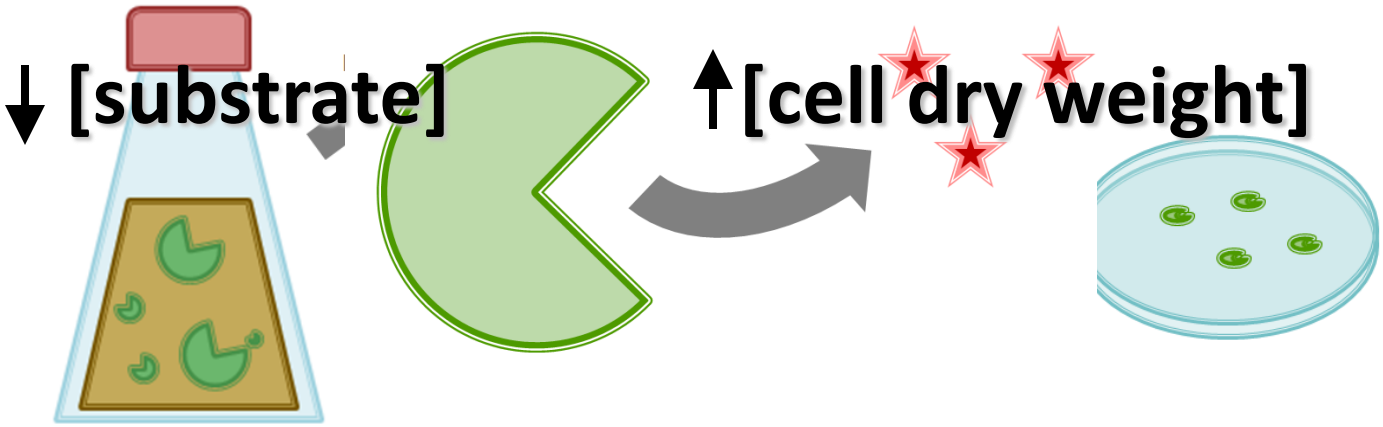


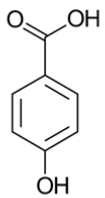
Vanillic acid

p-Hydroxybenzoic acid

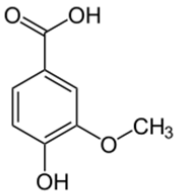
Fundamental Overview of Tracking Cell Growth

- Verifying that the cells are growing well on lignin as sole carbon source
  - Decreasing Concentration of the **Substrate**
  - Increasing Cell Dry Weight (**CDW**)
  - Track the number of "healthy cells" (aka Colony Forming Units, **CFU)**
  - Characterize the Generated Fats (Fatty Acid Methyl Esters, **FAME**)
    - Rupture and Transesterification → GC/MS

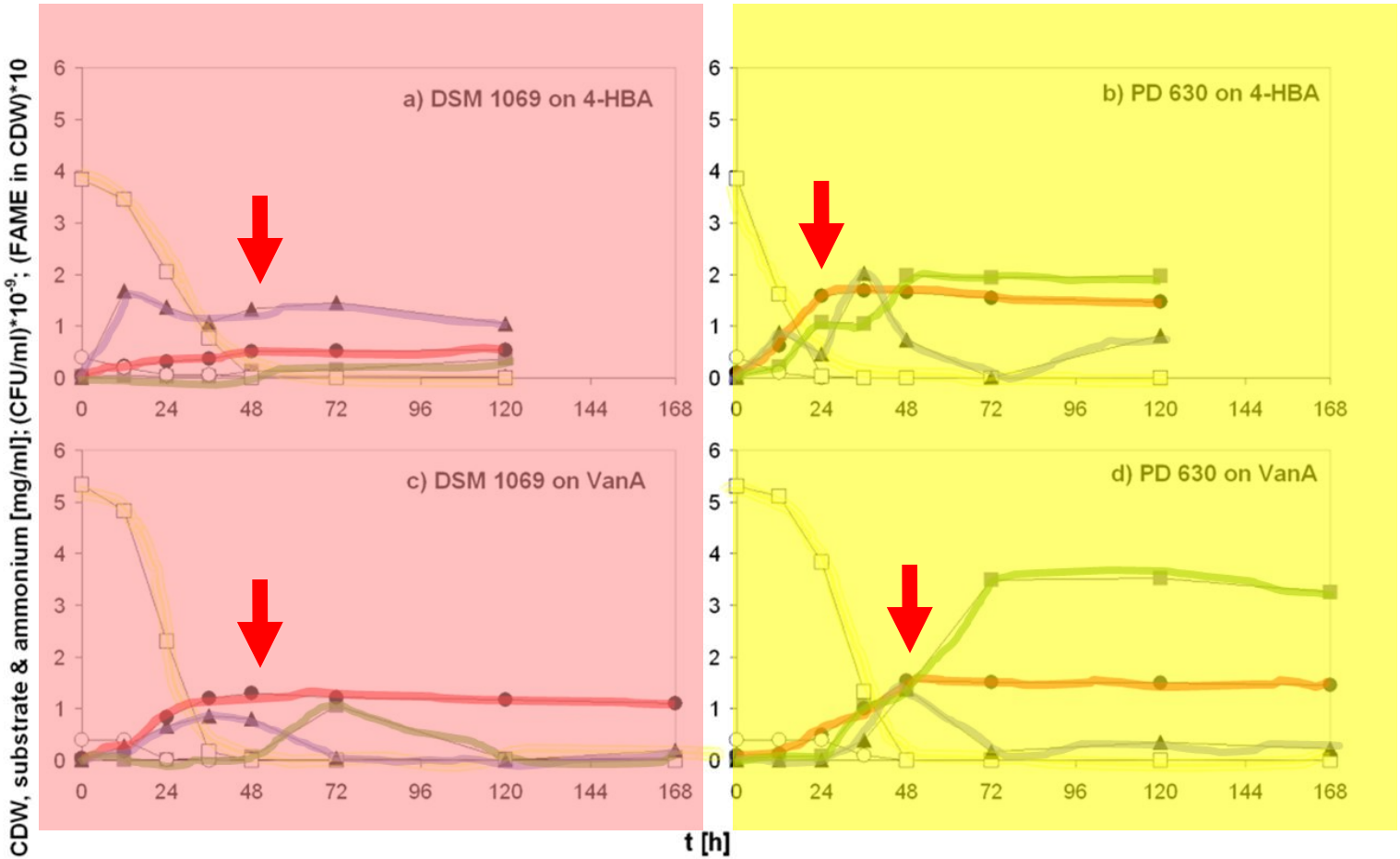




p-Hydroxybenzoic acid

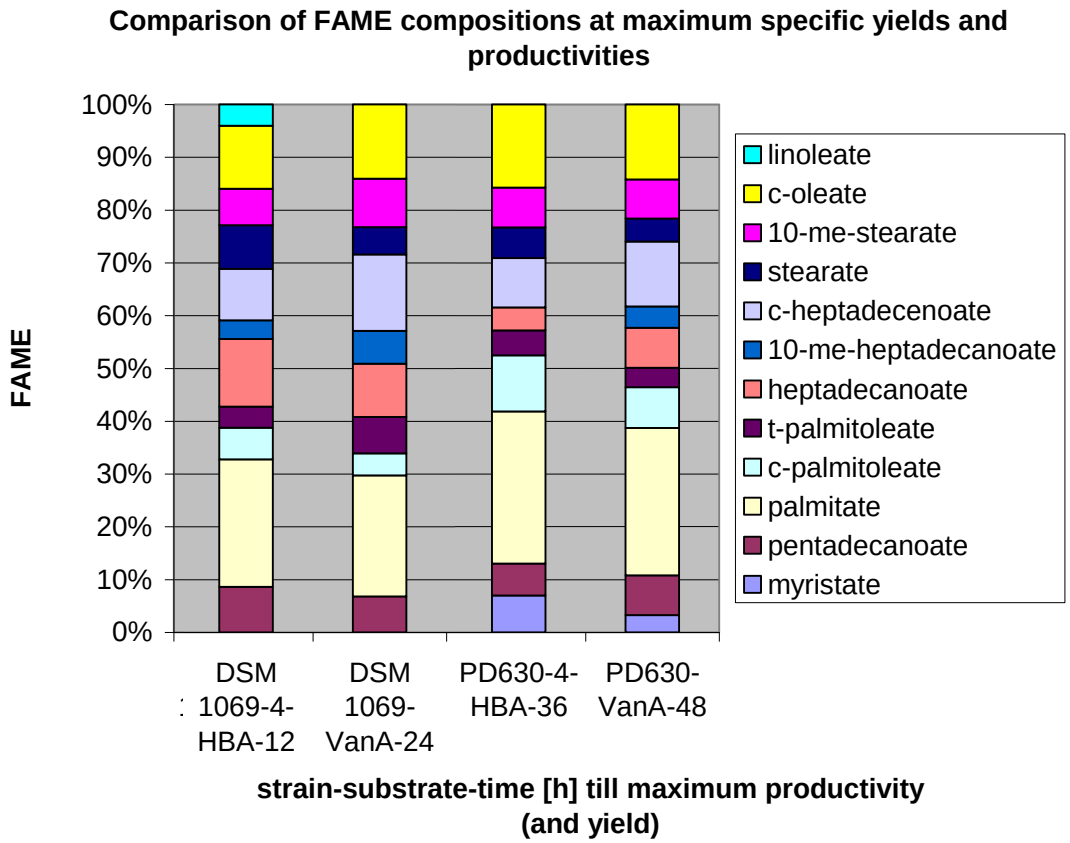


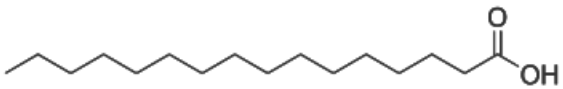
Vanillic acid



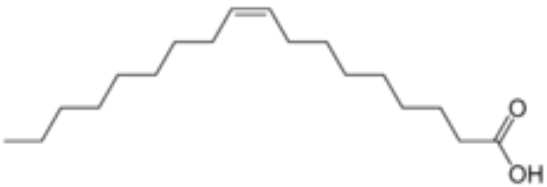
Kosa M, Ragauskas AJ (2011) Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol 29:53-61

Lipid Composition Within Bio-Oil

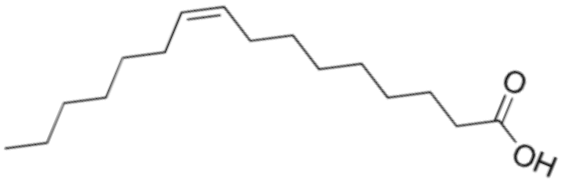




palmitic acid

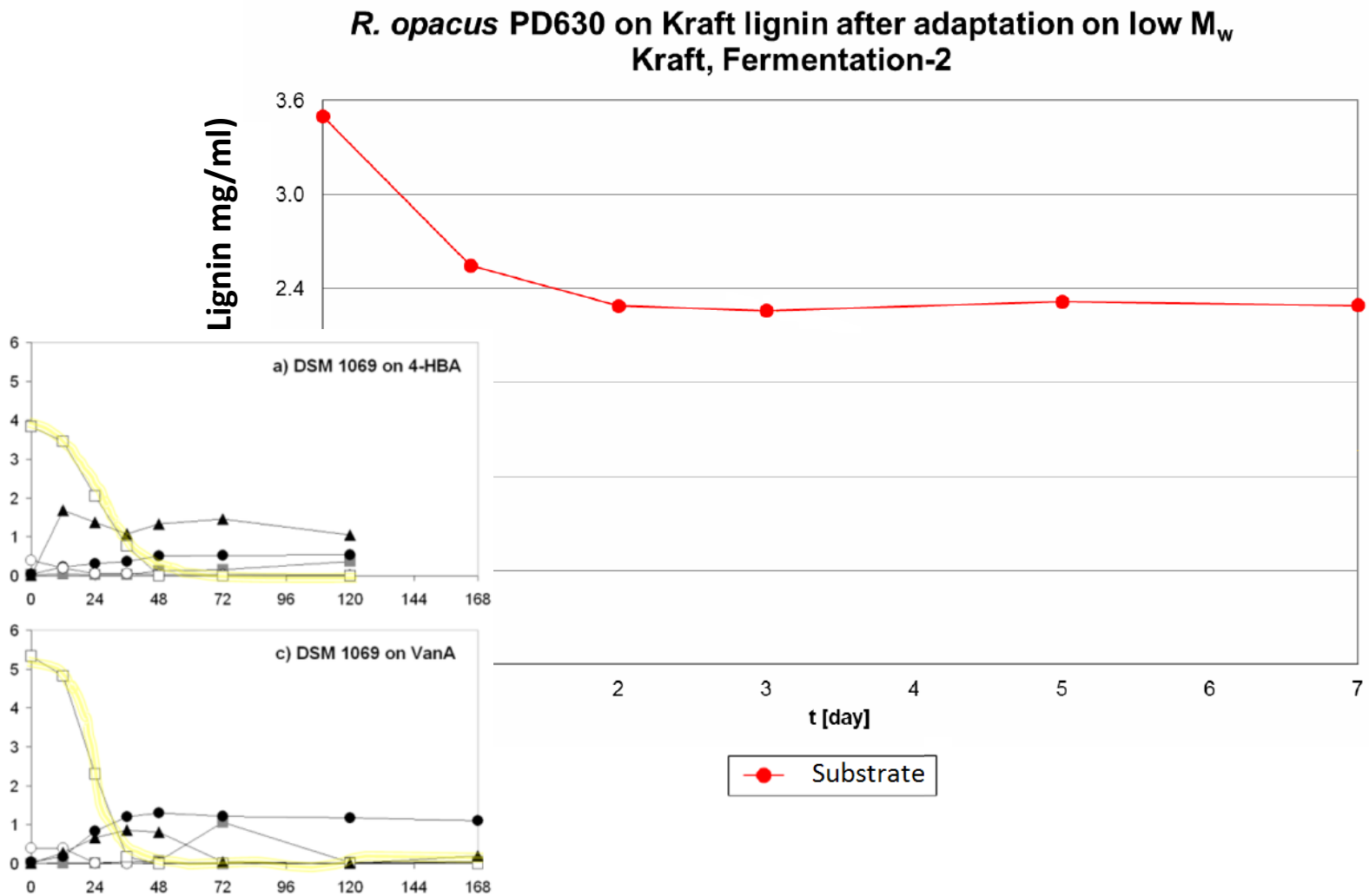


c-oleic acid



c-palmitoleate acid

Kosa M, Ragauskas AJ (2011) Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol 29:53-61



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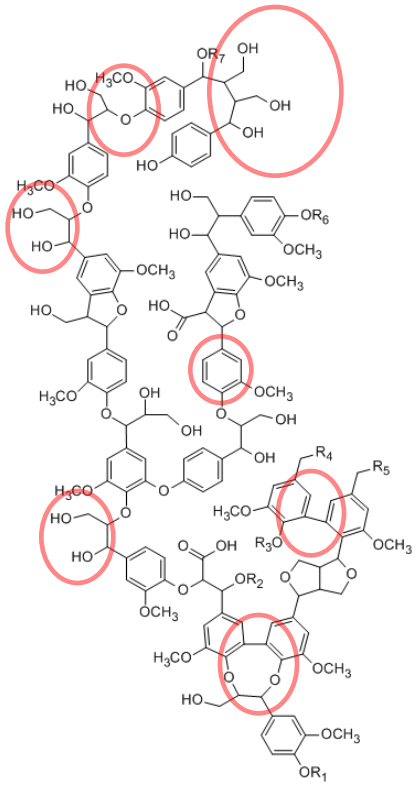

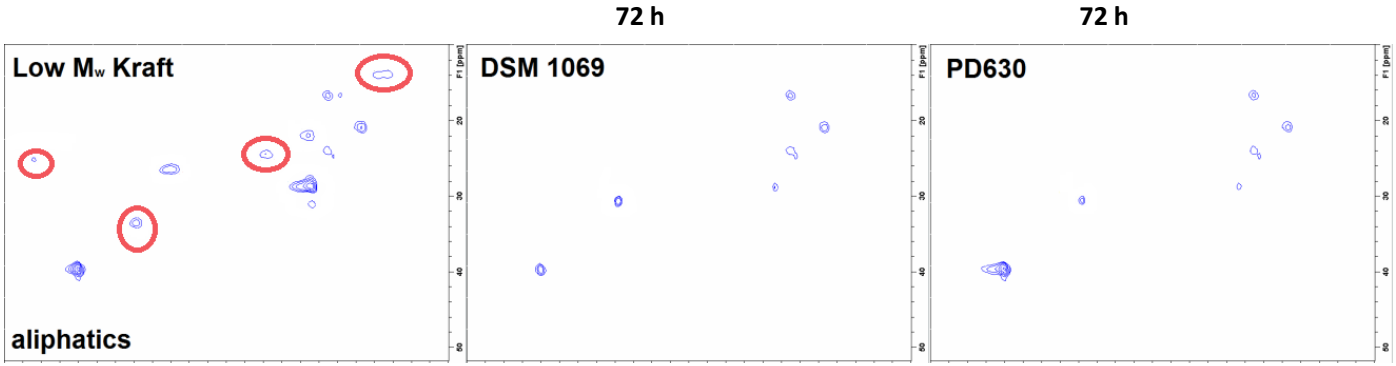

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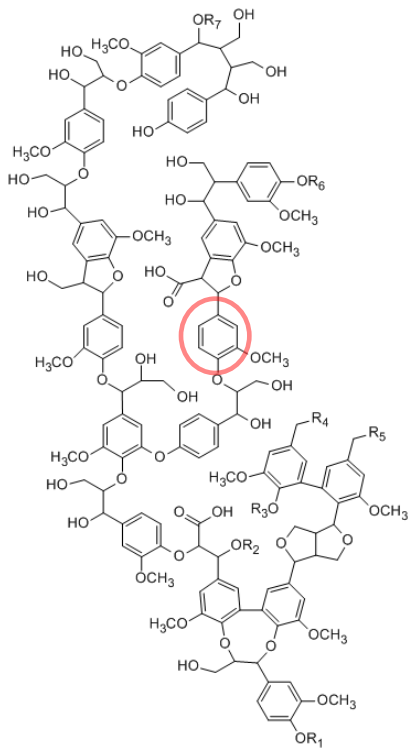

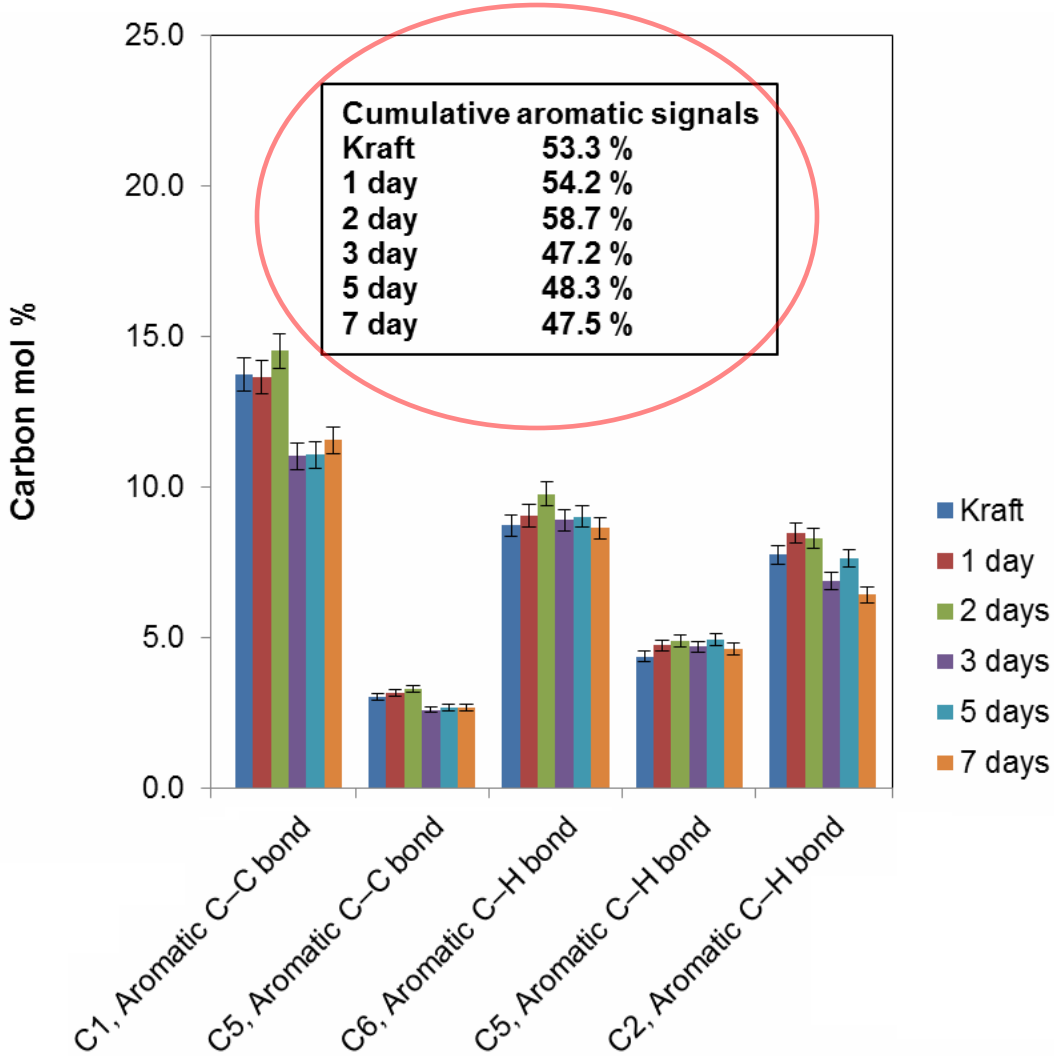

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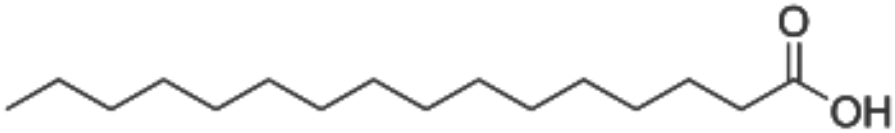
R. opacus	Extractive composition
PD630	8.4 % C14:0 21.5 % C16:1 44.6 % C16:0 25.5 % C18.1
DSM 1069	TBA



palmitic acid

Conclusions

- Oil-producing bacteria can metabolize aromatic biomass and produce bio-oil
- This process has potential as a supplemental energy platform

Current Accomplishments

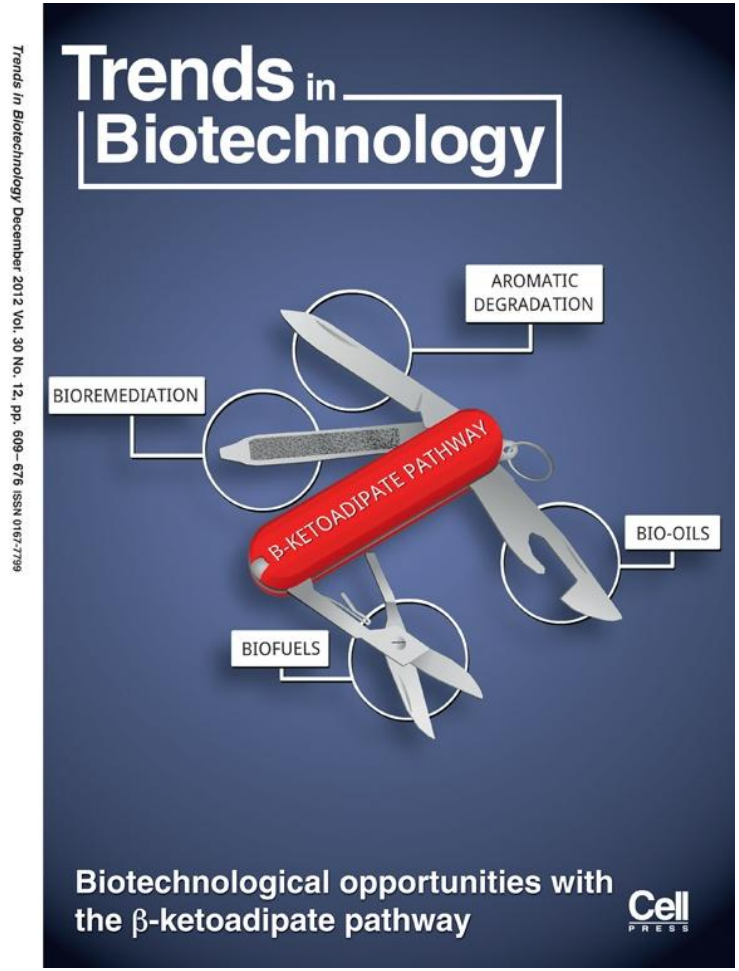
- Successfully generated bacterial bio-oils from:
  - Model compounds (G and H-type monolignol analogs)
  - Low M_w Softwood Kraft lignin

Future Work

- Adapt the bacteria to higher M_w Kraft lignin
- Alternative lignocellulosic derivatives

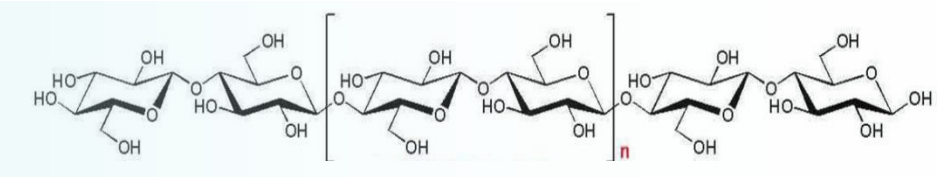
Trees into Fat

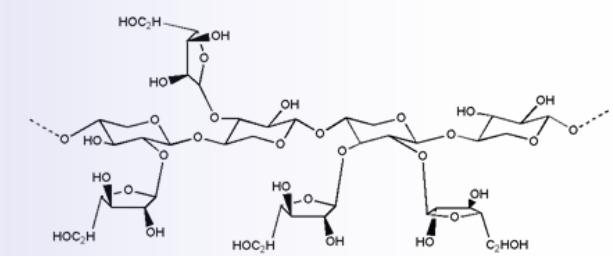
- Acknowledgement
  - Arthur Ragauskas
  - Matyas Kosa
  - DOE Biorefinery Project

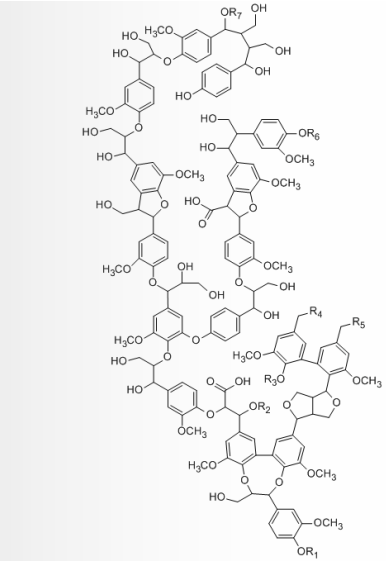


Review of Lignocellulosic Biomass

- Cellulose
  - Hardwood 40-44%
  - Softwood 40-44%
- Hemicellulose
  - Hardwood 25-35%
  - Softwood 20-32%
- Lignin
  - Hardwood 20-25%
  - Softwood 25-35%
  - Complex biomacromolecule
  - Monolignols
    - *p*-Hydroxyphenyl (H), Guaiacyl (G), Syringyl (S)
  - 3D polyaromatic macromolecule
    - Recalcitrant
  - Significantly less applications





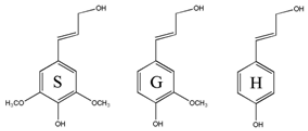


Horst H. Nimz, Uwe Schmitt, Eckart Schwab, Otto Wittmann, Franz Wolf "Wood" in Ullmann's Encyclopedia of Industrial Chemistry 2005, Wiley-VCH, Weinheim.

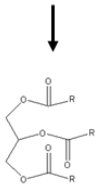
Optimizing the Use of Lignin

- Lignin
  - (>90%) Kraft lignin burnt as a non-optimized fuel
  - Opportunity for recovery-boiler limited mills
- Alternative Uses of Lignin
- Lignin → Biodiesel
  - Bacterial treatment
  - Sustainable supplemental fuel platform

LIGNIN ARTIFACTS

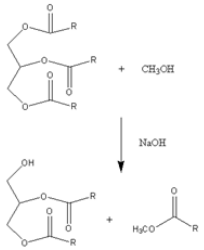


↓ (Aromatic Degradation)



TRIACYLGLYCEROLS

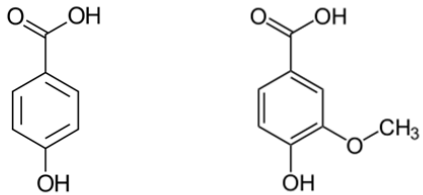
↓ (Transesterificaiton)



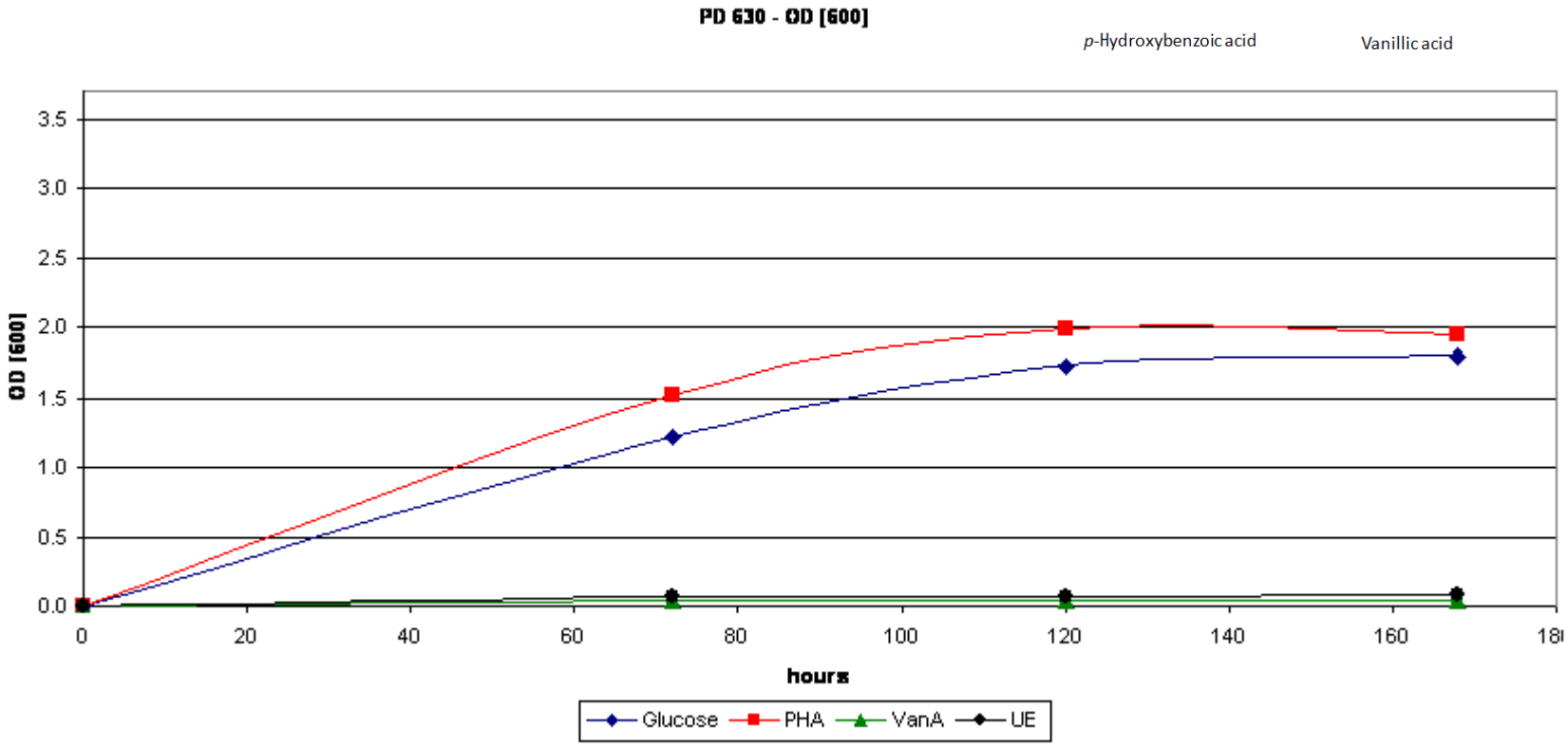
BIODIESEL

Kosa M, Ragauskas AJ (2011) Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol 29:53-61
Areskogh, J. Li, G. Gellerstedt, G. Henriksson, Investigation of the molecular weight increase of commercial lignosulphonates by laccase catalysis, Biomacromol. 11 (2010) 904-910.
Pu Y, Kosa M, Kalluri UC, Tuskan GA, Ragauskas AJ (2011) Challenges of the utilization of wood polymers: how can they be overcome? Appl Microbiol Biotechnol, 91:1525-1536

- Fermentation Growth with PD 630
  - Calculated via OD at 600 nm, 1 w/V%



p-Hydroxybenzoic acid Vanillic acid



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- Fermentation Growth with PD 630
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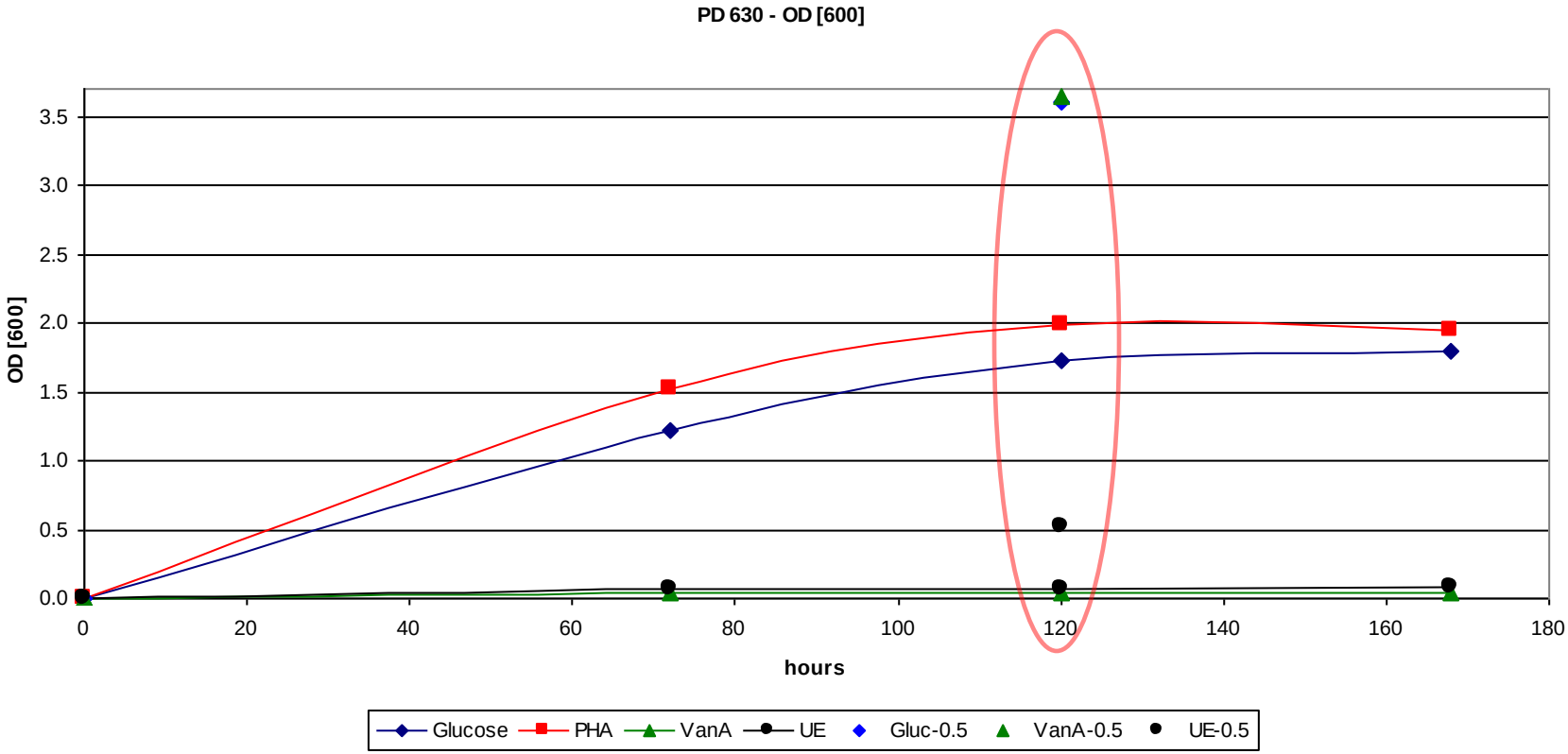

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- Fermentation Growth with DSM 1069
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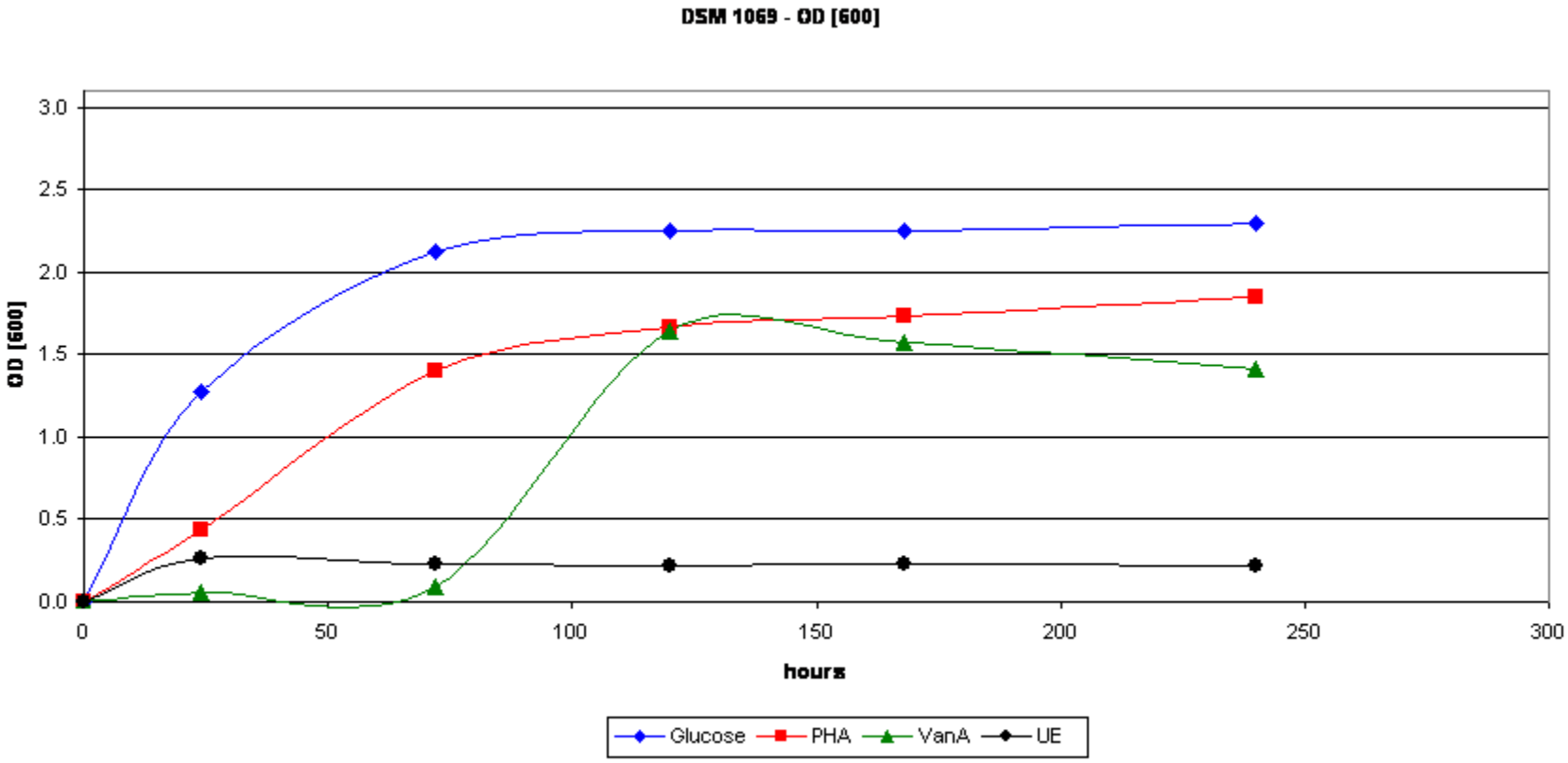

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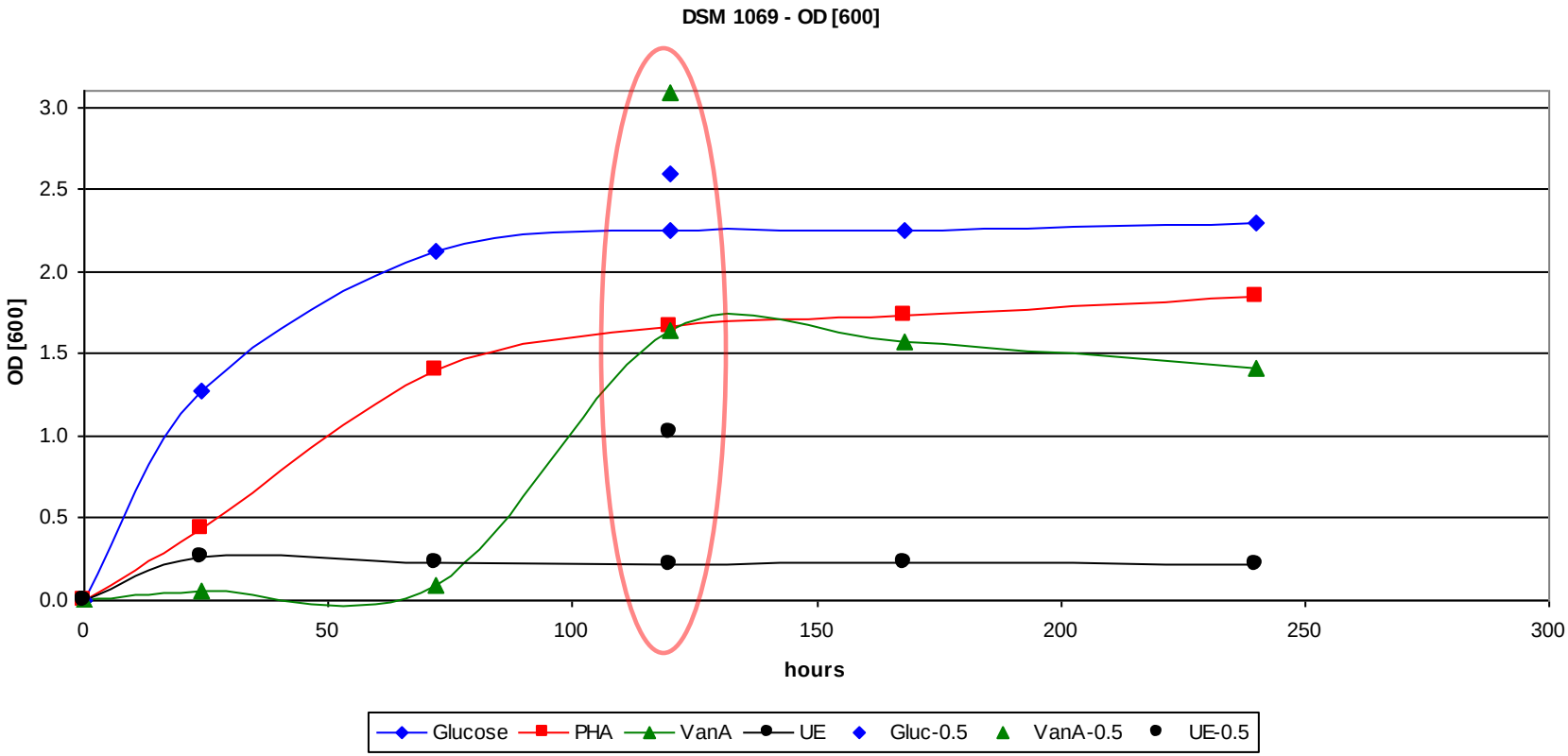

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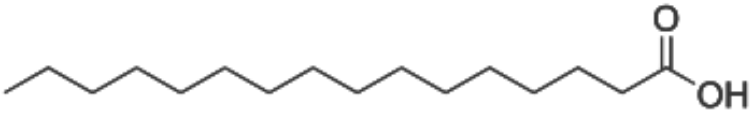
Strain	Carbon source	FA [%CDW]	t [h]
DSM 1069	glucose	13.8	12
	4-HBA	16.8	12
	VanA	8.7	36
PD630	glucose	19.8	12
	4-HBA	20.3	36
	VanA	14.6	48

Kosa M, Ragauskas AJ (2011) Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol 29:53-61

Strain	Carbon source	Relative proportion of FA [w/w%]					
		C14:0	C15:0	C16:0	C16:1^{cis}	C16:1^{trans}	C17:0
DSM 1069	glucose	0.0	8.3	25.8	6.5	5.1	10.6
	4-HBA	0.0	8.6	24.2	6.0	4.0	12.8
	VanA	0.0	8.4	32.1	7.2	4.1	10.0
PD630	glucose	0.0	4.3	31.1	6.4	4.5	5.7
	4-HBA	7.0	6.0	28.8	10.6	4.7	4.4
	VanA	3.3	7.6	27.9	7.7	3.7	7.6



palmitic acid

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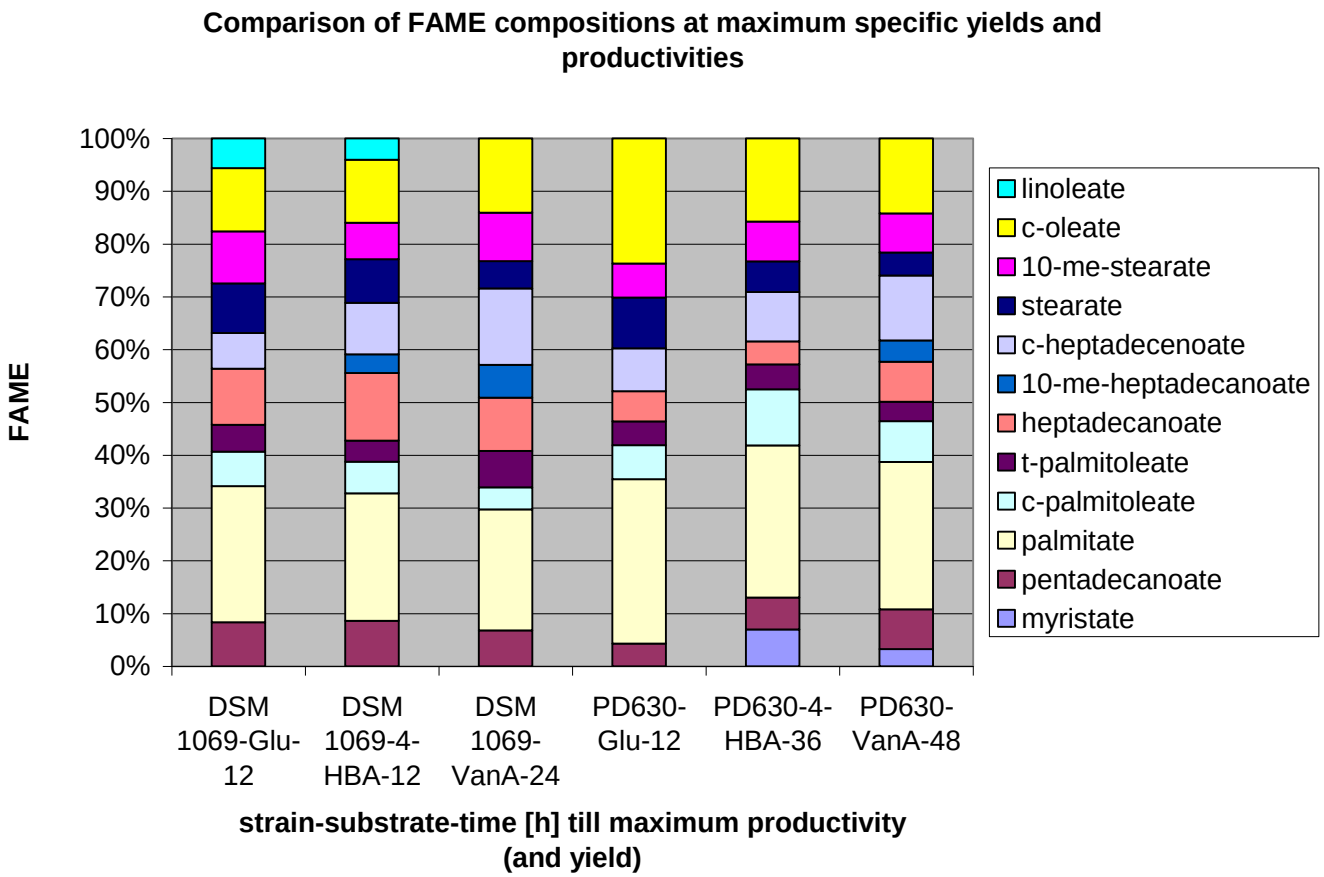
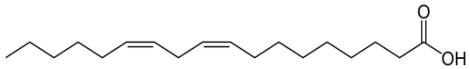
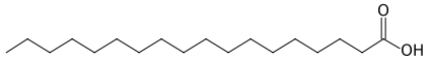
Lipid Composition Within Bio-Oil

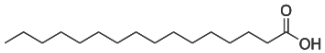




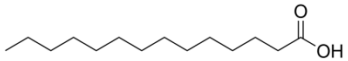
linoleic acid



stearic acid

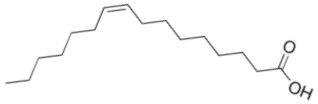


palmitic acid



myristate acid

c-oleic acid



c-palmitoleate acid

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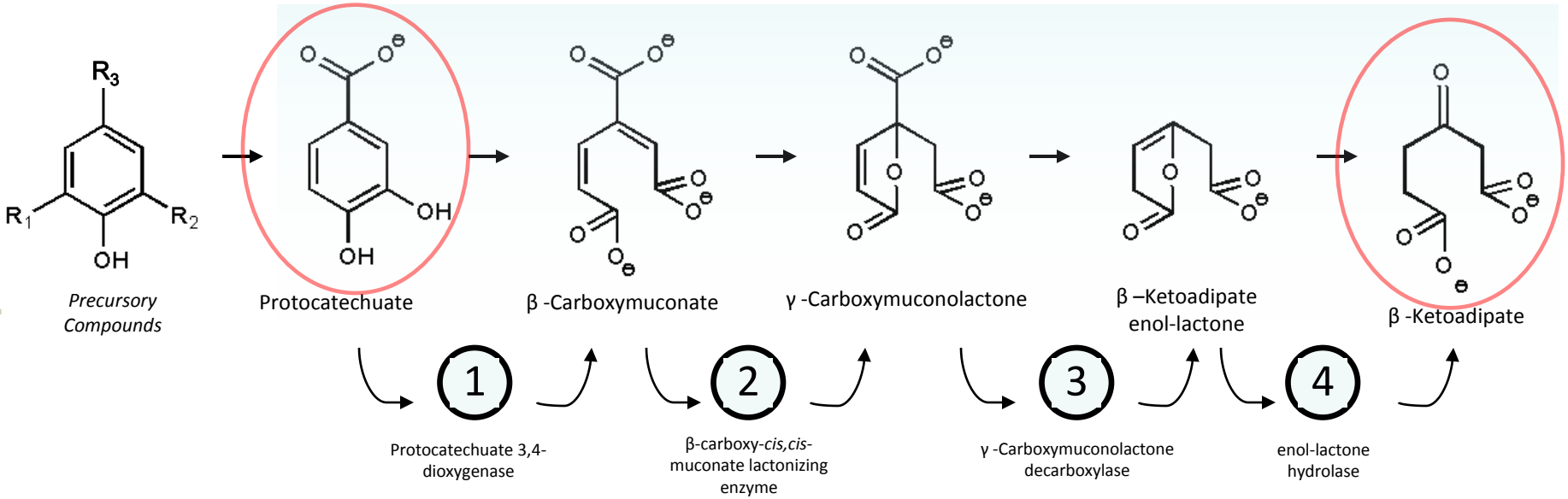
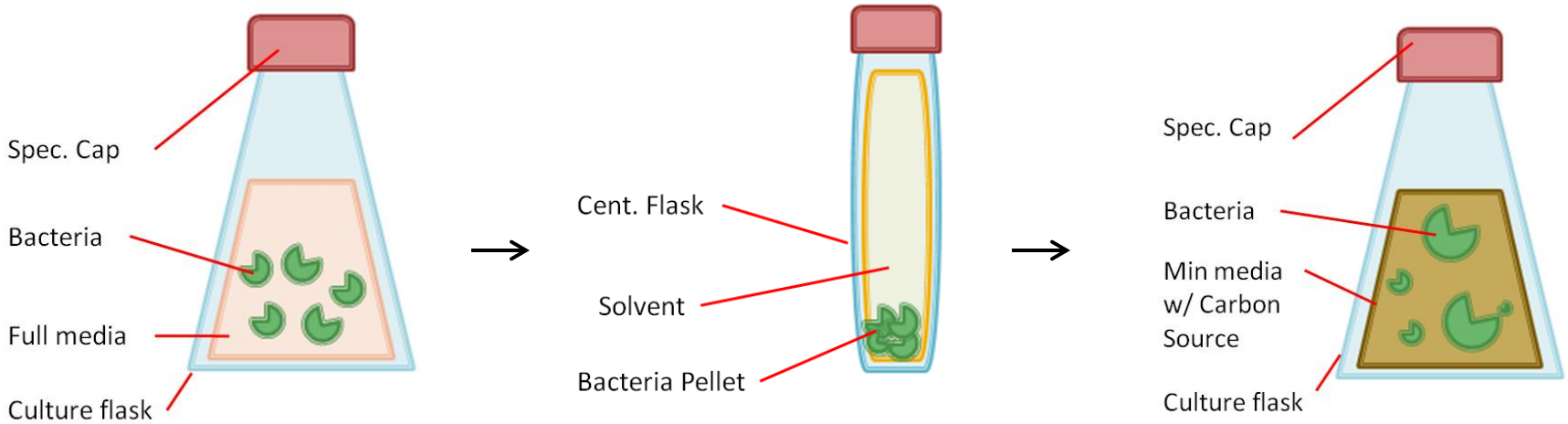
The β-KAP



Wells, T. and A.J. Ragauskas, *Biotechnological opportunities with the beta-ketoadipate pathway.* Trends in Biotechnology, 2012. **30**(12): p. 627-637

Experimental

- Adaptation Process
  - Cell proliferation (full media)
  - Centrifugation
  - Cell proliferation (minimal media + new carbon source)
    - Lipid accumulation (reducing nitrogen content of minimal media)



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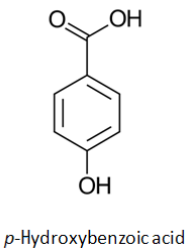
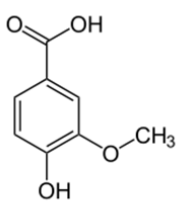
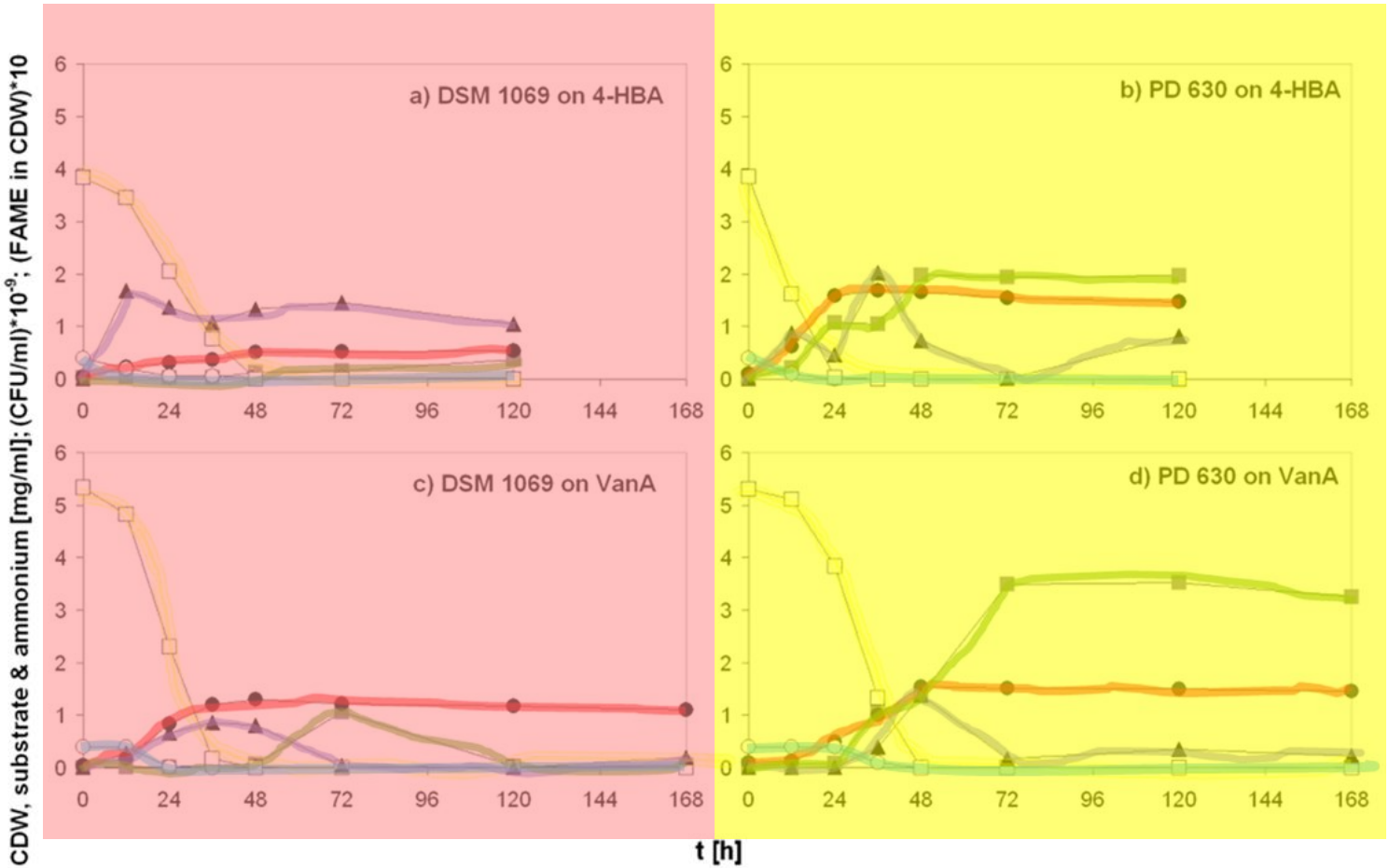


p-Hydroxybenzoic acid



Vanillic acid



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