

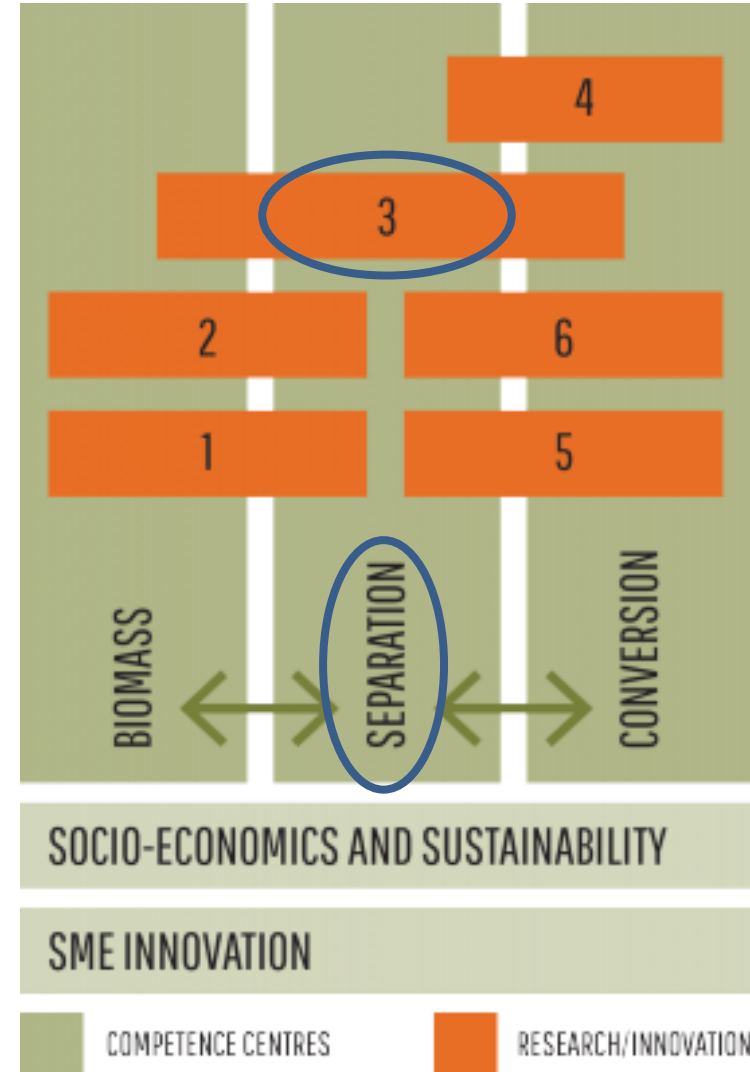


# Smart Separation of Biorefinery Streams

- **Separation**
- Embraces all 6 research projects
- **Project 3** Upgraded “sugar” streams from biomass

Anne S. Meyer

Dept. of Chemical and Biochemical Engineering  
 Technical University of Denmark

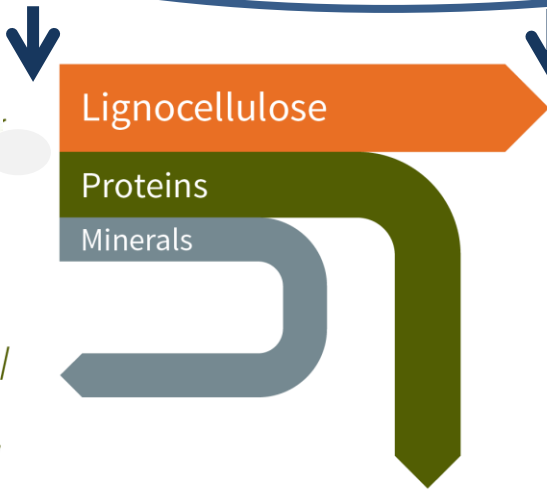




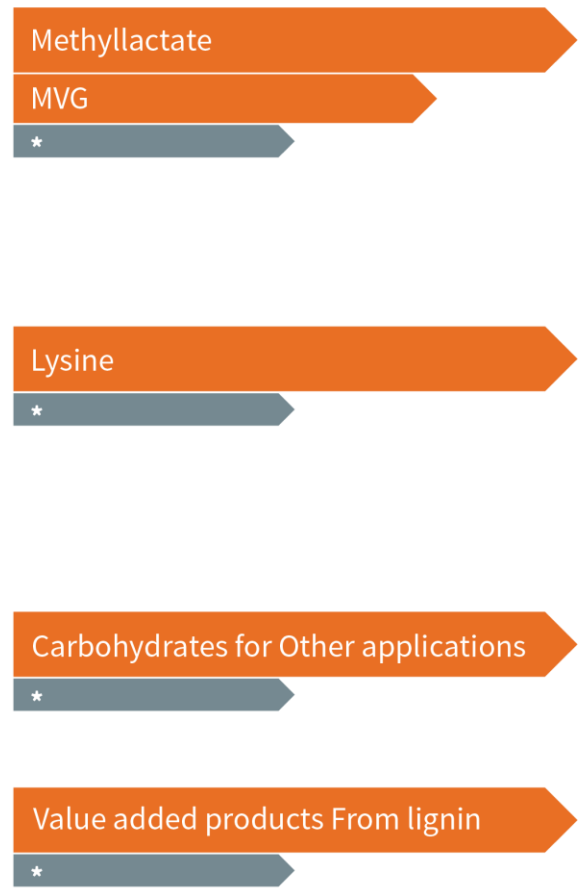
Separation is a key part of biorefining



yellow biomass / wheat straw  
Green biomass / perennial grasses



FEED

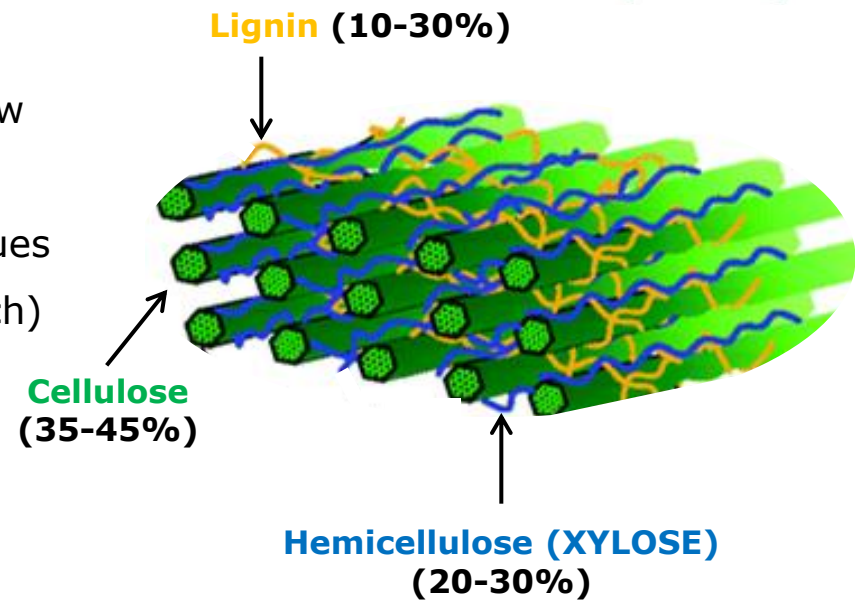


\* other biomass derived intermediates for further refining or energy



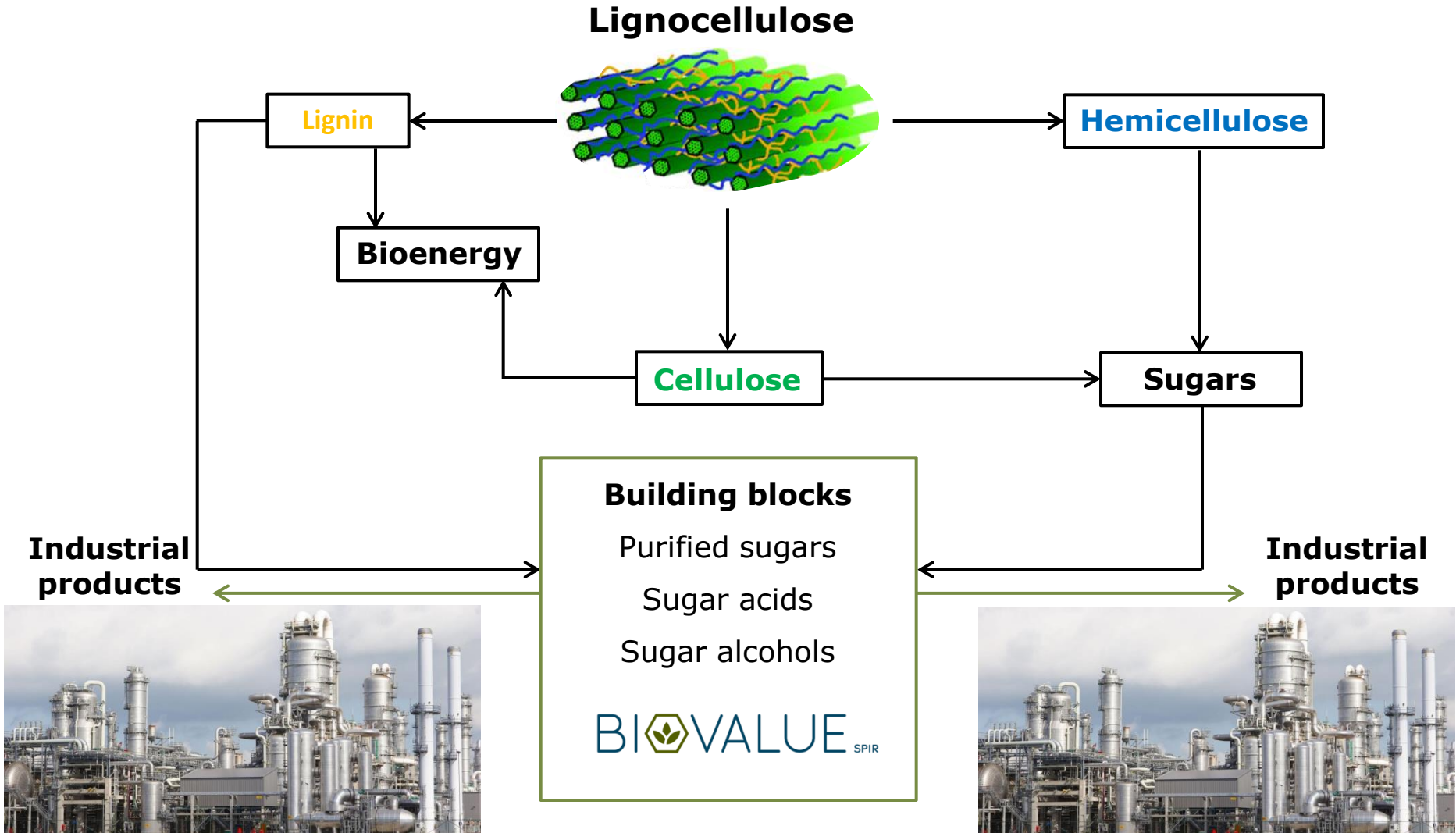
## Agroindustrial Residues Lignocellulose

- Renewable
- Non-food feedstock
- Abundant
  - Agricultural residues wheat straw
  - Green grass crops
  - Large scale agroindustrial residues e.g. KMC potato pulp (pectin rich)



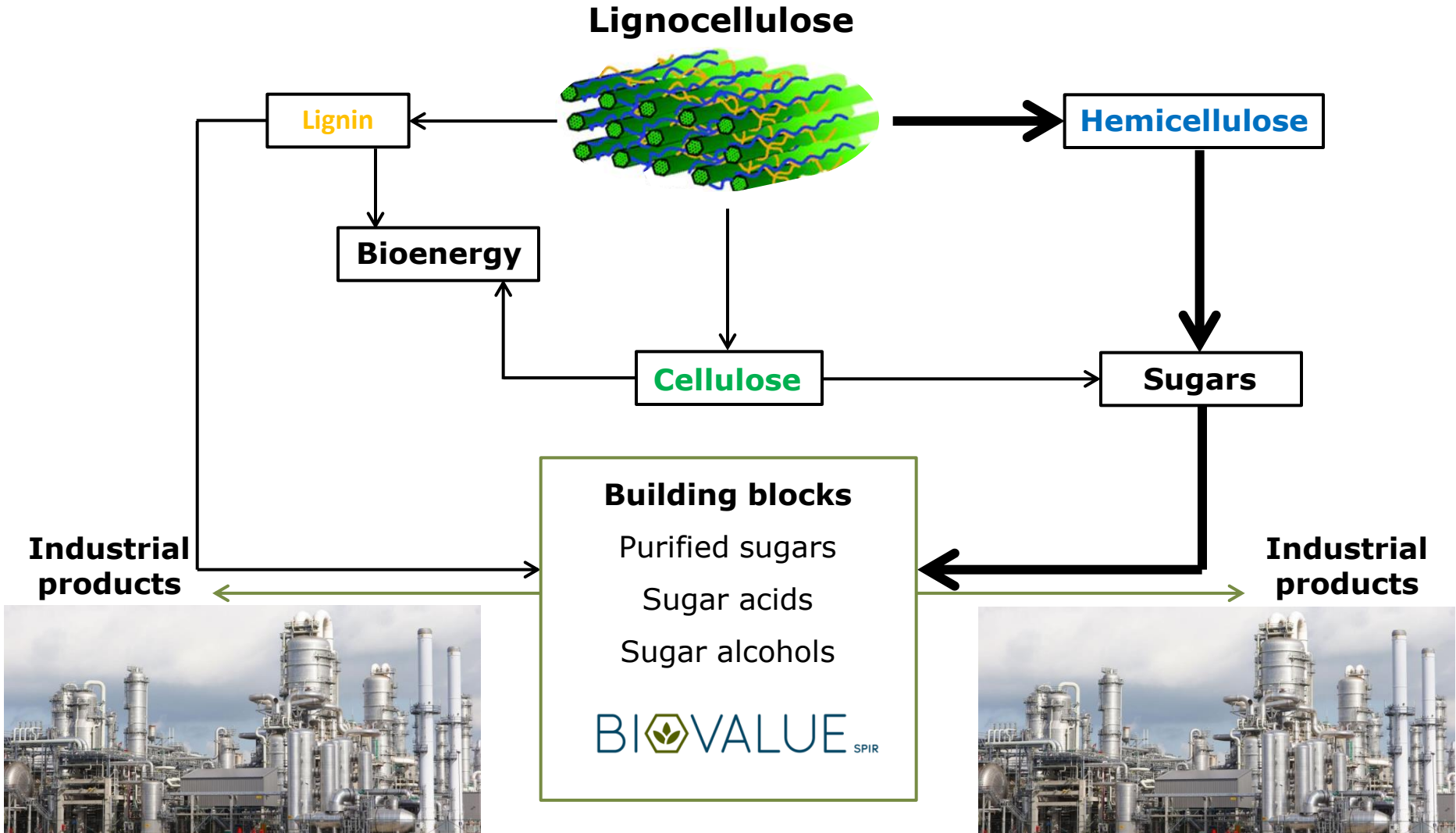


# General biorefinery concept





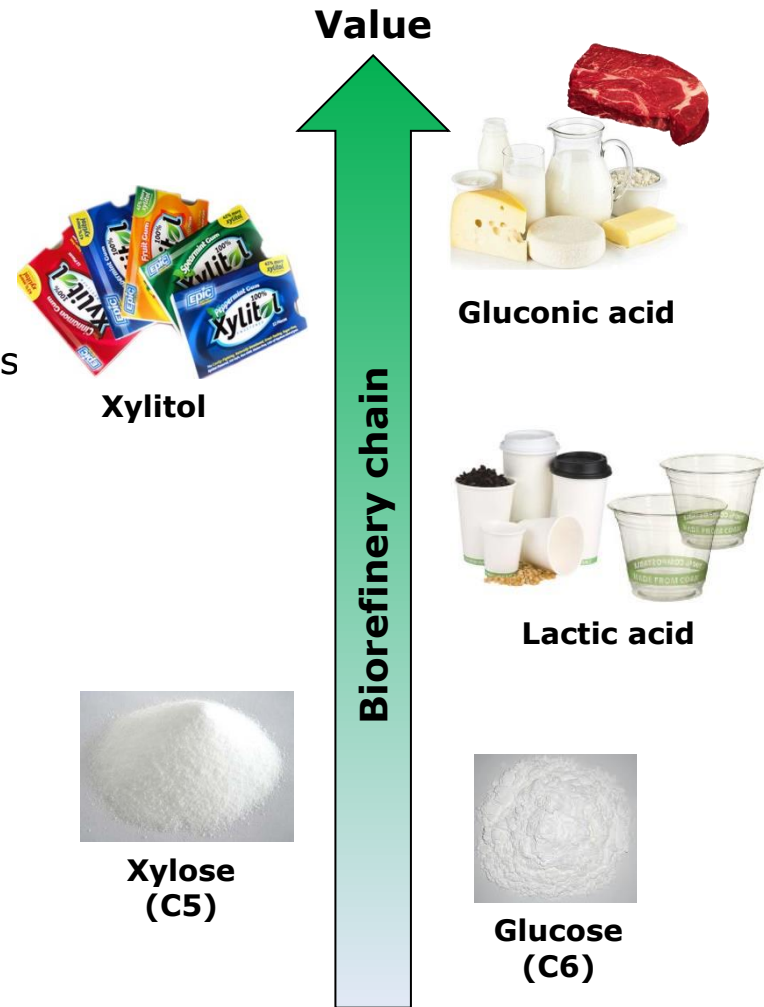
# General biorefinery concept





## Potential products

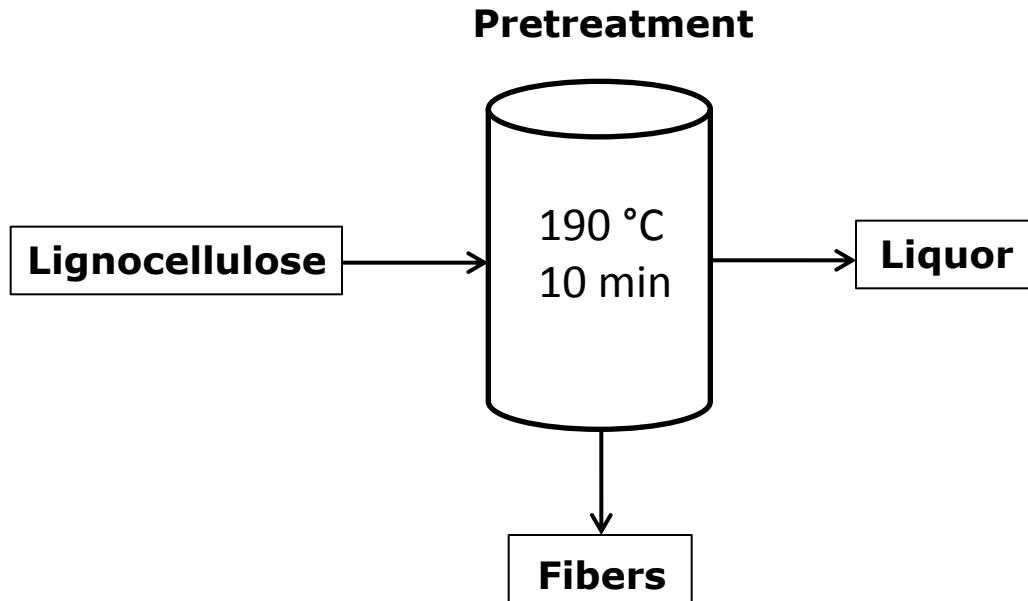
- Mixtures of C5 and C6 sugars from pretreated biomass
- Separation of C5 and C6 sugars
- Co-production of value-added products





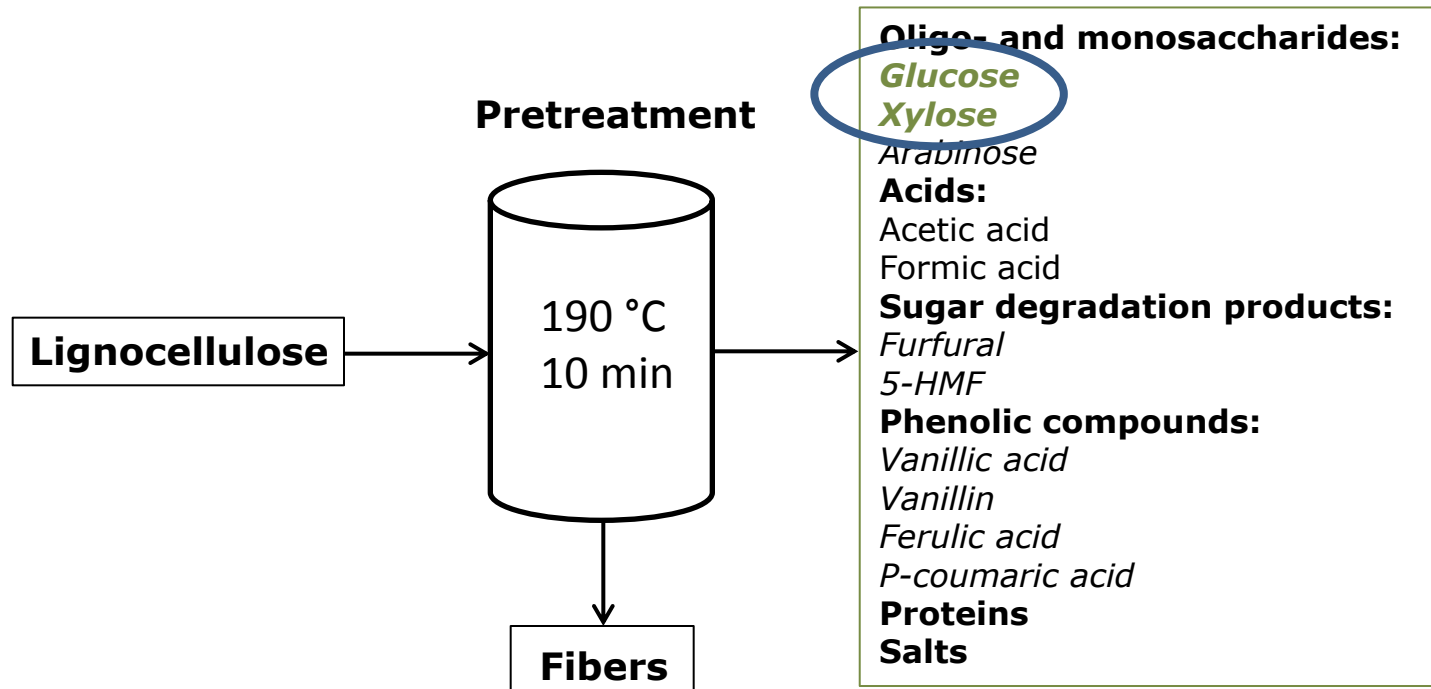
## Biomass pretreatment

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## Biomass pretreatment

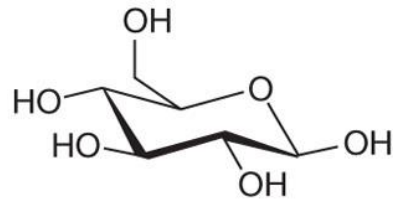




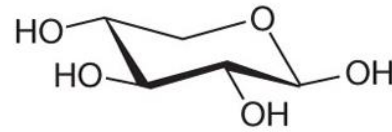


## Separation of xylose and glucose

- Xylose is the main sugar of the pretreatment liquor, there is some glucose there, too
- Xylose and glucose are quite similar and have similar properties



D-glucose

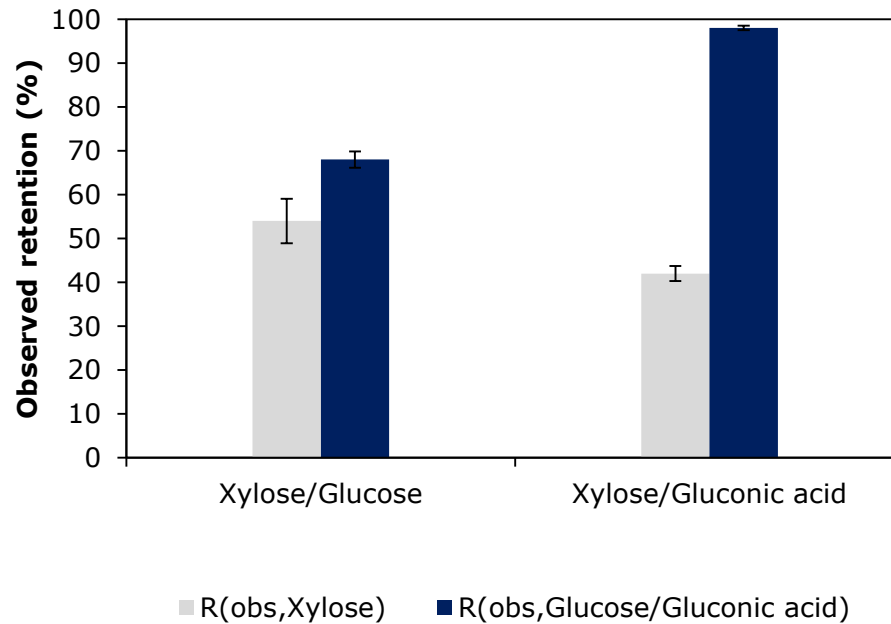


D-xylose

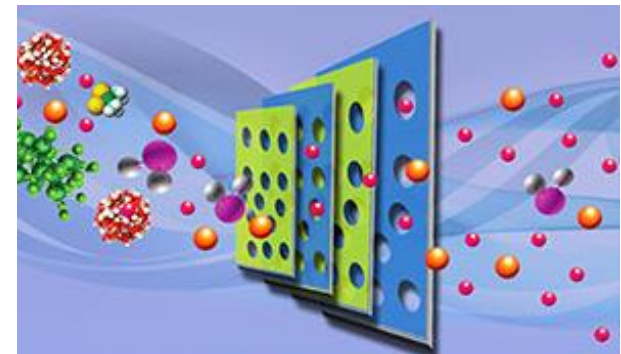


## Membrane technology nano filtration

- Separation can be carried out under mild conditions
- Simple
- Fast

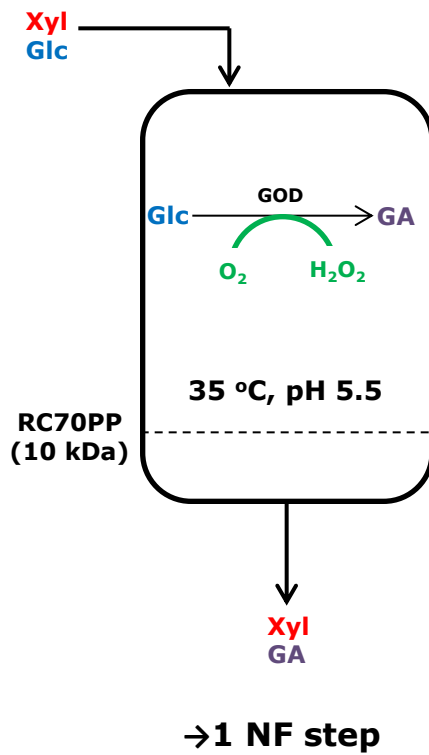


**NF270 membrane: 150-200 Da**





## Model system: GOD/CAT



### Abbreviations

Xyl: Xylose

Glc: Glucose

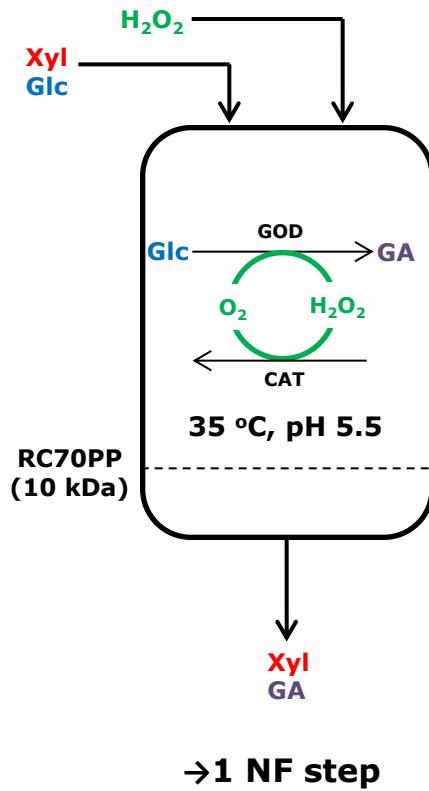
GA: Gluconic acid

GOD: Glucose oxidase



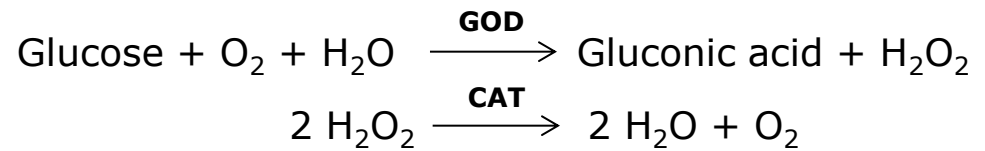


## Model system: GOD/CAT



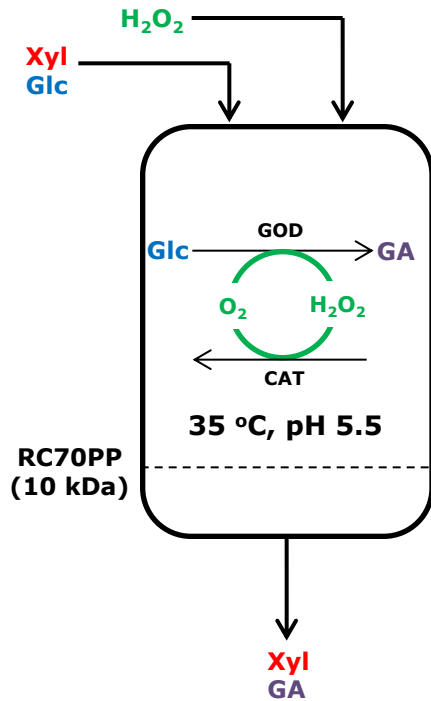
### Abbreviations

Xyl: Xylose  
 Glc: Glucose  
 GA: Gluconic acid  
 GOD: Glucose oxidase  
 CAT: Catalase

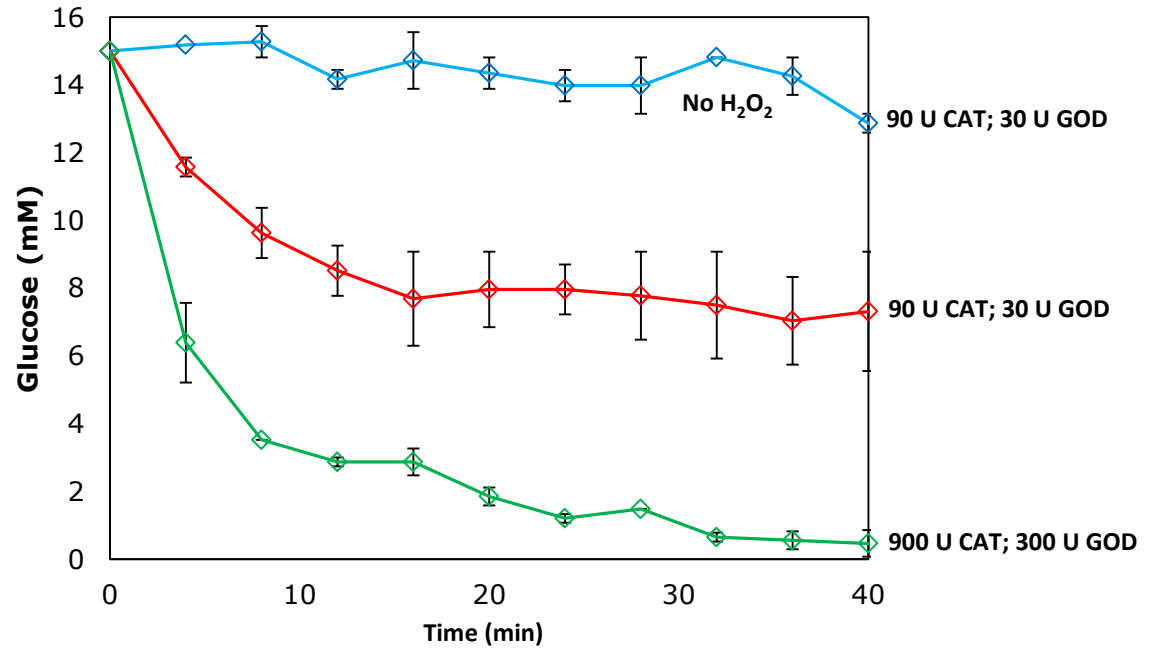




# Model system: GOD/CAT O<sub>2</sub> supply

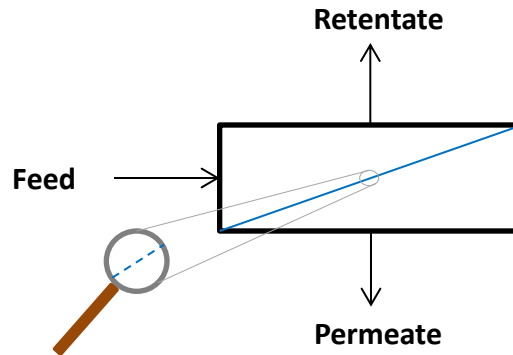


→1 NF step





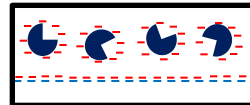
# Model system: GOD/CAT Reactor configurations



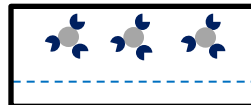
## Membrane as barrier



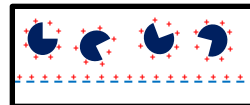
Size exclusion



Negative charge repulsion

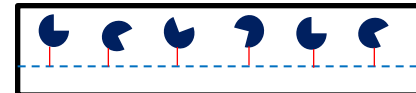


Immobilization onto carrier  
-> enhanced size exclusion

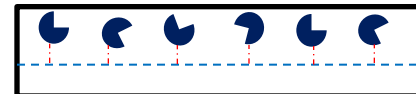


Positive charge repulsion

## Membrane as barrier & support



Immobilization by covalent bonding



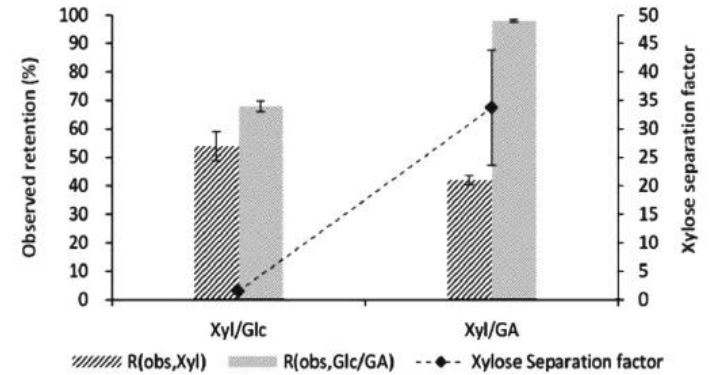
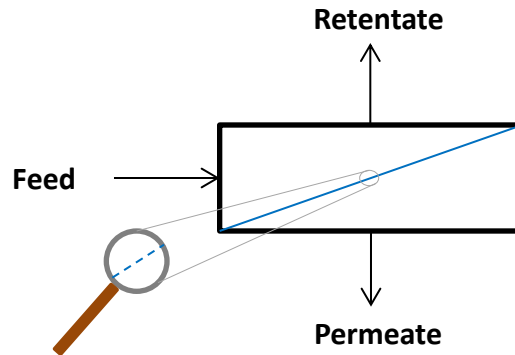
Immobilization by non-covalent bonding



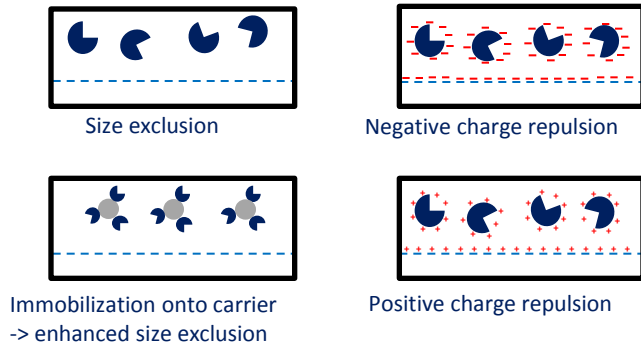
Immobilization by entrapment



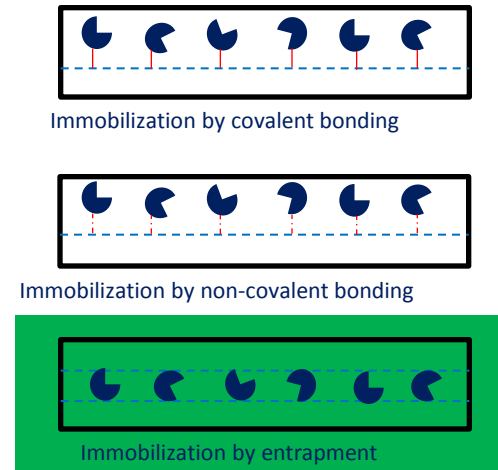
# Model system: GOD/CAT Reactor configurations



## Membrane as barrier



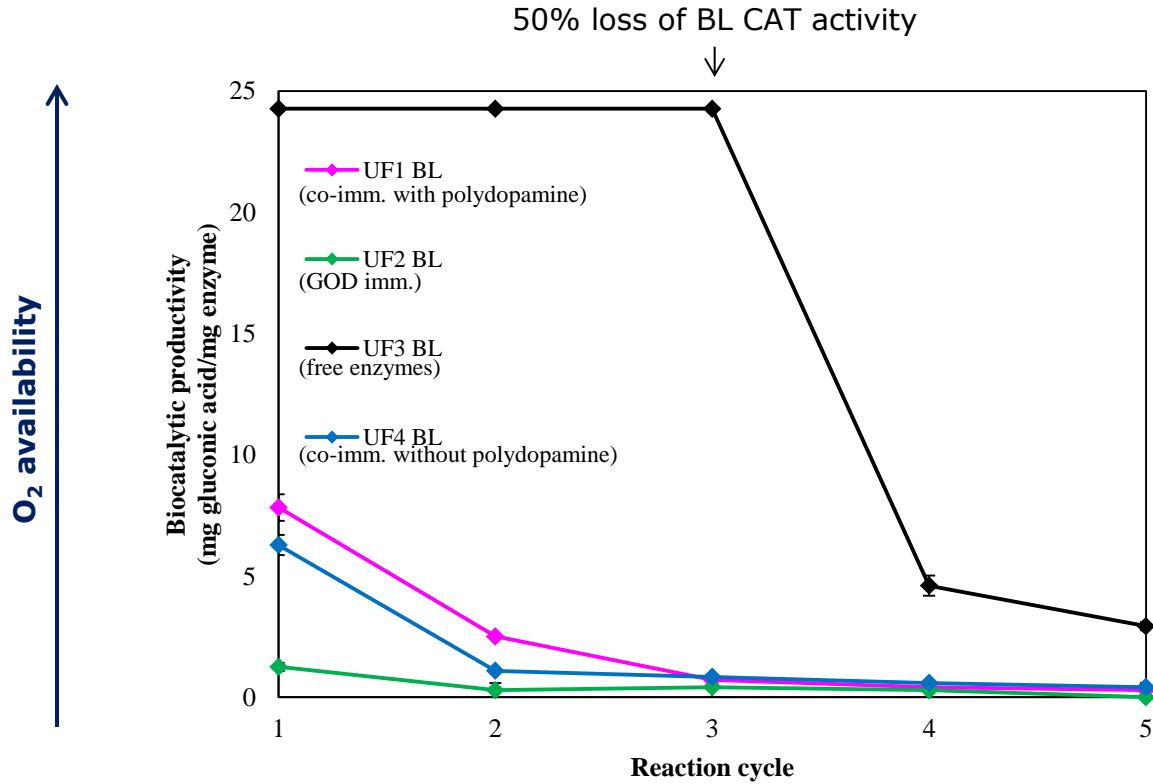
## Membrane as barrier & support





# Model system: GOD/CAT

## Biocatalytic productivity: Reactor configurations

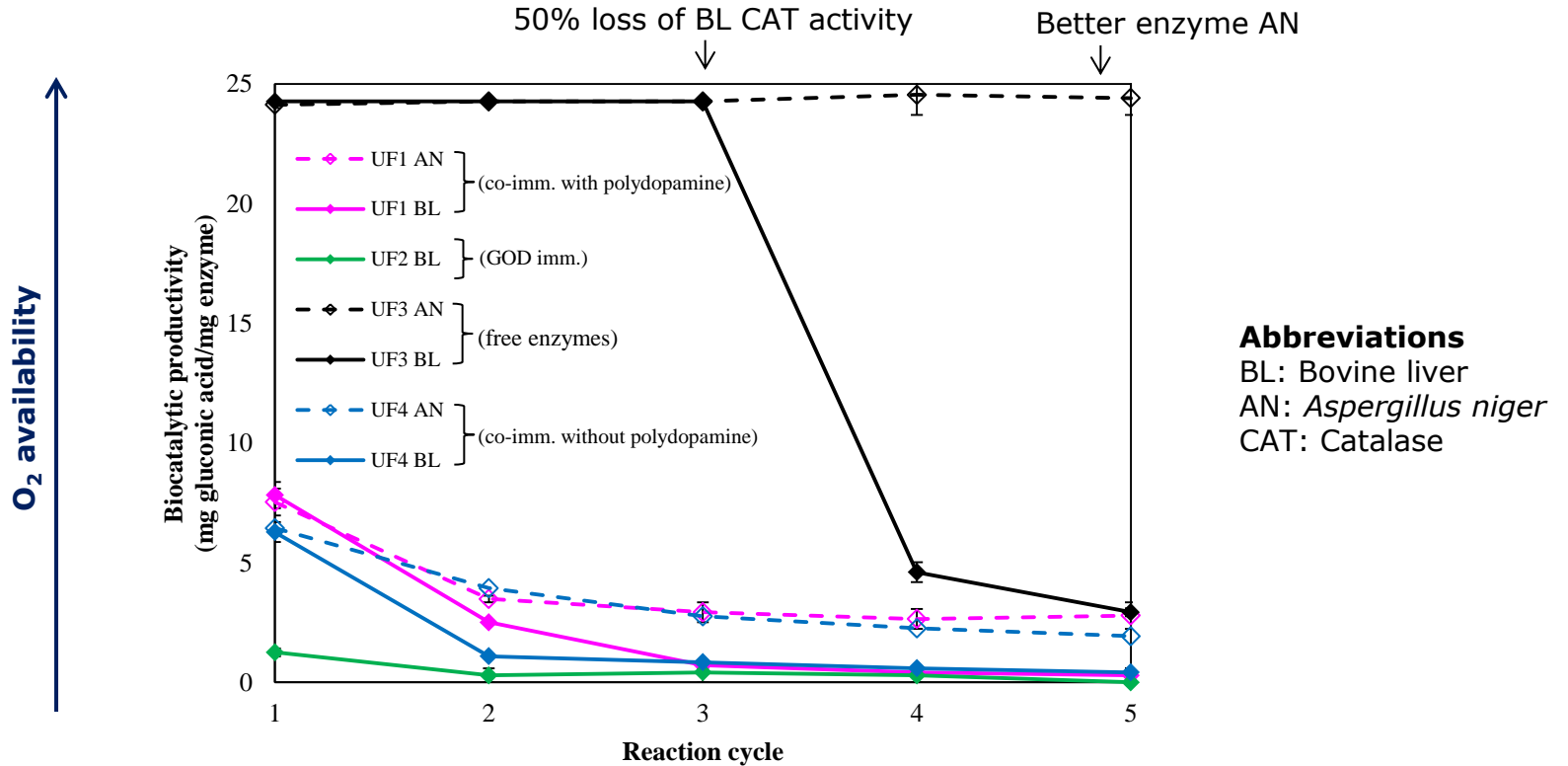






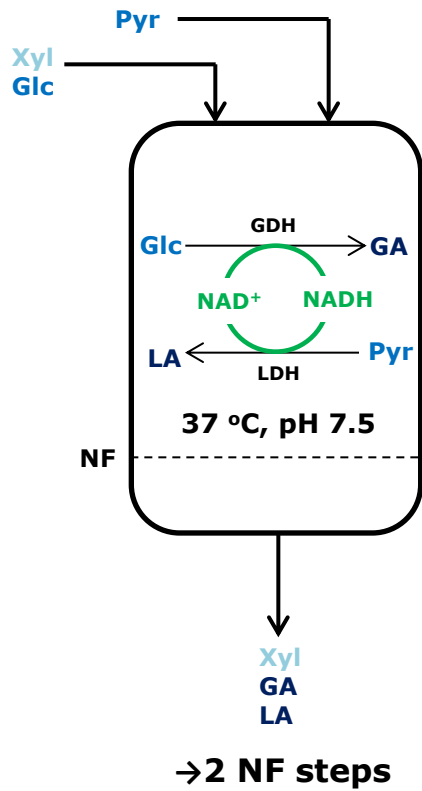
# Model system: GOD/CAT

## Biocatalytic productivity: Catalase origin



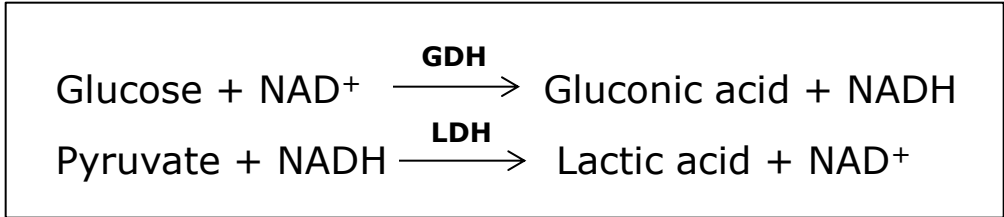


## Model system: GDH/LDH



### Abbreviations

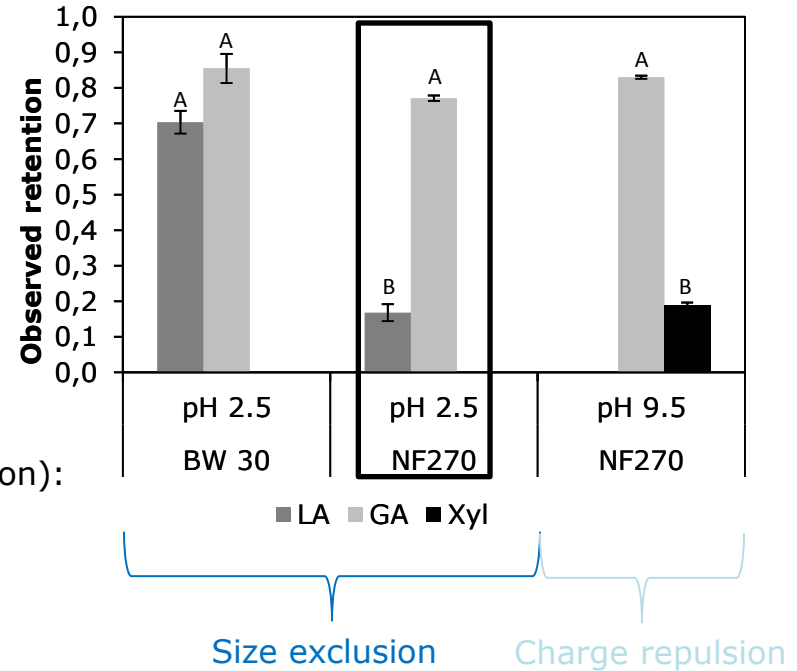
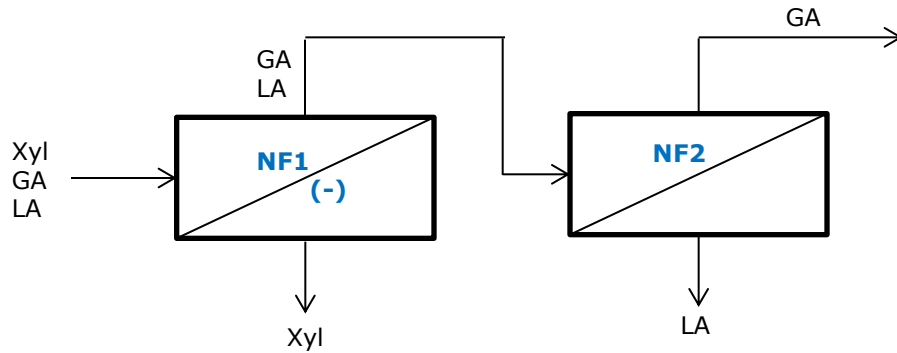
Xyl: Xylose  
 Glc: Glucose  
 GA: Gluconic acid  
 GDH: Glucose dehydrogenase  
 LDH: L-lactic dehydrogenase  
 Pyr: Pyruvate  
 LA: Lactic acid





# Model system: GDH/LDH Product recovery

## 1. Charge repulsion -> Size exclusion



- NF270, pH 2.5 (size exclusion) and pH 9.5 (charge repulsion): Similar separation performance
- Since charge repulsion preferred in NF1  
→ size exclusion selected in NF2  
**sequence selected for downstream purification**  
→ Xylose purity: 74%

→ 1.



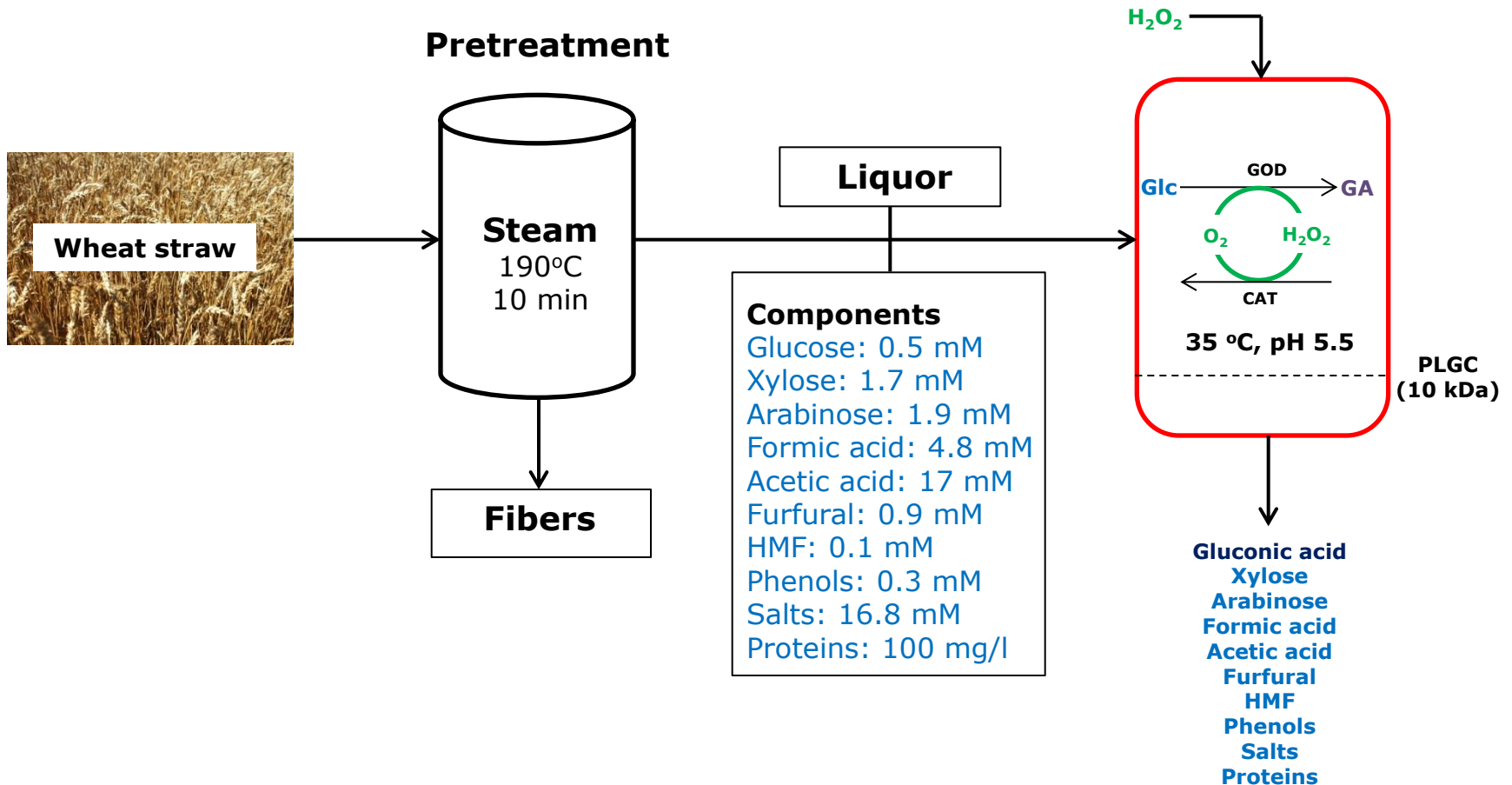
## Comparison of model systems

	No enzymes 1:9	GOD/CAT 1:9	GDH/LDH 1:1
Value-added products	0	1	2 (excl. XA)
Separation steps	1	2	3
Affinity for xylose	-	No	Yes
Yield (%)/purity (%) of xylose	28/59	41/99	<35/<74
Yield (%)/purity (%) of gluconic acid	-	98/20	<89/<60
Yield (%)/purity (%) of lactic acid	-	-	<42/<78
Biocatalytic productivity rate (mg product(s)/mg enzyme/min)	-	0.49	1.32
Throughput	High	High	Low
Fouling	Low	Low	High
pH adjustment steps	0	2	3
Enzyme cost (DKK/cycle)	0	4.2	15.7



# Biomass liquors

## Optimization of fouling and biocatalytic productivity





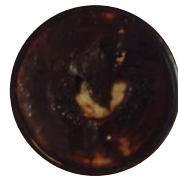
## Biomass liquors

### Optimization of fouling and biocatalytic productivity

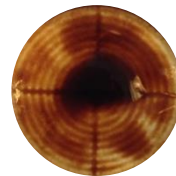
Condition	Filtration time (min)	WP loss (%)	Biocatalytic productivity (mg gluconic acid/mg enzyme)	Glucose (%)
100 rpm	140±0 <sup>A</sup>	26.6±6.5 <sup>A</sup>	0.52±0.04 <sup>C</sup>	73 <sup>B</sup> ±4.9
250 rpm	52.5±12.5 <sup>B</sup>	20.3±3.5 <sup>AB</sup>	0.60±0.01 <sup>C</sup>	84 <sup>AB</sup> ±1.2
250 rpm (cent.)	36.5±3.5 <sup>B</sup>	20.9±2.0 <sup>AB</sup>	0.56±0.04 <sup>C</sup>	80 <sup>B</sup> ±0
250 rpm (hydr./cent.)	7.8±1.3 <sup>C</sup>	2.4±2.4 <sup>B</sup>	14.4±0 <sup>B</sup>	95 <sup>A</sup> ±0
250 rpm (hydr./spiked/cent.)	9±1 <sup>C</sup>	9.1 <sup>AB</sup>	22.8±0.22 <sup>A</sup>	95 <sup>A</sup> ±0.9



100 rpm



250 rpm



250 rpm (cent.)



250 rpm (hydr./cent.)



250 rpm (hydr./spiked/cent.)

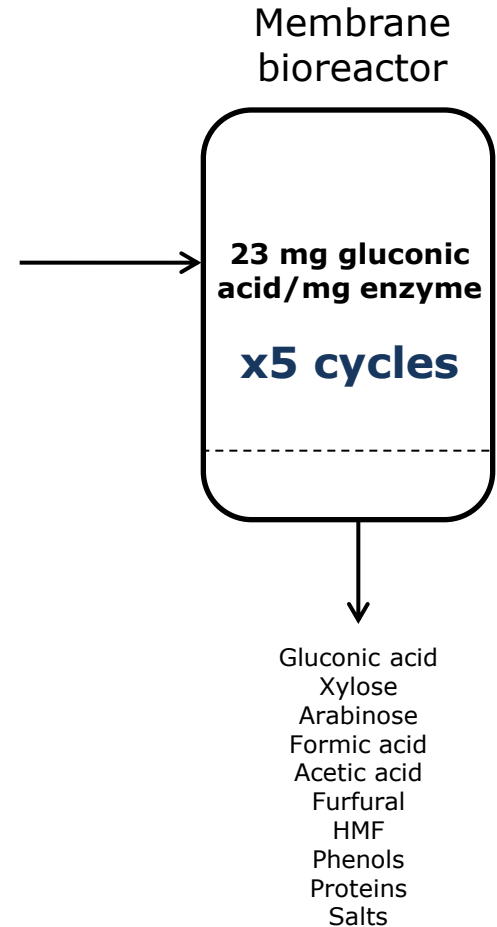


## Biomass liquors

5 consecutive cycles in the membrane bioreactor

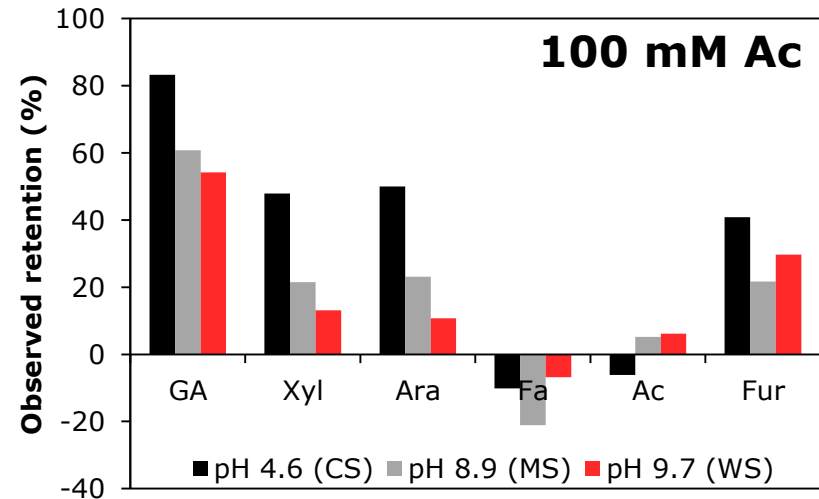
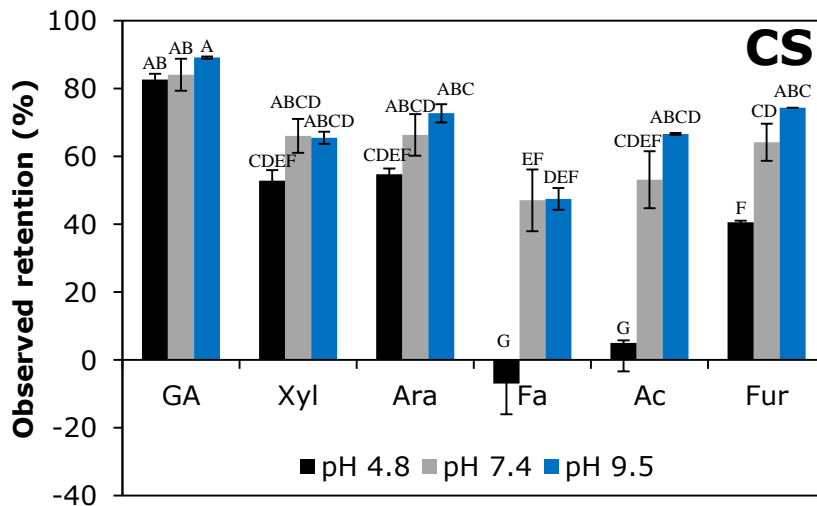
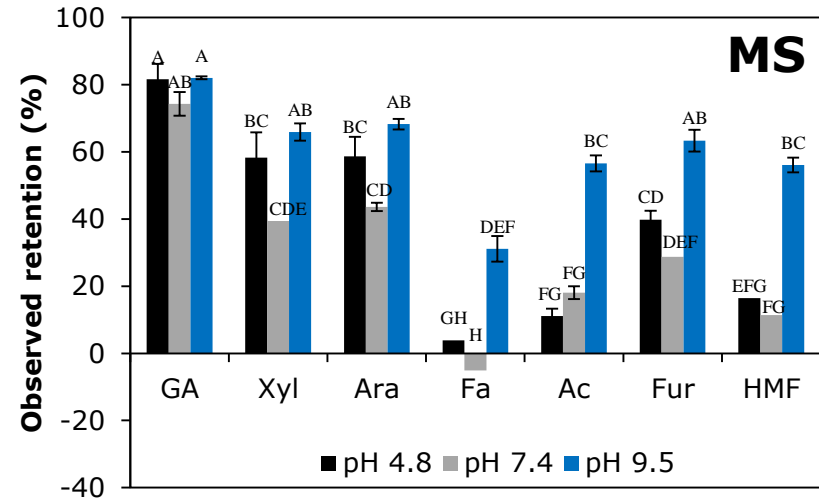
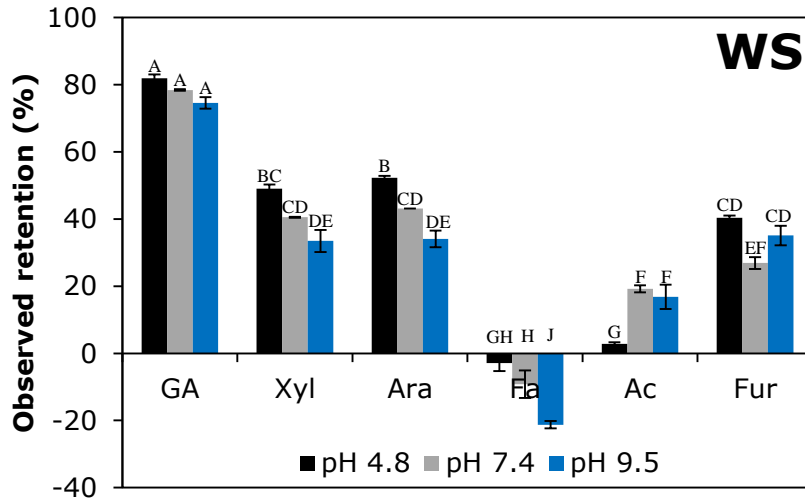
### Concentrations before adjustment of the feed solution

Dilute acid post hydrolysis liquors	WS	CS	MS	Model solution
Glucose (mM)	15 (9.4)	15 (14.1)	15 (18.4)	15
Xylose (mM)	135 (46.8)	135 (48.8)	135 (30.8)	135
Arabinose (mM)	6.8	8.2	3.1 (3.8)	0
Formic acid (mM)	5.6	6.2	3.8 (4.6)	0
Acetic acid (mM)	24.8	27.2	16.2 (19.9)	0
Furfural (mM)	5.3	2.3	2.0 (2.4)	0
5-HMF (mM)	0.6	0.2	1.6 (2.0)	0
Phenols (mM)	0.3	0.7	0.3 (0.4)	0
Protein (mg/l)	100	60	140	0
Salt	-	-	-	0





## Biomass liquors Purification: Addition of acetic acid







## Biomass liquors

### Wrap-up: Enzymatic reaction and xylose purification

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- Acid pre-sep removed fouling and maximized flux and biocatalytic productivity in the membrane bioreactor
- Separation factors decreased to 2.7 for wheat straw, 2.5 for corn stover and 2.2 for Miscanthus stalks vs. 34 for the model solution
- Biocatalytic productivity after 5 cycles similar for biomass liquors (wheat straw, corn stover and Miscanthus stalks) and model solution data, i.e. 24 mg gluconic acid/mg enzyme
- High complexity of the biomass liquors resulted in several complex separation phenomena  
NF: Donnan effect, sieving, electrostatic repulsion
- Separation may be improved by identifying new membrane materials capable of maintaining their zeta potential at high ionic strength



## Conclusions and perspectives

### REACTIVE MEMBRANE SEPARATION WORKS

***Integrating enzymatic reaction and nanofiltration presents new options for separating compounds with similar chemical properties by membrane technology***

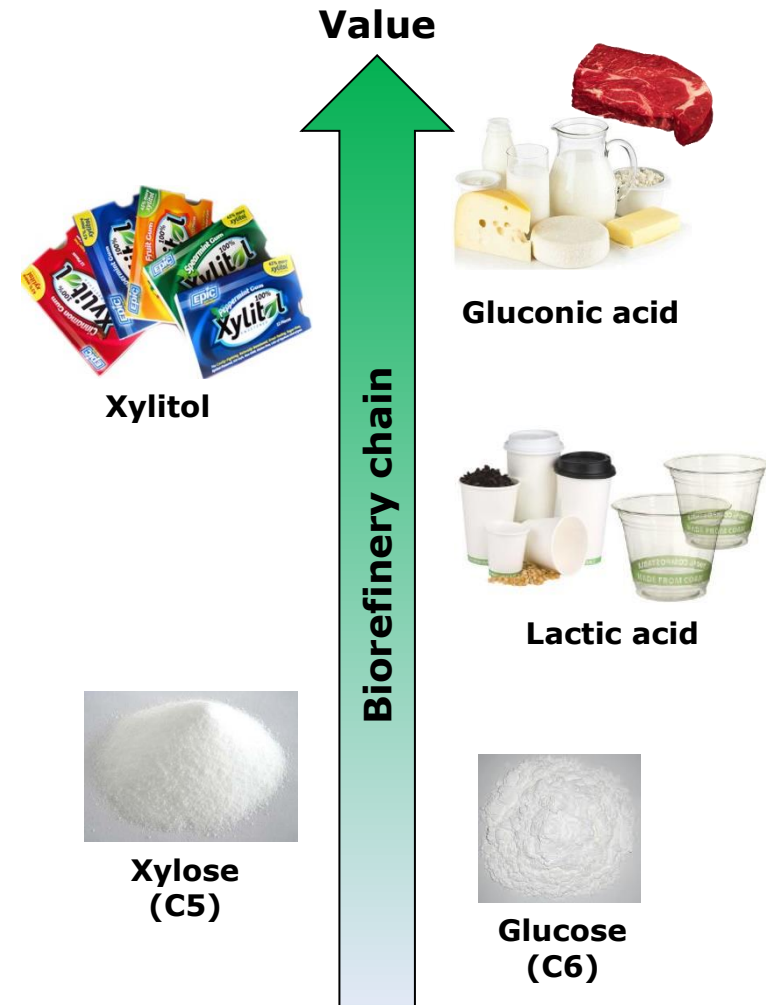
- ✓ Xylose can be recovered from biomass pretreatment liquors by enzyme-assisted membrane separation
- ✓ The efficiency of enzyme-assisted xylose separation can be enhanced by optimizing the enzyme system and reactor configuration





## Potential products

- Separation of C5 and C6 sugars
- Co-production of value-added products



## Acknowledgements

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### **Co-authors and other collaborators**

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Jacob-Holm Petersen

Annette E. Jensen

Demi Tristan Djajadi

Mohd Shafiq Sueb



### **Project partners in Bio-Value**

### **Funding**

Bio-Value Strategic Platform for Innovation and Research



## Follow our work

- 
- Morthensen ST, Sigurdardóttir SB, Meyer AS, Jørgensen H, Pinelo M. **Separation of xylose and glucose using an integrated membrane system for enzymatic cofactor regeneration and downstream purification.** J Mem Sci 523 (2017) 327–335.
  - Morthensen ST, Meyer AS, Jørgensen H, Pinelo M. **Significance of membrane bioreactor design on the biocatalytic performance of glucose oxidase and catalase: Free vs. immobilized enzyme systems.** Biochemical Engineering Journal 117 (2017) 41–47.
  - Morthensen ST, Luo J, Meyer AS, Jørgensen H, Pinelo M. **High performance separation of xylose and glucose by enzyme assisted nanofiltration.** Journal of Membrane Science 492 (2015) 107-115.
  - Luo J, Zeuner B, Morthensen ST, Meyer AS, Pinelo M. **Separation of phenolic acids from monosaccharides by low-pressure nanofiltration integrated with laccase pre-treatment.** Journal of Membrane Science 482 (2015) 83-91.
  - Sueb MSM, Zdarta J, Jesionowski T, Jonsson G, Meyer AS, Jørgensen H, Pinelo M. **High performance removal of acids and furans from wheat straw pretreatment liquids by diananofiltration.** Sep Sci Technol, 11 (2017) 1901-1912
  - Sueb MSM, Meyer AS, Jørgensen H, Pinelo M. **Impact of the fouling mechanism on enzymatic depolymerization of xylan in different configurations of membrane reactors** Sep Pur. (2017) 178: 154-162
  - .....mp@kt.dtu.dk or am@kt.dtu.dk