

# Carbohydrate Analysis of Seaweed in the Biorefinery to Chemicals and Fuel Context

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# Take home messages

- Seaweed specific protocols needed for:
  - Accurate mass balances of biorefineries
  - Identification of the process bottle necks and opportunities for improvement
- Color reactions: (lack of) specificity
- Protocols are converging
- Single run HPAEC-PAD possible

# What is ECN?

- ECN develops market driven technology and know-how to enable a transition to a sustainable energy society
- Business units:
  - Biomass & energy efficiency
  - Solar energy
  - Wind energy
  - Policy studies
  - Environment & energy engineering



## ECN

- Independent research institute
- ~500 employees
- Locations:
  - *Petten (HQ)*
  - *Amsterdam*
  - *Eindhoven*
  - *Kuala Lumpur*
  - *Beijing*

# The pilot/DEMO stage

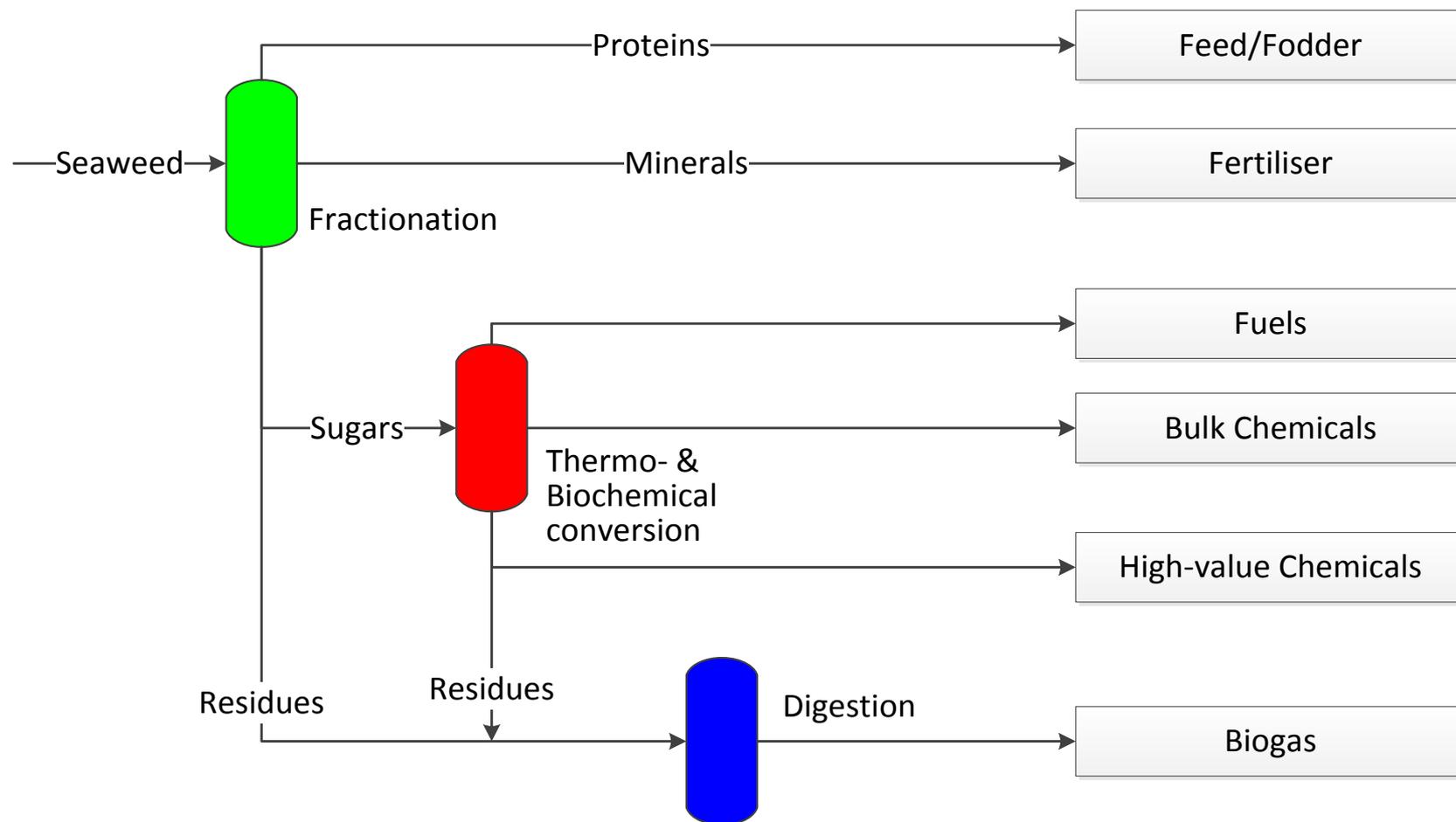


# Bio-Offshore



- Seaweed cultivation area 5,000 km<sup>2</sup> (<10 % of the NL area of the North Sea @ 57,000 km<sup>2</sup>)
- Integration with off-shore wind parks & (other) aquaculture operations
- Energy potential up to 350 PJ<sub>th</sub> (25 Mton dry biomass per year)
- Report: ECN-C—05-008
- Other plans: Crown Estate, 15,000 km<sup>2</sup> for biogas

# Seaweed biorefinery



# Compositional analysis of seaweed



- Critical evaluation of existing protocols
- Fundamental differences between seaweeds and lignocellulosic biomass
- Large differences between species
- Large seasonal variations
- Different recalcitrance of carbohydrates during hydrolysis
- Identification of unknown carbohydrates in the HPAEC (High Performance Anion Exchange Chromatography) chromatogram

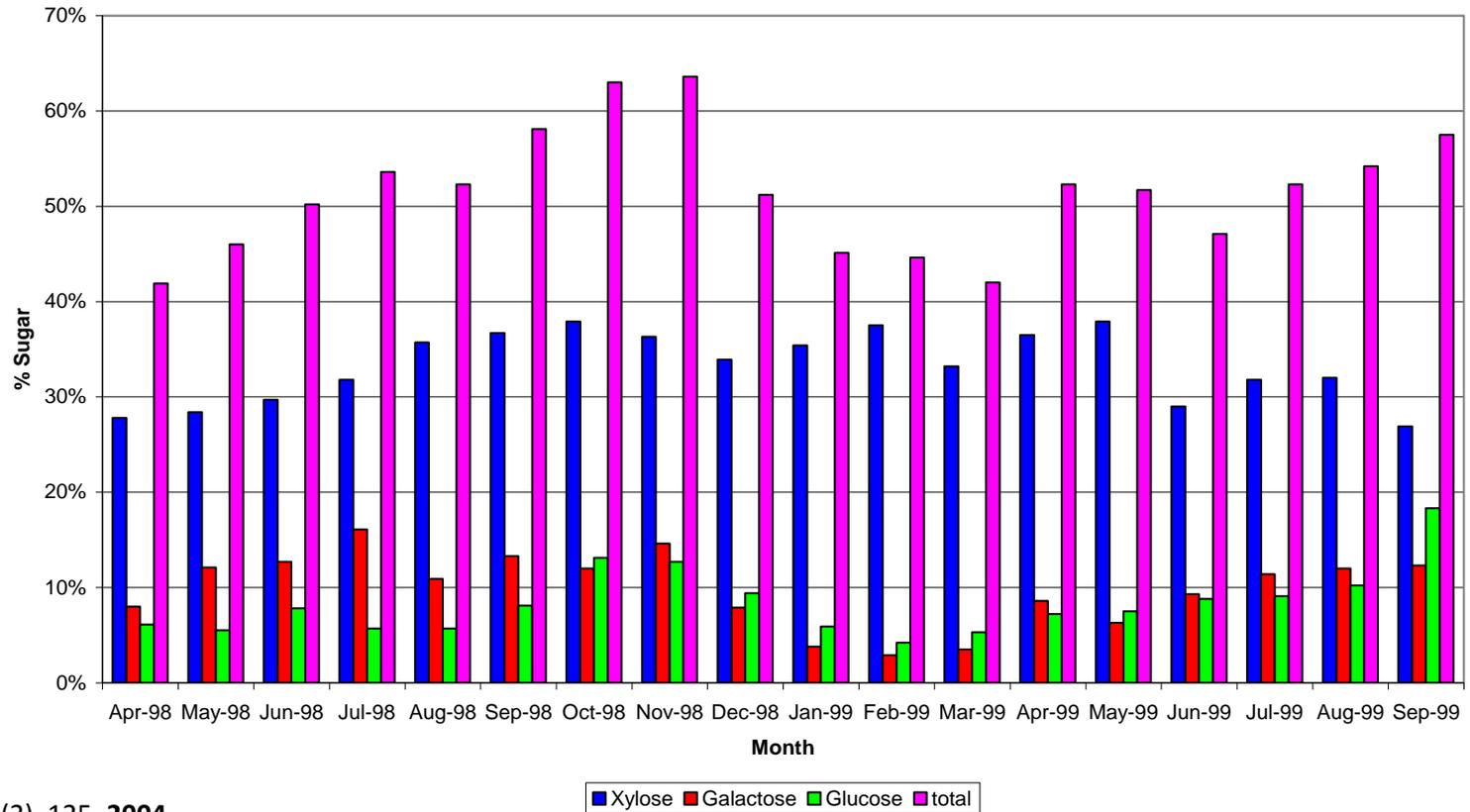


# Major carbohydrate components

Seaweed	Brown	Red	Green
Example species	<i>Laminaria digitata</i>	<i>Palmaria palmata</i>	<i>Ulva lactuca</i>
Carbohydrates in seaweed	Mannitol	Floridoside	Ulvan
	Alginate	Xylan	Cellulose
	Laminarin	Cellulose	
	<i>Fucoidan</i>		
	<i>Cellulose</i>		
Expected components in hydrolysate	Mannitol	Galactose (floridoside)	Rhamnose (ulvan)
	Glucose (laminarin/cellulose)	Glycerol (floridoside)	Glucuronic acid (ulvan)
	<i>Fucose (fucoidan)</i>	Xylose (xylan)	Iduronic acid (ulvan)
	Mannuronic acid (alginate)	Glucose (cellulose)	Glucose (cellulose)
	Guluronic acid (alginate)		Xylose

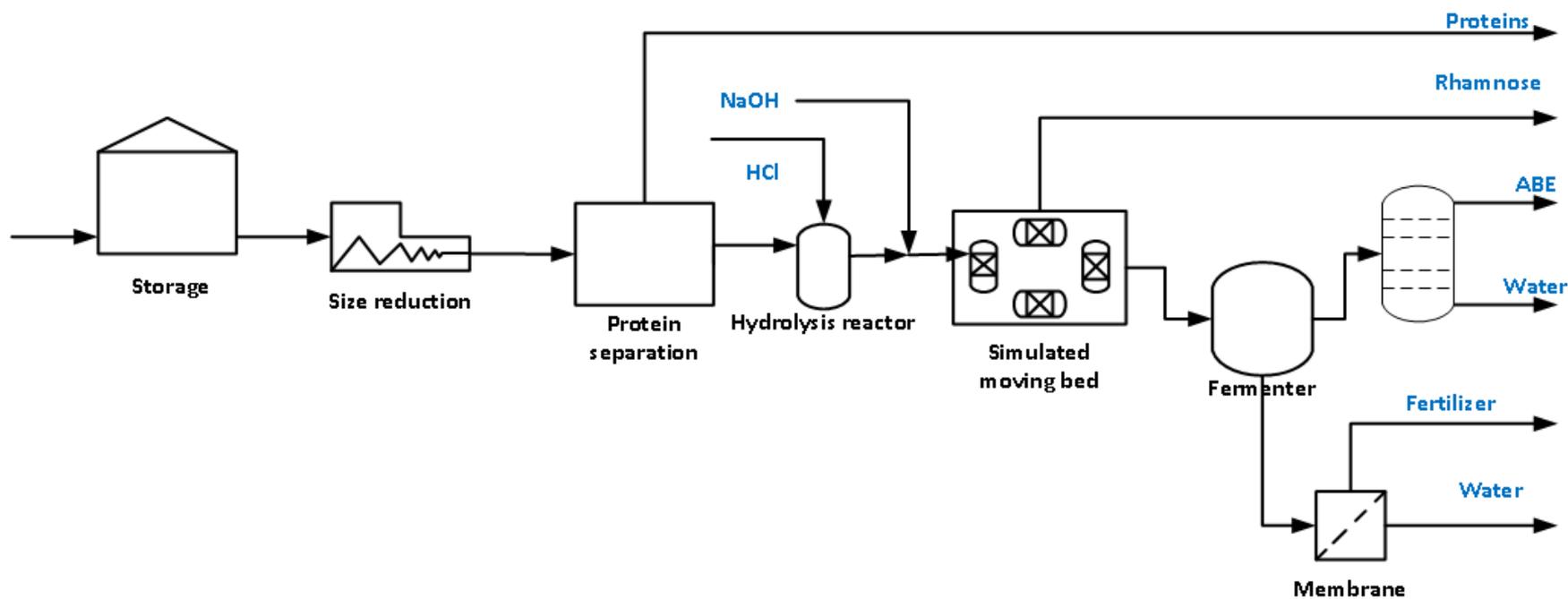
# Example of seasonal variation

Total Carbohydrate composition of *Palmaria Palmata*



*Bot. Mar.* 47(2), 125, 2004

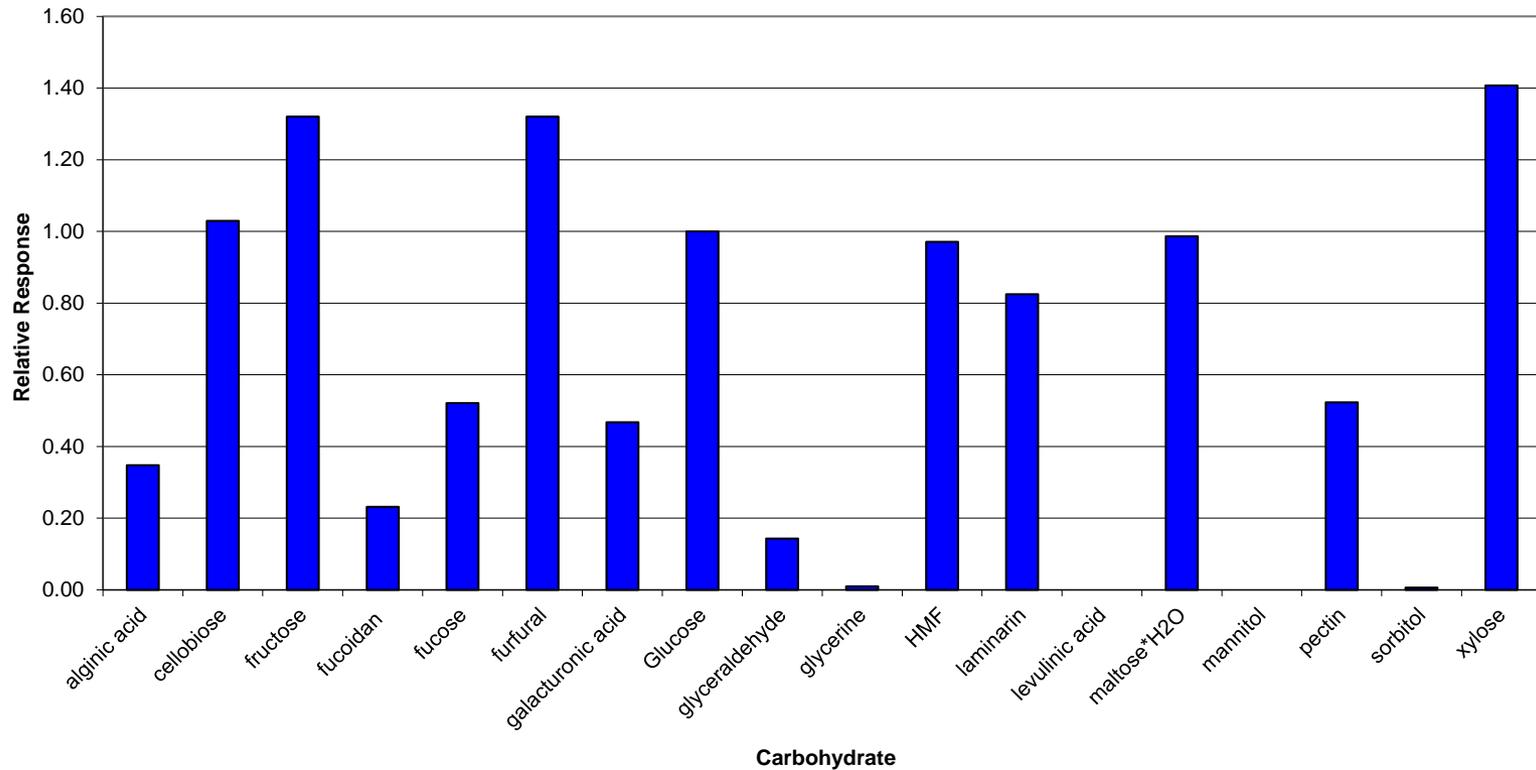
# Ulva biorefinery



<https://www.ecn.nl/publications/ECN-E--16-026>

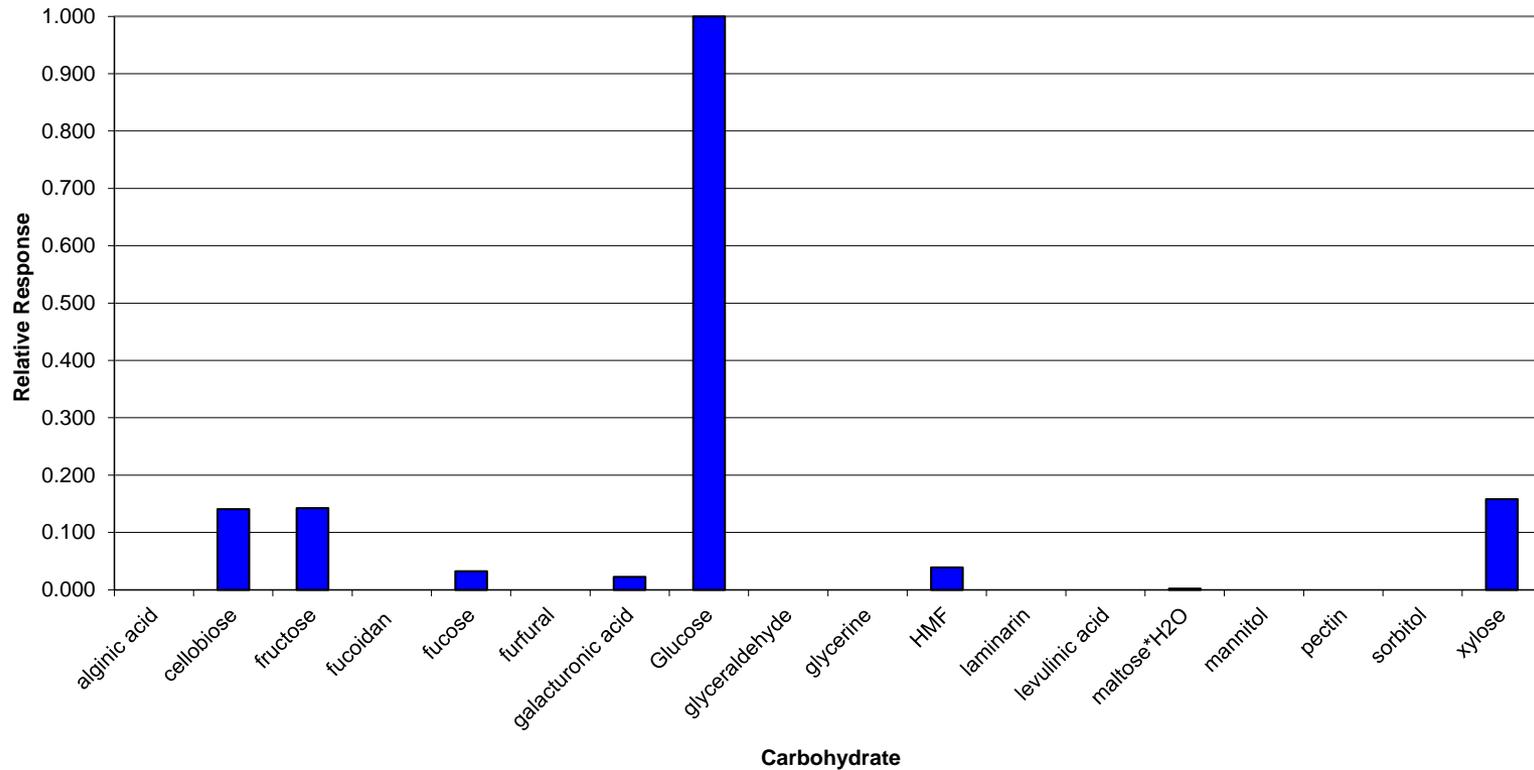
# Limitation of color reactions

Relative Response to Glucose for Phenol/H<sub>2</sub>SO<sub>4</sub> total sugar assay



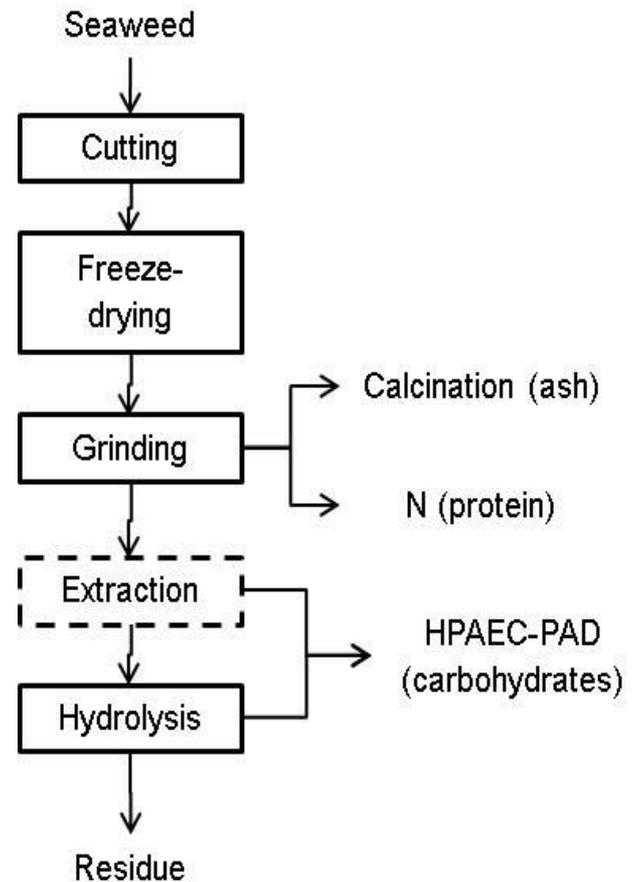
# Limitation of color reactions

Relative Response to glucose for toluidine glucose assay

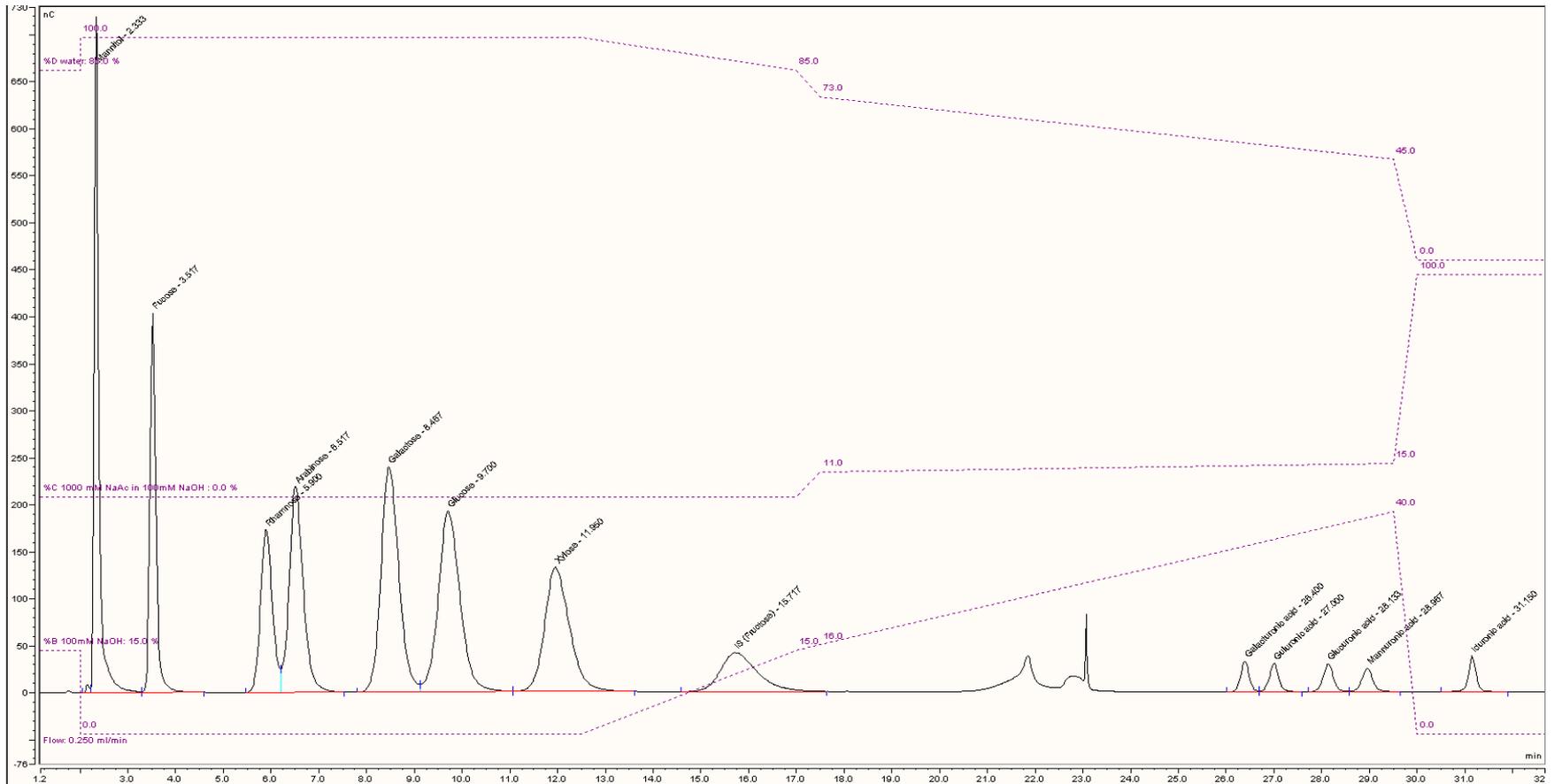


# Seaweed analysis protocol

- Uniform analysis protocol for carbohydrate composition of seaweeds
- Specific hydrolysis conditions optimized per type of seaweed.
- Starting point (protocol LC):
  - Pre-hydrolysis: 12M  $\text{H}_2\text{SO}_4$ , 30 °C, 1 hr.
  - Hydrolysis: 1.2M  $\text{H}_2\text{SO}_4$ , 100 °C, 3 hr.
  - Neutralization.
  - Analysis of monomeric carbohydrates HPAEC-PAD (Pulsed Amerometric Detection)
- **Research questions:**
  - Optimum hydrolysis time(s) for each class of seaweeds (brown, green and red)?
  - Pre-hydrolysis step required for seaweeds?

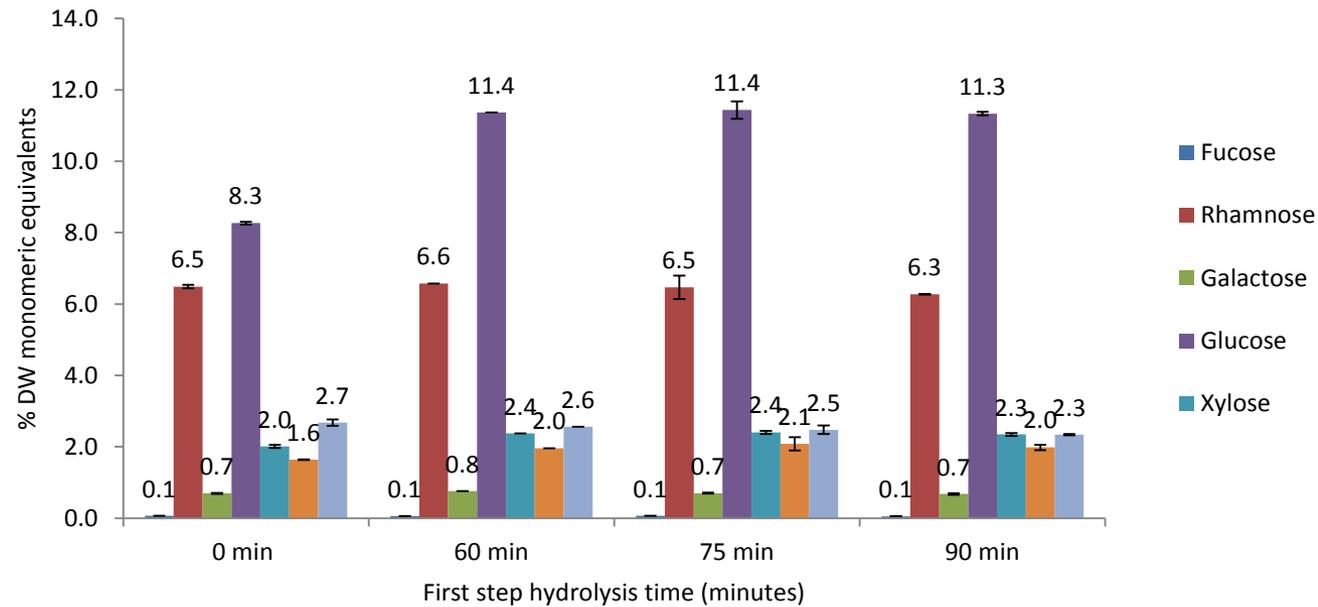


# Optimized HPAEC analysis

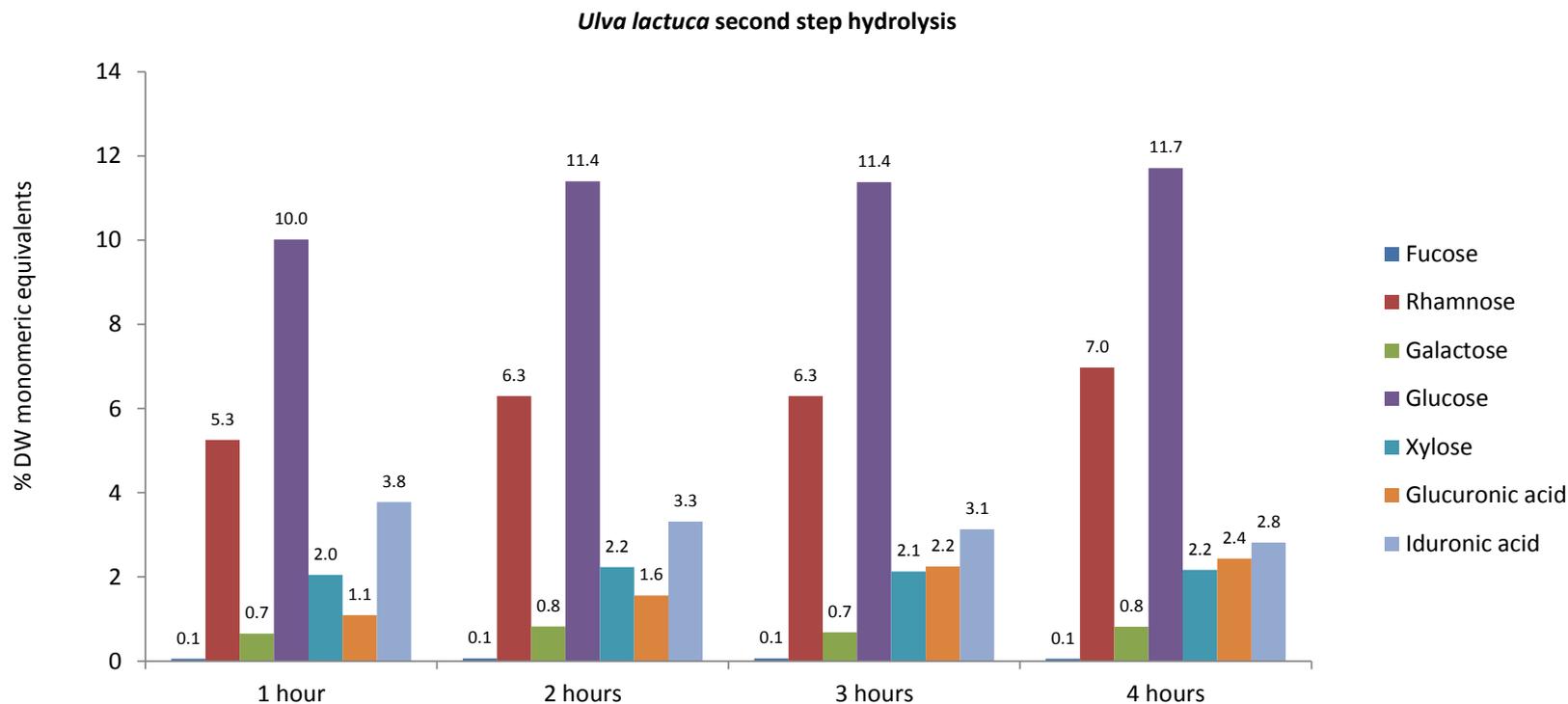


# Optimized hydrolysis Ulva, 1<sup>st</sup> step

## Ulva lactuca first step hydrolysis



# Optimized hydrolysis *Ulva*, 2<sup>nd</sup> step



# Preliminary conclusions

- Pre-hydrolysis step required for sufficient hydrolysis of seaweeds (particularly brown and green).
- Recommended pre-hydrolysis and hydrolysis times:
  - Brown (*Laminaria*): 1 hr. & 4 hr.
  - Red (*Palmaria*): 0.5 hr. & 2 hr.
  - Green (*Ulva*): 1 hr. & 4 hr.
- Analysis of all types of monosaccharides in single HPAEC run.

# Biorefinery

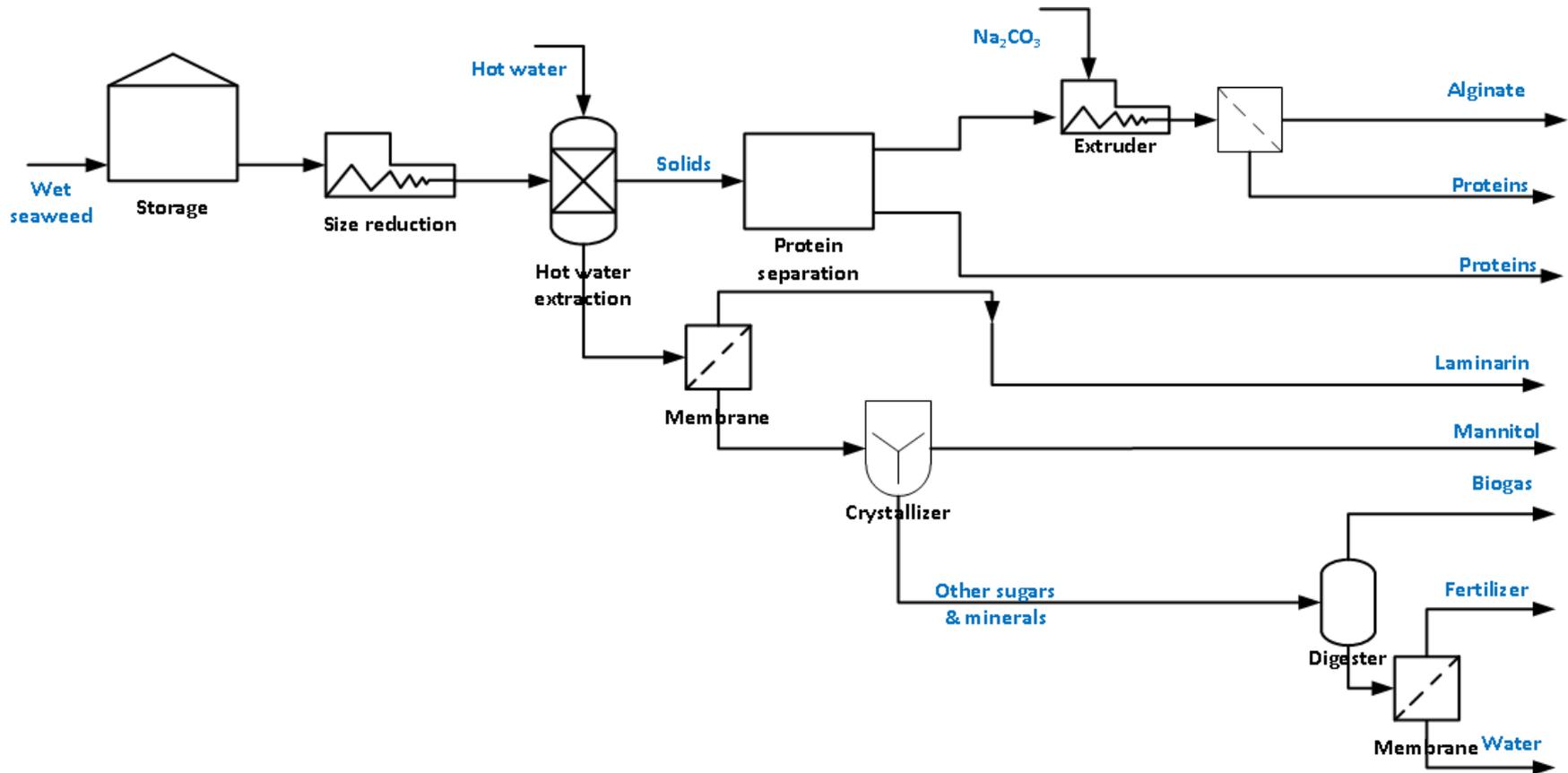
## Fresh Ulva



## Isolated monomeric Sugars



# Biorefinery Kelps



<https://www.ecn.nl/publications/ECN-E--16-026>

# From fresh Kelp to isomannide



# Outlook

- Work ongoing:
  - Extension work to other Kelps (*Alaria*, *Fucus*, *Saccharina*) & carrageenan / agar-containing red seaweeds (*Gracilaria*).
  - Pre-extraction prior to hydrolysis for determination of non-structural carbohydrates (brown: mannitol, red: floridoside).
- Converging towards reliable protocols
- Verification of the use of color reactions for example for quick screening enabled

# Acknowledgement



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# Thank you for your attention

Publications: [www.ecn.nl/publications](http://www.ecn.nl/publications)  
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