Optimal utilization of seaweeds in integrated biorefineries

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Background

Seaweeds are interesting feedstocks for biorefineries. The green seaweed *Ulva lactuca* contains a wide variety of carbohydrates and large amounts of protein and ash and can therefore be used for the production of biofuels, chemicals or animal feeds in integrated biorefineries [1, 2]. Rhamnose, one of the main sugars in *U. lactuca*, can be fermented by *Clostridium beijerinckii* to acetone, butanol and ethanol (ABE) and 1,2-propanediol (1,2-PD) [2]. The catabolic pathway of rhamnose, however, is not yet well studied in this organism.

General objectives

- Characterization of metabolic routes in *C. beijerinckii* from seaweed sugars towards ABE and 1,2-PD.
- Fermentations with *C. beijerinckii* on seaweed fractions to characterize the yield, product ratio and productivity.
- Improve the strain by genetic engineering to increase the economic viability of seaweed-based biorefineries.

The green seaweed *Ulva lactuca*

Next to proteins and ash, *U. lactuca* contains glucose, rhamnose and xylose that can be fermented by Clostridial species. In a biorefinery, all components in biomass are utilized. A biorefinery scheme for *U. lactuca* is shown in Figure 1.

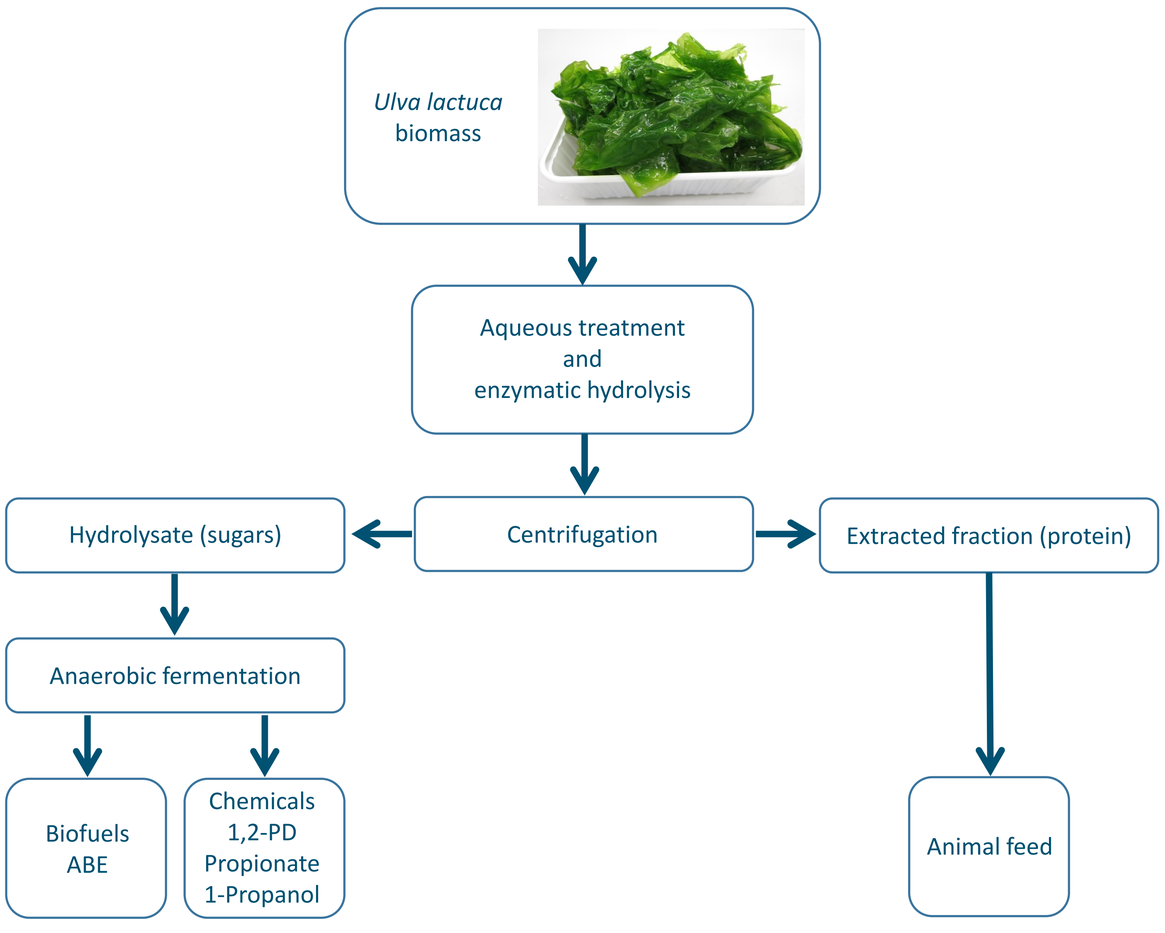


Figure 1. A biorefinery concept using the green seaweed *U. lactuca* as feedstock (modified from [1]).

Rhamnose metabolism in *Clostridium beijerinckii*

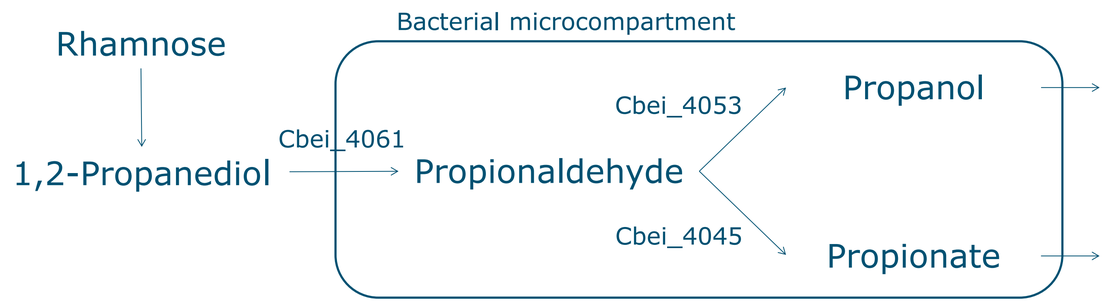
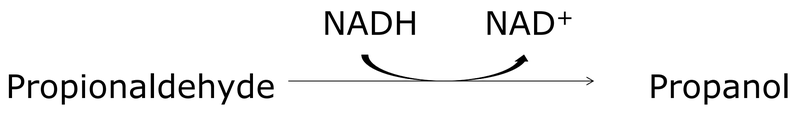


Figure 2. Proposed rhamnose metabolic pathway and the reactions within BMCs [3].

C. beijerinckii ferments rhamnose into ABE and 1,2-PD. In *C. phytofermentans* the presence of a metabolic route for the further metabolism of 1,2-PD has been described [3] (Figure 2).

In *C. beijerinckii*, a cluster of genes homologous to that described in *C. phytofermentans* for the conversion of 1,2-PD into 1-propanol and propionic acid is present in the genome. This cluster consists of 22 genes, including genes encoding enzymes, structural proteins and two encode for proteins with unknown function. The function of this cluster in *C. beijerinckii* is being investigated.

Enzymatic activity of propanol dehydrogenase (Cbei_4053)



The protein encoded by *Cbei_4053* has been overexpressed in *E. coli*. Propanol dehydrogenase activity in the cell-free extracts has been detected (Table 1)

Table 1. Enzymatic activity of Cbei_4053 in *E. coli* cell free extracts.

	Specific activity [U mg^{-1}]
E. coli BL21 [pET100_Cbei4043]	4.20 ± 0.29
E. coli BL21 [pET100]	0.09 ± 0.02

Future aspects

- The enzymatic activity of other enzymes involved in the metabolism of rhamnose and 1,2-PD will be determined.
- The substrate range of the enzymes will be investigated.

Acknowledgements

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References

[1] Bikker *et al.* (2016). Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels. *Journal of Applied Phycology*, 28(150).
[2] van der Wal *et al.* (2013). Production of acetone, butanol, and ethanol from biomass of the green seaweed *Ulva lactuca*. *Bioresource Technology*, 128, 431-437.
[3] Petit *et al.* (2013) Involvement of a bacterial microcompartment in the metabolism of fucose and rhamnose by Clostridium phytofermentans. PLOS One (8)1.

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