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Production of acetone, butanol, and ethanol from biomass of the green seaweed *Ulva lactuca*

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HIGHLIGHTS

- *Ulva lactuca* was characterized as feedstock for the acetone, butanol and ethanol fermentation.
- Hydrolysates were obtained using mild pretreatment conditions and commercial cellulases.
- *Ulva lactuca* hydrolysate was used as substrate for fermentation by two different strains.
- Rhamnose was utilized by *C. beijerinckii* for production of 1,2-propanediol.

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ABSTRACT

Green seaweed *Ulva lactuca* harvested from the North Sea near Zeeland (The Netherlands) was characterized as feedstock for acetone, ethanol and ethanol fermentation. Solubilization of over 90% of sugars was achieved by hot-water treatment followed by hydrolysis using commercial cellulases. A hydrolysate was used for the production of acetone, butanol and ethanol (ABE) by *Clostridium acetobutylicum* and *Clostridium beijerinckii*. Hydrolysate-based media were fermentable without nutrient supplementation. *C. beijerinckii* utilized all sugars in the hydrolysate and produced ABE at high yields (0.35 g ABE/g sugar consumed), while *C. acetobutylicum* produced mostly organic acids (acetic and butyric acids). These results demonstrate the great potential of *U. lactuca* as feedstock for fermentation. Interestingly, in control cultures of *C. beijerinckii* on rhamnose and glucose, 1,2 propanediol was the main fermentation product (9.7 g/L).

1. Introduction

Different types of plant biomass, including energy crops, cereals and agricultural by-products, are promising starting materials for the production of fuels and chemicals, using the so-called biorefinery approach, in which the whole biomass is being valorized into a range of different products. The most developed biorefinery concepts are currently based on lignocellulosic feedstocks, where sugars are fermented into ethanol or other energy carriers, and other fractions (such as lignin) are converted into high value chemical additives (Cherubini, 2010). In order to meet the expected increasing demand for biofuels and biochemicals, and to diversify the feedstock and product portfolio of biorefineries, there is a need to find additional suitable biomass sources, in particular those that do not rely on using large amounts of agricultural land. This necessity has led to an interest into the use of biomass from aquatic environments, such as seaweeds (macroalgae) (Subhadra and Grinson, 2010; John et al., 2011; Kraan, 2011).

Seaweeds are fast growing marine plants that may reach large sizes, for example, 3–20 m length for certain kelp species (Lüning, 1993). Growth rates and yields of material per surface area that can be obtained in seaweeds forests are significantly higher than those reported for terrestrial plants (Gao and McKinley, 1994; Horn et al., 2000), mostly due to the lower energy required for the production of supporting tissue compared to that for land plants and a higher capacity of nutrient uptake over their entire surface. Seaweeds have a wide range of commercial applications, traditionally as feed or food, and soil fertilizer, and nowadays as source of hydrocolloids for the food industry, personal healthcare and pharmaceutical industry (McHugh, 2003). Seaweeds are classified into three broad groups based on pigmentation: Brown (Phaeophyceae), red (Rhodophyceae) and green (Chlorophyceae). Green seaweeds have

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traditionally been a part of local diets due to their high protein content and nutritional value (Bobin Dubigeon et al., 1997), however, hydrolysates from this algae can also serve as feedstock for the production of butanol (Potts et al., 2012). Pre-treatment and hydrolysis methods needed for the solubilization of seaweed sugars are less severe than those needed for pre-treatment of lignocellulosic materials, most probably due to the absence of lignin in their cell walls and the relatively lower crystalline of sugar polymers.

Some species of *Clostridium* are able to produce acetone, butanol and ethanol (ABE) by anaerobic fermentation from a wide variety of sugars, both hexoses and pentoses, a process known as the ABE fermentation. Solvent-producing clostridial species are able to utilize sugars in a variety of substrates, including (lignocellulosic) hydrolysates derived from plant biomass (Qureshi et al., 2010). Since seaweeds are composed of a mix of different sugars, these organisms are expected to efficiently convert most sugars into an ABE mixture (Huesemann et al., 2012). The ABE process is being commercially re-introduced for the production of biologically derived butanol (biobutanol) to be used as biofuel or to replace petrochemically produced butanol in the bulk chemical market (López-Contreras et al., 2010; Green, 2011). Some species of green seaweeds, including *Ulva lactuca*, are rich in the deoxysugar rhamnose (Lahaye, 1991), that can be fermented anaerobically into commercially interesting 1,2 propanediol by a number of microbial species, including some clostridial strains (Forsberg et al., 1987; Saxena et al., 2010).

In the present study, *U. lactuca* L., harvested along the Dutch coast of the North Sea was biochemically characterized as fermentation feedstock. Solubilization of sugars in the biomass was carried out using different pre-treatments followed by enzymatic hydrolysis. Hydrolysates of *U. lactuca* were used as medium for the production of acetone, butanol and ethanol by two different well-studied clostridial strains.

2. Methods

2.1. Source and storage of Ulva samples

U. lactuca was collected along the coast of Zeeland (The Netherlands) in the month of December 2008 and stored at −80 °C for several weeks before being freeze-dried lyophilized and stored at room temperature.

2.2. Analytical protocols

2.2.1. Chemical composition of U. lactuca

Freeze-dried *Ulva* was milled over a 2 mm screen. Ash content was determined by incineration at 575 °C for 4 h. Solvent and water extractives were determined by successive extraction with toluene/ethanol (2:1 v/v), 95% ethanol and boiling water. Sugar content was determined on the residue after solvent extraction and the whole biomass after hydrolysis with 72% w/w H_2SO_4 at 30 °C for 1 h, followed by dilution to a H_2SO_4 concentration of 1 M and hydrolysis for 3 h at 100 °C. The hydrolysate was neutralized with bariumcarbonate and analyzed for neutral sugars on a HPAEC (High Performance Anion Exchange Chroamtography) equipped with a CarboPac PA1 (250 × 4 mm) column with a CarboPac PA1 (50 × 4 mm) guard-column (Dionex), and pulsed amperometric detection, using fucose as internal standard (no fucose was found in *Ulva* samples), as described previously (van den Oever et al., 2003). Uronic acids in this hydrolysate were determined spectrophotometrically by the *m*-hydroxydiphenyl assay using galacturonic acid as standard (Blumenkrantz and Asboe Hansen 1973).

2.2.2. Elemental analysis

Elemental analysis (CHNS) was carried out on a Thermo Quest EA 1110 elemental analyzer. For determining total phosphorus *Ulva* was subjected to sulfuric and nitric acid treatment according to the Dutch Standard Method NEN-6662. Phosphorus was detected spectrophotometrically using molybdate blue at 885 nm according to Dutch Standard Method NEN-6479.

2.2.3. Aminoacids analysis

Amino acid analysis (free or protein-bound amino acids) was performed by Ansynth BV (Roosendaal, The Netherlands) using an Alpha Plus II, Biochrom 20 or Biochrom 30 amino acid analyzer. Standard methods were used for the determination of Tryptophan (AOAC, 1993) and other amino acids (AOAC, 1990).

2.3. Pre-treatment and enzymatic hydrolysis of Ulva

2.3.1. Pre-treatment

Freeze-dried *U. lactuca* was milled over a 2 mm screen and resuspended at 10% (w/w) in water. Mild alkaline pre-treatment was carried out by addition of NaOH at 6% (wt NaOH/wt dry matter) and incubation of the slurry at 85 °C for 4 h. Acid pre-treatment was carried out at pH 2 by addition of H_2SO_4 (7.5% wt H_2SO_4/wt dry matter) and incubation of the slurry at 150 °C for 10 min. Pre-treatment at 85 °C was performed under atmospheric conditions in a stirred stainless steel vessels. For pre-treatment at 150 °C, closed 316L stainless steel cylindrical reactors fitted with thermocouples, were submerged in a silicon oil Haake B bath equipped with a Haake N3 temperature controller (Thermo Fisher Scientific, Waltham, MA, USA) The sample core temperature was recorded (Picotech data collector and software, Picotech, UK), the core temperature was held at 150 °C for 10 min and the cylinders were immersed in an ice bath. Samples were centrifuged at 10,000*g* for 15 min at room temperature. The sugar composition of pellet and supernatant was determined as described in Section 2.2.1. The supernatant was analyzed before and after hydrolysis with 1 M H_2SO_4 at 100 °C for 3 h to distinguish between soluble oligomeric and monomeric sugars and enable the determination of total sugars.

2.3.2. Enzymatic hydrolysis of the pre-treated material

The insoluble fractions of the pre-treated material (pellets) were subjected to enzymatic hydrolysis by a commercial cellulase cocktail (GC220, Genencor). Pellets were dispersed in 50 mM sodium acetate buffer, pH 5.0, containing the antibiotic Pen Strep (Sigma–Aldrich) at 0.02 mL/g of *Ulva*. GC220 was added at 0.3 mL/g of pre-treated *Ulva* (35 FPU/g (Kabel et al., 2006)). Enzymatic hydrolysis was performed at 50 °C for 96 h and the supernatant was analyzed for sugar composition.

2.3.3. Preparation of hydrolysate for fermentation

A hydrolysate was prepared by re-suspending milled *Ulva* in milliQ water at 15% DM (w/w) and incubating the suspension at 150 °C for 10 min in a pressurized 2-L vessel**.** This vessel mixes by head over tail rotation and was equipped with a thermocouple to record the core temperature (Picotech data collector and software, Picotech, UK). Heating of the vessel was achieved by circulation silicon oil with a Haake B bath equipped with a Haake N3 temperature controller (Thermo Fisher Scientific, Waltham, MA, USA).

Enzymatic hydrolysis of the *Ulva* slurry obtained after pretreatment was carried out as described in Section 2.3.2 at 50 °C for 24 h, but without the addition of antibiotics. The pH of the hydrolysate was 5.8. The hydrolysate was centrifuged at 10,000*g* for 15 min, at 4 °C, after which the supernatant was stored at −20 °C.