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Laminaria digitata as a potential carbon source for succinic acid and bioenergy production in a biorefinery perspective

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ABSTRACT

A novel biorefinery concept utilizing macroalgae *Laminaria digitata* to produce succinic acid, and direct the process residues for feed and energy production, is investigated in the present study. Enzymatic hydrolysis was performed at high solid loading (25% w v^{-1}) resulting in solubilization of the carbohydrates to soluble sugars, which accumulated in the liquid hydrolysate. The overall sugar recovery in the macroalgae hydrolysate was 78.23%. *Actinobacillus succinogenes* 130Z was able to ferment macroalgae hydrolysate to succinic acid with a yield of 86.49% (g g^{-1} of total sugars) and an overall productivity of 0.50 g L^{-1} h^{-1}. Removal of carbohydrates from the macroalgal biomass through enzymatic hydrolysis resulted in up-concentration of protein and lipid fractions in the post-hydrolysis solid residue (PHSR). Energy recovery of PHSR and fermentation broth through anaerobic digestion corresponded to 298 and 285 NmL CH_4 g^{-1} VS_{added}, respectively. PHSR could potentially be used for: dietary food additive, fish feed, bioenergy production and added value products. This study opens possibility to conceive different biorefinery scenarios in which the efficient use of the macroalgal biomass fractions can provide numerous added-value bio-based products and energy.

1. Introduction

One of the proposed solutions to reduce the dependency of the worldwide economy on fossil fuels and petrochemicals is to replace oil-refinery derived products with bio-based derived products by means of the so-called biorefineries. A biorefinery can be conceived as the facility or a cluster of biobased facilities that integrate biomass conversion processes and technologies in a sustainable and efficient way to produce a palette of marketable products (food, feed, chemicals, and materials) and energy (biofuels, power and/or heat) from biomass [1]. In this context, a biorefinery, particularly based on aquatic biomass, offers an excellent opportunity to displace fossil fuels and oil-refinery based products. One of the advantages of using aquatic biomass – particularly macroalgae – as feedstock for biorefineries is that during their production the use of arable land and fertilizers, is unnecessary, compared for instance to energy crops or lignocellulosic biomasses, thereby minimizing competition over land, food and feed production [2]. Another advantage is that different macroalgae species (e.g. *Laminaria digitata* and *Saccharina latissima*) can reach carbohydrate content up to 60% DM [3], which makes them suitable substrates for the production of building block chemicals and bioenergy through a biorefinery approach. Building block chemicals are molecules with multiple functional groups that possess the potential to be transformed into new families of useful molecules [4].

Succinic acid has been recognized as one of the twelve most promising building block chemicals that can be produced from sugars via biological or chemical conversions [4]. At present, succinic acid is mainly produced by petrochemical-based process from *n*-butane/butadiene via maleic anhydride utilizing the C4-fraction of naphtha [5]. Succinic acid is used as precursor for the production of numerous commodities in agricultural, food, chemical, and pharmaceutical industries. When succinic acid is produced via fermentation-based processes using a renewable substrate, it is referred to as biosuccinic acid or bio-based succinic acid. Currently however, biosuccinic acid production is not competitive with the petrochemical-based process, mainly due to its high production cost [5,6]. Hence, there is a need to develop cost-effective conversion technologies to produce succinic acid from inexpensive renewable resources as, for example, macroalgal biomass. Furthermore, succinic acid production via fermentation consumes CO_2, which can definitely improve the sustainability indicators of the biorefinery process.

Therefore, the primary aim of this study was to evaluate the brown macroalgae *L. digitata* as a potential feedstock for advanced biorefinery scenarios to produce the building block succinic acid. Utilization of all the organic content to useful products would increase the sustainability of a biorefinery approach. The anaerobic digestion process has a versatile preference for organic compounds, where carbohydrates, proteins,

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Nomenclature

DM	dry matter
PHSR	post-hydrolysis solid residue
BMP	biochemical methane potential
FAME	fatty acid methyl esters
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
MUFA	monounsaturated fatty acids
VFA	volatile fatty acids

lipids, volatile acids, and other compounds can easily be converted to biogas. Therefore, we selected to convert the residues from the succinic acid's fermentation to biogas. Thus, a secondary aim was to determine the biochemical methane potentials (BMPs) of the leftover residues after the enzymatic hydrolysis (post hydrolysed solid residue-PHSR) and the fermentation broth after succinic acid production, as well as of the macroalgal biomass itself. Finally, the PHSR was characterized in terms of protein and lipid content to evaluate its potential use as feed or supplementary purposes.

2. Materials and methods

2.1. Chemicals and gases

All chemicals and enzymes used in this study were of analytical grade and were purchased from Sigma Aldrich ApS (Brøndby, Denmark) and gases were supplied by AGA A/S (Copenhagen, Denmark).

2.2. Sample collection and preparation

L. digitata samples were collected in early August 2012 at Hamborg Strand (north of Hanstholm at the Danish North Sea coast) and stored at −20 °C until analysis and experiments were performed. Part of the *L. digitata* material was dried in a Lytzen Oven at 50 °C until the material was crunchy, not elastic. During the drying process, the material was turned frequently to allow all material to dry efficiently. After four days, all the material was dried until moisture content was <10%. Retsch SM 2000 cutting mill was used to reduce the particle size of the dried macroalgae material to <2 mm which was used for experiments. For all analysis, the material was ground into powder using a Siebtechnik Screening disc mill TS 250.

2.3. Preparation of macroalgae hydrolysate

Dried and ground macroalgae material was weighted and mixed with distilled H_2O (250 g L^{-1}) for high substrate loading. The pH of the mixture was adjusted to 4.8, followed by sterilization at 121 °C for 20 min. Enzymes used for hydrolysis were: Celluclast 1.5 L (cellulase), Novozyme 188 (β-glucosidase) for hydrolysis of laminarin and alginate lyase for hydrolysis of alginate to reduce viscosity. Enzyme loadings were, Celluclast 1.5 L: 40 U gDM^{-1}; Novozyme 188: 40 U gDM^{-1}; and Alginate lyase; 10 U gDM^{-1}. Enzymatic hydrolysis was performed in a shaker at 50 °C and 150 rpm for 48 h. All enzymes used in this work were purchased from Sigma Aldrich ApS (Brøndby, Denmark). After enzymatic hydrolysis the hydrolysate was poured into 50 mL Falcon tubes and centrifuged at 10.000 G-force for 15 min. The supernatant (liquid hydrolysate) was collected and stored at −20 °C prior to use. The post-hydrolysis solid residue (PHSR) left over from the centrifugation step was washed with water and centrifuged again. This procedure was repeated three times, in order to remove any residual liquid hydrolysate from the solid residue. PHSR was dried at 105 °C overnight and ground into powder using a screening disc mill for further characterization.

2.4. Microorganisms, medium and seed culture growth

The strain of *Actinobacillus succinogenes* 130Z (DSM 22257) was obtained from DSMZ. The culture stock was stored in glycerol at −80 °C prior to use. Seed culture medium was composed of (g L^{-1}): glucose (10.0), yeast extract (5.0), $NaHCO_3$ (10.0), $NaH_2PO_4 \cdot 2H_2O$ (9.6), $K_2HPO_4 \cdot 3H_2O$ (20.3). Medium was sterilized at 121 °C for 20 min. Seed culture was cultivated at 37 °C and 150 rpm in 50 mL sealed anaerobic bottles containing 30 mL medium and inoculated with 1 mL of −80 glycerol stock culture. Pure N_2 gas was used to establish anaerobic conditions in the bottles.

2.5. Cultivation and fermentation of macroalgae L. digitata hydrolysate

Two sets of batch fermentations were carried out at different working volumes. First, small batch fermentations of macroalgae *L. digitata* hydrolysate were conducted in triplicates in 200 mL sealed anaerobic bottles filled with 100 mL of fermentation medium, containing the following (g L^{-1}): yeast extract (10.0), K_2HPO_4 (3.0), $MgCl_2$ (0.2), $CaCl_2$ (0.2), NaCl (1.0), $MgCO_3$ (30.0). Sterile macroalgae *L. digitata* hydrolysate (20 mL) was injected into sterile batch bottles. Bottles were flushed with pure N_2 to create anaerobic conditions and inoculated with 5% (v v^{-1}) of exponentially growing inoculum. Following, bottles were incubated in shaker at 37 °C and 150 rpm for 48 h.

Subsequently, batch fermentation of macroalgae *L. digitata* hydrolysate was conducted in duplicates in two identical 3-L fermenters (Sartorius BIOSTAT Aplus, Germany) with an initial working volume of 1.5 L. The fermentation was conducted by mixing sterile *L. digitata* hydrolysate and synthetic medium at 1:1.5 ratio. The synthetic medium was composed of (g L^{-1}): yeast extract (16.7), K_2HPO_4 (5.0), $MgCl_2$ (0.3), $CaCl_2$ (0.3), NaCl (1.7), $MgCO_3$ (67.5). Medium was sterilized at 121 °C for 20 min prior to mixing. All batch fermentations in 3-L bioreactors were inoculated with 5% (v v^{-1}) of exponentially growing inoculum at 37 °C, 200 rpm for 48 h. Prior to the start of batch fermentation, pH was adjusted to 6.8 using 50% phosphoric acid and 0.05 mL of sterile Antifoam 204 (Sigma Aldrich) was added. Sodium hydroxide solution (8 M) was added automatically to maintain the pH at 6.8. N_2 gas was used to create anaerobic conditions in the fermenters.

2.6. Biochemical methane potential assay

Biochemical methane potential (BMP) was determined according to Angelidaki et al. [7] in 320 mL glass vessels (batch reactors). A thermophilic (53 ± 1 °C) methanogenic inoculum derived from Snertinge centralized biogas plant in Denmark, was used (80 mL) in the batch reactors. The inoculum was allowed to degas for seven days in an incubator prior to use. The basic characteristics of the inoculum used in the BMP assay are given in Supplementary data (Table S1). Two different concentrations of the biomasses (5 and 2 g VS L^{-1}, respectively) were tested separately in distinct batch reactors and water was added up to final working volume of 100 mL. Avicel® PH-101 cellulose (Sigma Aldrich) was used (2 g VS L^{-1}) to validate the accuracy of the BMP assay process. Batch reactors only with inoculum and water (blanks) were included to determine the residual methane production from the inoculum. Finally, the batch reactors was flushed with a N_2/CO_2 (80/20% v v^{-1}) gas mixture, closed with rubber stoppers and aluminum caps, and incubated for a minimum of 30 days. All BMP experiments were performed at least in triplicates.

2.7. Analytical methods

Total solids (TS) or dry matter (DM), volatile solids (VS) and ash contents were determined as described in Standard Methods [8]. Total