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Valorization of macroalga *Saccharina latissima* as novel feedstock for fermentation-based succinic acid production in a biorefinery approach and economic aspects

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ABSTRACT

This study aimed to evaluate the potential of the macroalga *Saccharina latissima* as feedstock for fermentation-based succinic acid production in a biorefinery approach. Seasonal variations in the content of carbohydrates, and fermentable sugars, had a significant impact on the succinic acid yield and titer. A maximum succinic acid yield of 91.9% (g g^{-1} of total sugars) corresponding to 70.5% of the theoretical maximum yield was achieved when a blend of macroalgal biomass cultivated over two growing seasons and harvested in July and August was used as feedstock. A succinic acid titer of 36.8 g L^{-1} with a maximum productivity of 3.9 g L^{-1} h^{-1} was achieved. The high content of total phenolic compounds (TPCs) in the macroalgal biomass (July–August: 5–1% DM), and high concentration of macro- (Ca, K, Na, Mg, P, N and Fe) and micronutrients in the solid residue recovered after enzymatic hydrolysis (PHSR), makes co-production of antioxidants (i.e. phenolics) and fertilizer very attractive. Finally, a simplified economic assessment showed that for the analyzed scenarios the main product's selling price (succinic acid) can be lowered significantly by coproducing added value products (fertilizers) and high added value-lower volume products (antioxidants).

1. Introduction

Biorefinery has been defined as the facility or a cluster of facilities that integrate biomass conversion processes and technologies to produce a palette of marketable products (food, feed, chemicals, and materials) and energy (biofuels, power and/or heat) from biomass in a sustainable and efficient way [1]. Biorefinery has the potential to partially or totally displace oil-refinery through the production of equivalent bio-based fuels and chemicals. Moreover, biorefineries aim to play a key role in promoting bio-based economy. The biorefinery product portfolio includes besides biofuels and biochemicals, also platform molecules or building blocks which can be converted to a number of other useful chemicals through chemical reactions [2].

Succinic acid is recognized as one of the top building blocks by US Department of Energy [3]. Succinic acid is mainly used as an ion chelator, a surfactant and as additive in pharmaceuticals and foods [4] and its current global market ranges from 30,000 to 50,000 tons annually [6]. This building block chemical can be used as a platform for the production of a number of commodities, especially chemicals, but also amino acids, vitamins, pigments, etc. [7]. Some of the chemicals in high demand that could be produced using succinic acid as a precursor include 1,4-butanediol, ethylene diamine disuccinate, diethyl succinate and adipic acid [4]. Moreover the theoretical global market value for succinic acid was estimated at US$ 14.1 billion, considering a scenario where it replaces 100% of its petrol derived equivalents in their end use application [5].

Currently, however, succinic acid is mainly produced via petrochemicals from butane through maleic anhydride contributing to greenhouse gas emissions [8]. Environmental concerns along with the predictable increase demand for succinic acid has driven the attention to fermentation-based succinic acid as a sustainable alternative to petrol derived succinic acid [9,10]. While using renewable biomass as carbon source, fermentation-based succinic acid production also consumes CO_2 [4]. Indeed, it has been estimated that production of bio-succinic acid as an alternative to petro-based succinic acid would result in a CO_2 emission savings of approximately 4 to 4.5 tons per ton of succinic acid produced [11,12]. However, bio-succinic acid production is still struggling to become competitive with the production cost of petro-based succinic acid. In this context, some of the key challenges fermentative succinic acid production has to overcome include: utilization of inexpensive carbon sources; high succinic acid yield and titer (120–150 g L^{-1}) with none or very little by-product formation, maximizing sugar utilization and reducing the cost of product extraction/purification; and increase productivity from the current 1–2 g L^{-1} to approximately 2.5–5 g L^{-1} h^{-1} [4,13]. Moreover, succinic acid production in a biorefinery approach with extraction of high value products has the potential to improve the economic indicators of the overall process.

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Nomenclature

DM	dry matter
PHSR	post-hydrolysis solid residue
TES	total extractable substances
TPCs	total phenolic compounds

Actinobacillus succinogenes 130Z is considered one of the most promising succinic acid producers due to its ability to produce succinic acid naturally as major product from a wide variety of carbon sources (e.g. crude glycerol, lignocellulosic biomass, macroalgae) [10,14,15], and its tolerance to high initial sugar concentrations (up to 143 g glucose L^{-1}) [16] and high product titer (80 g L^{-1}) [17].

Macroalgae constitute an abundant, renewable resource with a high carbohydrate content (up to 60% of dry matter), but also, a wide spectrum of bioactive compounds such as vitamins, minerals, pigments, proteins, lipids and polyphenols [18], which makes them a very attractive feedstock for fermentation process and biorefinery in general. Macroalgal production does not require agricultural land, fresh water, or fertilizers and thus does not compete for resources with land-based food/feed crops. Moreover, mass-cultivation of macroalgae is possible using available farming technology developed over decades in Asian countries [19]. Additionally, macroalgae do not contain lignin, or only to a negligible extent, making harsh pretreatments of the biomass prior to the saccharification process unnecessary, which represents obvious economic and environmental benefits. The chemical composition of macroalgae, including the carbohydrate content, varies seasonally [20,21]. Thus harvest time will have an important effect on the fermentable sugar concentration after hydrolysis and titer of the fermentation product. A previous work has shown the suitability of macroalgae as biorefinery feedstock but the importance of seasonality and cultivation period (i.e. age) was not addressed, which can have an important impact in the techno-economic performance of the biorefinery [15].

This study aimed to evaluate the potential of the macroalga *Saccharina latissima* as feedstock for fermentation-based succinic acid production in a biorefinery approach. The effect of harvest time (season) and cultivation period (age) in the carbohydrate content and concentration of fermentable sugars after enzymatic hydrolysis, and succinic acid yield and titer were accessed. The leftover residues after enzymatic hydrolysis (post-hydrolysis solid residue-PHSR) were characterized for total phenolic compounds, a high value commodity which should improve the cost efficiency of the proposed biorefinery. Moreover, the protein content and amino acid composition, and mineral content of the PHSR were determined to evaluate its potential to be used as ingredient for feed, and/or fertilizer. Finally, a simplified economic assessment was performed for advanced macroalgal biorefinery scenarios considering co-production of succinic acid, fertilizer, polyphenols and methane as well as CO_2 savings — due to biogas upgrading — and credits for CO_2 consumption.

2. Materials and methods

2.1. Chemical 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 of macroalgal biomass

S. latissima biomass was harvested from a commercial cultivation area granted to Hjarnø Havbrug A/S located just outside of Horsens Fjord (55°47.529′ N, 10°03.027′ E). The cultivation system consisted of 200-meter longlines to which seeded cultivation lines (droppers; 4 m in length) were attached approximately 1 m apart from each other. *S. latissima* biomass was sampled monthly from May 2013 to May 2014. Samples were collected from three randomly selected cultivation lines. For more details see Marinho et al. [22]. If present, sand and debris were removed from samples, but not the epiphytes. Prior to chemical analyses and experiments, macroalgal biomass was frozen (−20 °C), freeze-dried and milled using a Siebtechnik Screening disc mill TS 250.

2.3. Characterization of macroalgal biomass

As starting point a seasonal carbohydrate (glucose and mannitol) and total phenolic compound (TPC) profiling was conducted in macroalgal samples cultivated over one growing period. In addition, these compounds were also quantified in some macroalgal samples cultivated over two growing seasons (July S2 and August S2).

2.4. Preparation of macroalgal hydrolysate

From the seasonal profiling results, macroalgal samples with the highest glucose and mannitol content were identified and used to perform a first set of enzymatic hydrolysis experiments with a substrate loading of 15% (w w^{-1}). Performance of enzymatic hydrolysis was evaluated based on the efficiency calculated as follows:

$$Hydrolysis\ \ efficiency(\%) = \frac{Glu\,cose_{Released}}{Glu\,cose_{Feedstock}} \times 100$$

where:

$Glucose_{Released}$ — the amount of glucose released after 48 h of enzymatic hydrolysis;

$Glucose_{Feedstock}$ — the total amount of glucose in freeze-dried macroalgae.

Based on the enzymatic hydrolysis efficiency and concentration of fermentable sugars, the best biomass (July S2) was selected to perform a second set of enzymatic hydrolysis experiments. Three different substrate loadings were tested 15, 20 and 25% (w w^{-1}), resulting in three different qualities of hydrolysate. Finally, in order to increase the harvesting period and the biorefinery portfolio, enzymatic hydrolysis of a mix of macroalgal biomass harvested in July 2S and August 2S (mass ratio 1:1) was performed in a 3-L fermenter.

In all enzymatic hydrolysis experiments the pH was adjusted to 4.8, followed by vat pasteurization at 70 °C for 15 min. Enzymes used for hydrolysis were: Cellulase and β-glucosidase for hydrolysis of laminarin, and alginate lyase for hydrolysis of alginate to reduce viscosity. Enzymatic loadings were, Cellulase: 40 U g DM^{-1}; β-glucosidase: 25 U g DM^{-1} (10 U g DM^{-1} for the 3-L-fermenter trial); Alginate lyase: 10 U g DM^{-1}. Samples were incubated in a platform shaker at 50 °C and 150 rpm for 48 h. After completion of enzymatic hydrolysis, the macroalgal slurries were poured into 50 mL Falcon tubes and centrifuged at 10,000*g* for 15 min. The liquid fractions (macroalgal hydrolysates) were collected and stored at −20 °C prior to use while the post-hydrolysis solid residues (PHSR) left over from the centrifugation step were freeze-dried and ground into powder using a Siebtechnik Screening disc mill TS 250 for further characterization.

2.5. Preparation of macroalgal and post-hydrolysis solid residue (PHSR) extracts for total phenolic compound (TPC) determination

Preparation of macroalgal and PHSR extracts was performed according to Wang et al. [23] with some modifications. Freeze-dried and ground macroalgal and PHSR aliquots (400 mg dry weight) were extracted with 10 mL of 70% aqueous acetone solution. Samples were incubated in a platform shaker for 24 h at 200 rpm and at room temperature in the darkness. Afterwards, samples were centrifuged