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Monitoring complex monosaccharide mixtures derived from macroalgae biomass by combined optical and microelectromechanical techniques

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ABSTRACT

To foster the development of macroalgal biomass for biorefinery applications, we tested two orthogonal techniques for rapid phenotyping of the green macroalga *Ulva* based on its glucose, rhamnose, xylose and glucuronic acid contents as derived for reference by acid hydrolysis. Partial Least Squares (PLS) regression analyses, calculation of slopes and correlations across different spectral ranges/frequencies were used to predict the monosaccharide contents using two complementary methods: near infrared reflection spectroscopy (NIRS) and microelectromechanical systems (MEMS) resonating membrane vibrometry. Both methods were found to perform sufficiently well in monosaccharide mixtures and to enable quantitative assessment of different monosaccharide contents with the relative Root Mean Square Error of Prediction (%RMSEP) ranging from 8 to 16% (with similar accuracy when using PLS analyses). The best estimation was found for rhamnose and glucose contents, whereas xylose and uronic acid content predictions were found to be less accurate using PLS analyses. For the two latter components, slopes across different spectral ranges and frequencies at certain signals provided better estimates for their concentrations (e.g. for NIRS slopes: R^2 values in the range 0.55–0.66 and with higher accuracy for MEMS: between 0.75 and 0.90). This result is pivotal for opening new perspective to the construction of simple, multi-functional sensors for biomass downstream processing control in biorefinery and biometric applications.

1. Introduction

Bioeconomy provides a possible solution for the demand on the natural resources by substitution of the nonrenewable resources with resources derived from biomass [1]. A fundamental unit that will enable the bioeconomy implementation is biorefinery. Biorefinery is a collective term for the complex system that includes biomass production, transportation, conversion into products and distribution. A key component of biomass is its monosaccharide content. Monosaccharides can be directly used in food, cosmetic and industrial applications, such as batteries and paper, or, alternatively, they can be fermented by microorganisms to advanced products including biofuels. Biomass composition is very diverse and each of the composing monosaccharides has its own industrial applications and market values. In addition, the structure and relative abundance of the monosaccharides predicate the downstream processing for fractionation, purification and fermentation [2]. Because of the vast diversity in the chemical composition of the macroalgae biomass feedstock for biorefinery [3], there is a clear need for methods for rapid quantification of monosaccharides in the biomass or its derived intermediate products. Upon widespread availability, these methods will enable rapid process adaptation for the variation in the raw material input, thus increasing energetic and environmental efficiency.

Reflectance spectroscopy of solid particles in the VIS-NIR-SWIR region (400–2400 nm) is a well known technique by which a material chemical composition can be rapidly and quantitatively assessed [4–6]. Since the late 1970s, qualitative and quantitative applications of near infrared spectroscopy (NIRS) in various fields including pharmaceutical [7], food [8], textile industries [9], and fresh plants [10] have grown dramatically. In macroalgae, spectral signatures based on absorption features are indicative of the macroalgal type and condition [11]. The synergy of multivariate statistical methods (such as Partial Least Squares Regression (PLS) and Principle Component Analyses (PCA)) is very useful for extracting quantitative information from NIR spectroscopy. For example, dry matter, nutrient content, oil, protein, salinity and plant diseases have been accurately estimated in fresh vegetation samples. NIR spectral range (700–2500 nm) in combination with regression analyses was implemented to study sugar concentrations in

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fruits (e.g. apples, mango, passion fruit), vegetables (e.g. potato, sugar beet) and dry cakes [12–16]. Interestingly, Oliveira et al. [14] found that NIR spectroscopy was superior in determination of total acid content, while the best results for glucose, fructose, sucrose, total sugar and citric acid contents were obtained using mid-infrared spectral range. Rady and Guyer [15] highlighted on a potential of using selected wavelengths to estimate sugar content in potatoes. In the last decade, many studies also focus on the applicability of using NIRS for biomass analyses and biomass processing monitoring [17–19]. For example, [19] built multispecies feedstock models for composition, as well as monosaccharide release and yield.

Extensive research has been also conducted in using light spectroscopy in the field of macroalgae biomass characterization (e.g. [10,20–22]). Robic et al. [23] applied PLS regression using reflectance measurements to characterize the chemical composition of Ulvan, a major polymer of *Ulva*, common cosmopolitan green macroalgae, with functional properties, making it viable for Ulvan industrial production. Shefer [24] explored the direct *Ulva* biomass monosaccharides phenotyping based on spectral slopes and spectral index analyses of monosaccharides, where low and high contents of glucose, rhamnose, xylose and glucuronic acid from fresh and dried tissues of *Ulva fascinata* were successfully distinguished.

Micro- and nano-electromechanical systems (MEMS/NEMS) devices operating near vibrational resonance are emerging tools in chemical analysis [25–27], where high sensitivity and selectivity to specific components is potentially unlimited [26,28–30]: for example, recently in-line piezoelectric MEMS cantilever resonators have been used to monitor the sugar composition in wine fermentation process [31]. Surface defined chemical characterization can thus provide for a powerful tool augmenting far-field optical characterization techniques. While very large scale integration (VLSI) of MEMS/NEMS resonator arrays for fast chemical analysis has been proven as viable [32], some caveats still exist. The most central practical problem with the deployment of MEMS/NEMS devices, specifically occurring in dynamic rather than static wet ambient operation [33], is the strong dependence of the measurement on the cleanliness of the sensor surface, limiting both actuation efficiency and quality factors below their theoretical limits [34,35]. This has been overcome with the use of open-gap (single-film co-located electrode) [36] rather than close-gap [37] membrane resonator geometry. In the latter geometry for micro-membranes, operation in harsh aqueous conditions is achieved in single-layer membrane geometry [38]. In the current paper, rather than using multiplexed physical arrays of MEMS resonators with different characteristics (e.g., geometry or coating), a singular membrane is used in exposure to the sugar complexes, and the measured spectra are analyzed with advanced statistical methods in conjunction with the NIRS characterizations.

Macroalgae are an emerging sustainable feedstock for biorefineries [39]. However, marcroalgae feedstock show a large variation and diversity of the composing monosaccharides, making it a challenging feedstock for biorefinery. In the previous work we showed that adaptation of the microorganism number and type to specific monosaccharides composition of the fermenting media increases the efficacy of monosaccharides conversion to bio-ethanol [2]. However, rapid determination and quantification of monosaccharides in the macroalgae biomass hydrolysates, prepared for fermentation are lacking. In this work, we exemplify the application of two complementary methods on the monosaccharides determination in the green macroalgae *Ulva* hydrolysates, a potential input material for multiple fermentation processes [27]. Our goal is therefore to rapidly quantify the monosaccharide content in the acid hydrolysates of the *Ulva* biomass. Rapid quantification of the major monosaccharides in the hydrolysates will allow adaptation of fermentation processes to increase the conversion efficiency.

2. Materials and methods

We used two complementary methods to predict the monosaccharide contents: near infrared reflection spectroscopy (NIRS) and microelectromechanical (MEMS) resonating membrane vibrometry.

2.1. Monosaccharide and sugar acid content determination with ion chromatography

Macroalgae from *Ulva* genus were collected in Tel Aviv, Israel in May 2015. The biomass dried in an oven at 40 °C until constant weight. The dried biomass was made brittle by liquid nitrogen and then grinded into powder manually in a mortar. The *Ulva* powder was sieved by 30 mesh sieve to make sure all particle sizes are smaller than 0.5 mm. All chemicals and standards were purchased from Sigma–Aldrich (Israel) if not otherwise mentioned.

Thermochemical deconstruction for monosaccharides release was conducted in 10 mL centrifuge tubes (Nalgene™ Oak Ridge High-Speed PPCO, Thermo-Fisher Scientific, CA) in autoclave (Tuttnauer 2540MLV, Netherlands). For each batch, dried samples of *Ulva* were weighed on analytical balance (Mettler Toledo, Switzerland), sulfuric acid (Sigma–Aldrich, Israel) was injected into the tube and the mix was vortexed to make the powder well distributed in acid. After deconstruction, the hydrolysates were neutralized by sodium hydroxide (Sigma–Aldrich, Israel). All the solid/liquid ratio, acid concentrations, hydrolysis time and temperature are shown in Table 1.

Dionex ICS-5000 platform (Thermo Fischer Scientific, MA, USA) was used to quantify released monosaccharides in hydrolysate. We used standard HPLC method for sugar analysis in the biomass hydrolysate [40]. Carbopac MA1 and its corresponding guard column were used for separation. An electrochemical detector with AgCl was used as reference electrode for detection. A trinary solvent system was used for elution as shown in Table 2. The column temperature was kept at 30 °C and the flow rate was set to 0.4 mL min^{-1}. Calibration curves were produced for rhamnose, glucose, xylose and glucuronic acid on gradient to determine the concentration of corresponding substances in the hydrolysate. All uronic acid peaks were integrated and calculated accordingly using the calibration curve of glucuronic acid (UA) for estimation. The concentrations of the rest of the released monosaccharides were negligible. For spectral analysis 10 μL of hydrolysates was dried at 40 °C on the microscope glass.

2.2. Optical and mechanical measurements

For NIRS analyses, we conducted a preliminary study to determine the best background on which to trap the monosaccharide released

Table 1
Protocols used for *Ulva* biomass deconstruction to monosaccharides.

#j	T (°C)	Time (min)	%Acid	%Solid
1	100	30	0	5
2	100	45	0.5	15
3	100	60	2	25
4	100	45	5	5
5	121	30	0.5	25
6	121	45	0	15
7	121	60	5	5
8	121	30	2	15
9	134	30	2	25
10	134	45	5	25
11	134	60	0	15
12	134	60	0.5	5
13	134	30	5	15
14	121	45	2	5
15	100	60	0.5	15
16	134	45	0	25