Catalytic hydrogenation of seaweed for sugar alcohol production

Name course	:	BSc Thesis Biotechnology
Number	:	YBT-80324
Study load	:	24 ects
Date	:	15 August 2018
Student	:	Karlijn Hertsig
Registration number	:	961130330030
Study programme	:	BBT
Report number	:	108BCT
Supervisors	:	Elinor Scott, Tomas van Haasterecht
Examiner	:	Ton van Boxtel
Group	:	Biobased Chemistry and Technology
Address	:	Bornse Weilanden 9 6708 WG Wageningen The Netherlands

WAGENINGEN UNIVERSITY & RESEARCH

Catalytic hydrogenation of seaweed for sugar alcohol production

Abstract

Seaweed, hydrogenation, sugar alcohols, alginate

To release pressure on the system, applications of the upcoming feedstock seaweed are researched. The biomass composition of the researched seaweed is beneficial for biorefinery. According to Ban et al. (2017) alginate can successfully be catalytically hydrogenated into sugar alcohols. The highest yield found was 61%. Now the transition from component hydrogenation to biomass hydrogenation is attempted to be made with the aim to produce sugar alcohols for sweetener applications.

In this research alginate and biomass hydrogenation were compared and results were validated by HPLC analysis. Also, the influence of washing of the seaweed before hydrogenation was researched. Results showed alginate yields 49% sugar alcohols and seaweed 10%. Washed biomass results in 1% sugar alcohols. A variation of in reaction time and temperature did not have any influence on the sugar alcohol yield and resulted all in 10% sugar alcohols. To conclude, alginate hydrogenation is reproducible and all sugar alcohol yields found were originating from biomass composition and no sugar alcohols were produced during hydrogenation of seaweed.

HPSEC and LC-MS analyses confirmed during hydrolysis of seaweed hydrogenation. Therefore it is concluded the reaction is interrupted in the hydrogenation. Possible causes were researched. First, protease treatment to remove the protein content from the biomass was researched. It resulted in 0% sugar alcohol yield and was therefore not beneficial for sugar alcohol production. Nevertheless, a lot of unknown compounds were removed from the liquid phase. Second, the catalyst (Ru/C) performance was researched by a glucose hydrogenation experiment. The catalyst was negatively influenced by the presence of seaweed during glucose hydrogenation, yet the catalyst was still able to hydrogenate glucose into sugar alcohols. But in the end, it is suggested the catalyst is inhibited by the seaweed during hydrogenation.

Table of content

Introduction 1
- Seaweed as feedstock for biorefinery 1
- Seaweed composition 1
- Sugar alcohol production from seaweed 2
- Approach 2

Materials and Methods 4
- Materials 4
- Methods 4
  - Sample preparation 4
  - Pretreatment 4
  - Catalytic hydrogenation 4
  - Product separation 4
  - Analysis 5
    - HPLC organic acids - high pressure liquid chromatography organic acids 5
    - HPLC monosaccharides - high pressure liquid chromatography monosaccharides 5
    - GC - Gas chromatography 5
    - HPSEC - high pressure size exclusion chromatography 5
    - LC-MS - liquid chromatography with mass spectrometry 5

Results 6
- Alginate hydrogenation compared to seaweed hydrogenation 6
  - Alginate hydrogenation 8
    - Sugar alcohol yields 6
    - Solid residues 7
    - HPSEC analysis 7
    - LC-MS analysis 8
  - Seaweed hydrogenation 8
    - Sugar alcohol yields 8
    - Solid residues 9
    - HPSEC analysis 9
    - LC-MS analysis 10
- Effect of process parameters on seaweed hydrogenation 10
  - Effect of time variation on seaweed hydrogenation 10
  - Effect of temperature variation on seaweed hydrogenation 11
  - Stability of the liquid phase 11
- In depth hydrogenation experiments for a better understand of the reaction 11
  - Effect of protein content on the hydrogenation of seaweed 11
    - Sugar alcohol yields 12
    - HPLC analysis 12
  - Effect of seaweed presence on the hydrogenation of glucose 13
    - Sugar alcohol yields 13
    - HPLC analysis 13
    - Odor observation 14

Discussion 15
- Alginate hydrogenation compared to seaweed hydrogenation 15
  - Sugar alcohol yields 15
  - Solid residues 15
  - HPSEC interpretation 16
  - LC-MS interpretation 16
- Effect of process parameters on seaweed hydrogenation 17
- In depth hydrogenation experiments for a better understand of the reaction 17
  - Effect of protein content on the hydrogenation of seaweed 17
  - Effect of seaweed presence on the hydrogenation of glucose 17

Conclusion 19

Recommendations 20

References 21

Appendices 22

Introduction

Dear reader, in the world which you and I live today, we stand on the edge of a major transition. Because the human population keeps growing and the amount of resources keeps declining, the need for innovation and technical development is higher than ever. Competition for arable land, water and energy is growing and limits will be reached [1]. In an attempt to relieve pressure on the system, the seaweed feedstock is researched for applications in biorefinery [2][3]. In this thesis the sugar alcohols production from seaweed is examined as potential supply of chemical compounds as part of this quest.

Seaweed as feedstock for biorefinery

Seaweed biomass had been considered to be a promising renewable energy source for many years and is seen as a potential feedstock for biorefinery [4]. In 2014 seaweed aquaculture contributed 49% to the global mariculture production of 27.3 million tonnes and the sector is only expanding [5]. Figure 1 shows the expansion of the global seaweed aquaculture production over the past fifty years.

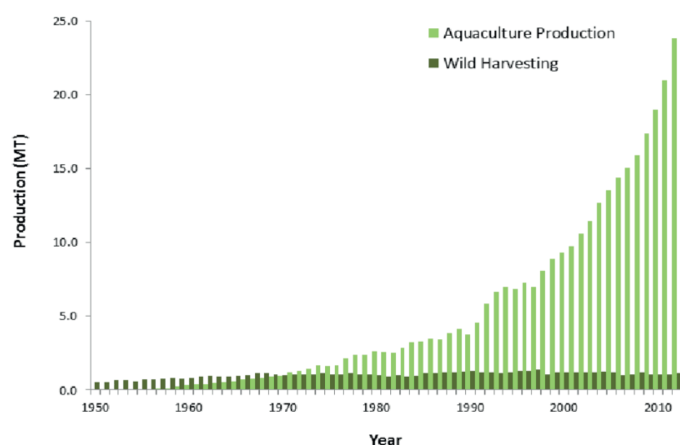


Figure 1, Global seaweed aquaculture production, FAO (2015)

The cultivation of seaweed is beneficial compared to terrestrial crops for several reasons [6]. Firstly, marine macro algae are cultivated in coastal areas and open sea. Therefore seaweed does not require arable land, fertilizer and fresh water supply to be cultivated. Hereby the competition with food crops cultivation is avoided in once [7]. Secondly, algae have a higher photon conversion efficiency and a higher CO_2 fixation rate than land plants [8][9], which indicates a higher growth rate and yield over time compared to agricultural crops.

To narrow down the scope of this thesis, the macro algae Alaria esculenta was chosen to be studied. This brown algae is also known as dabber lock or winged kelp. The species is unbranched and grows up to four meters long despite the harsh conditions of its natural habitat, which is profitable for large-scale cultivation [10]. However, this research is about the biotechnological application of the species, not the cultivation. Therefore, the biomass composition of the seaweed is more relevant.

Seaweed composition

Next are described the most important traits of the biomass composition of the seaweed. To start, macro algae in general lack hemicellulose and lignin, the major constituents of a common plant cell wall. Because seaweed floats in water, it does not need to fight gravity as much as terrestrial crops. Therefore it does not need much of a plant anatomy and practically all of the biomass consist of leaves. This makes the biomass relatively easy to depolymerize [11]. Meanwhile, brown algae accumulate big amounts of carbohydrates [8]. The species Alaria esculenta includes besides 30% of proteins and minerals, 70% carbohydrates. This latter, major part of sugar content is the most suitable to biotechnological processing. The sugar content includes the following saccharides: alginate, laminarin, mannitol and cellulose in the ratio 3:1:1:1 [12].

The chemical composition of the seaweed saccharides are described as follows. To begin with mannitol, a sugar alcohol with the formula $C^6H^{14}O^6$. Next are laminarin and cellulose, both polysaccharides of glucose units. But instead of the straight beta-1,4 linkages in cellulose, laminarin exists of beta-1,3 linked units with beta-1,6 branches [13] [14]. Lastly is alginate, or the so-called alginic acid, this polysaccharide is made up of beta-D-mannuronic acid (M) and alpha-L-guluronic acid (G). These units are linked together by 1,4 glycosidic bonds [15]. A visual representation of the two uronic acids and the saccharides can be found in Appendix 3.

Sugar alcohol production from seaweed

As mentioned before, mannitol is already present in the biomass. This sugar alcohol is seen as a chemical building block, together with the other sugar alcohols, including sorbitol and galactitol [11]. In Asia, mannitol is largely produced by isolation from seaweed. This process includes separation by Soxhlet extraction with yields of approximately 18% wt.

In contrast, outside Asia sugar alcohols are synthesized by catalytic hydrogenation of fructose [16]. The reaction mixture is formed from either starch or sucrose, in both cases a feedstock competing for food. The industrial hydrogenation is called a catalytic hydrothermal conversion, which stands for a thermochemical process taking place at elevated temperatures with water as reaction medium [17]. As can be seen in Figure 2, during hydrothermal conversion macromolecules are first hydrolysed, whereby the chemical bonds of the disaccharides are cleaved with the addition of water. Then the released monosaccharides are hydrogenated under influence of hydrogen gas and a catalyst into the stable end products mannitol and sorbitol. With the addition of an heterogeneous catalyst, more stable and valuable products are formed. Industrial production of sugar alcohols mostly use Nickel as catalyst [16].

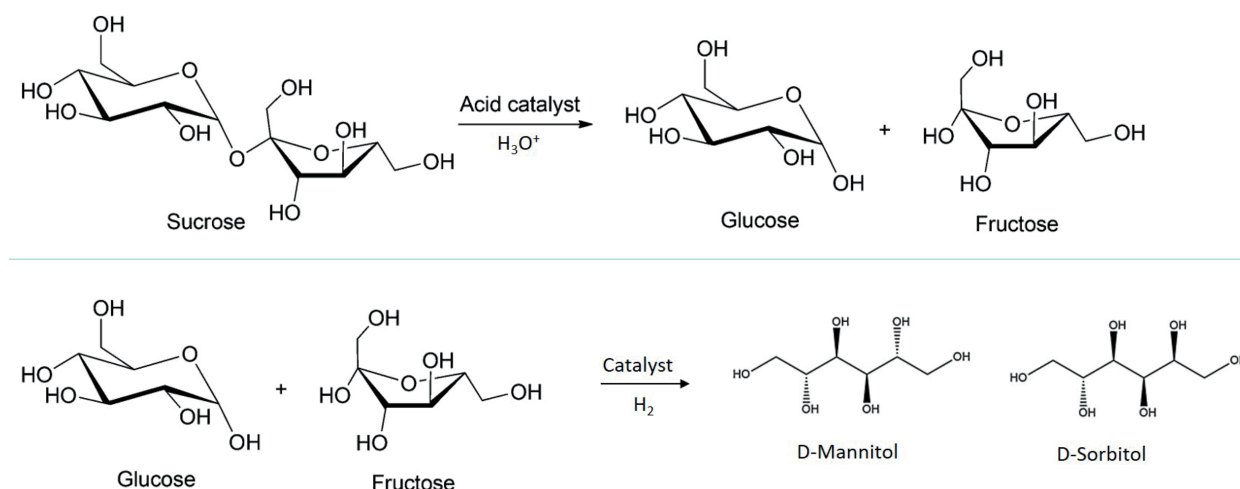


Figure 2, Reaction mechanism of industrial sugar alcohol production, hydrolysis and hydrogenation

According to Ban et al. (2017) [18] alginate can also be successfully catalytically hydrogenated in sorbitol and mannitol. The highest yield of sugar alcohols, 61%, was obtained at 150°C for 12 hours under 50 bar of hydrogen gas with Ruthenium on Carbon catalyst. The reaction mechanism proposed for the reaction is shown in Figure 3 together with cellulose hydrogenation. The different steps of hydrolysis and hydrogenation are again shown. In contrast to cellulose, alginic acid requires three hydrogenations steps instead of one. Meanwhile, the end products of alginate hydrogenation are sorbitol and mannitol compared to sorbitol solely in the cellulose pathway.

Approach

Combining the information above, it was a logical step to explore the sugar alcohol production from seaweed biomass. Therefore, the aim of this research was to get more insight in the catalytic hydrogenation of the complete macro algae biomass, combined with the goal to produce sugar alcohols. The reaction pathway proposed by Ban et al. (2017) was leading for understanding the reaction mechanism. Different aspects of the catalytic hydrogenation were examined.

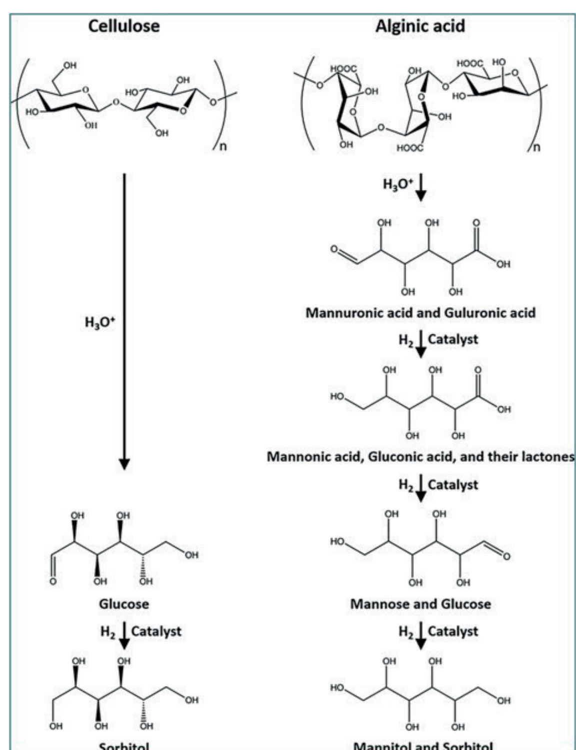


Figure 3, Reaction mechanism of alginic acid and cellulose hydrogenation into sugar alcohols

First, the alginate hydrogenation was compared to the seaweed hydrogenation. Second, the influence of washing the seaweed before hydrogenation was researched. Third, the influence of two process parameters, reaction time and reaction temperature, on sugar alcohol production for seaweed hydrogenation was examined. After those experiments, a redirection in approach was taken and the influence of the protein content of the seaweed biomass on the hydrogenation was researched by treating the biomass with protease before hydrogenation. Also, the performance of the catalyst Ru/C was examined with a glucose hydrogenation experiment.

The experimental results were validated by HPLC analysis of organic acids and monosaccharides, HPSEC and LC-MS as will be explained in the materials and methods.

Materials and methods

Materials

The seaweed species used in this study was the Alaria esculenta. It was farmed in Oban Scotland and harvested and dried in June 2017. One kilogram was provided for research through Jelle van Leeuwen. 100 grams of the biomass was washed and treated with cellulase and protease on forehand.

The reference hydrogenations were filled with sodium alginate, D-sorbitol and D-mannitol and D-glucose purchased at Sigma-Aldrich, together with the catalyst Ruthenium on Carbon, 5% wt loading. HPLC standards including galactitol, glucose, xylose, galactose and D-mannuronic acid were purchased at Sigma-Aldrich. L-Guluronic acid sodium salt, was purchased at Carbosynth. Protease, CAS number: 9036-06-0, was purchased at Sigma-Aldrich. Whatman filter paper Catalogus number 1441-055 was obtained from Sigma-Aldrich.

Methods

For this research, different types of experiments were performed. Following are five sections divided in sample preparation, pretreatment, catalytic hydrogenation, product separation and analysis.

Sample preparation

Before the seaweed was used for the hydrogenation, general pretreatment was needed to make homogeneous and attainable samples. The biomass was grinded for five minutes with the Tomado TM-5560 and sieved afterwards to the maximum size of 0.8 mm, recovering 40 – 60% of the biomass depending on the amount biomass added in the grinder. 5.0 grams of seaweed was added to 50 mL demi water together with 0.50 grams of Ru/C. This is the standard reaction mixture used for processing with pH 6.3.

Pretreatment

The influence of multiple pretreatments on the hydrogenation were researched. First treatment tested was washing the biomass. 5.0 grams sieved seaweed was placed it in a Büchner funnel with the Whatman filter paper. Under vacuum 100 ml of demi water was filtered. Afterward the seaweed was weighted and the additional weight (water) was subtracted from the amount of demi water that should be added for the reaction mixture. The same volume was thus used for all batches.

Next was the influence of addition of liquid nitrogen researched in an attempt to break the biomass structure. By adding approximately 5 ml liquid nitrogen to the standard reaction mixture and stirring it for 10 minutes, the pretreatment was completed.

100 grams of the supplied biomass was pretreated on forehand with protease and cellulase. This biomass was stored in the freezer and contained an unknown amount of water, therefore this seaweed was freeze dried for 16 hours. This biomass was further prepared as explained above.

Likewise, another protease pretreatment was performed during research. Therefore the standard reaction mixture was inoculated with protease, 4% protein loading, and incubated at 25 ^{O}C with 50 rpm for 66 hours. Afterwards the mixture was filtered with the Büchner funnel and Whatman filter paper under vacuum. Then the seaweed was weighted and the additional water volume was calculated to come back at the standard 50 ml reaction mixture.

Catalytic hydrogenation

The hydrogenation reactions of this research were performed with the 100 ml hydrogenation reactor (Parr Instrument Company, Illinois). Appendix 4, Turning on autoclave, was followed to start the hydrogenation. The reaction time was varied between 2 and 48 hours and the reaction time was varied between 140 and 180°C. Other initial reaction conditions 50 bar reaction pressure, catalyst Ru/C were taken from literature [18] and a stirrer speed of 1000 rpm and cooling water of temperature of 20°C were used. After completion of the reaction time the reactor was turned off again following Appendix 4, Turning off autoclave.

Product separation

To separate the liquid phase from the solids, the reaction mixture was directly filtrated from the reaction vessel with the Büchner funnel and filter paper. The liquid phase was measured for volume and divided in three fractions. Each stored at a different temperature, room temperature (20°C), fridge (4°C) and freezer (-18°C). The solid phase was weighed and dried in the 105°C oven till a constant weight.

Analysis

Multiple analysis methods were used to identify the content of the reaction product and quantify the desired sugar alcohol content of the liquid phase.

HPLC organic acids - high pressure liquid chromatography organic acids

The analysis method applied for general insight of the product content. When identification was complete, the analysis quantified the total sugar alcohol content and the uronic acids D-mannuronic acid and L-Guluronic acid. The HPLC was performed on the BioRAD HPX_87H 300x7.8 mm column with eluents H2SO4 5mM, flow 0.5 ml/min, temperature 50°C and detection was performed with UV 210 nm and RI.

HPLC monosaccharides - high pressure liquid chromatography monosaccharides

This analysis method distinguished and quantified the different monosaccharide forms. Including the sugar alcohols mannitol, sorbitol and galactitol. This method included the Phenomenex Rezex RPM-Monosaccharide Pb+ LC 300 x 7.8 mm column with eluents milliQ, flow 0.6 ml/min, temperature 75°C and detection with RI.

GC - Gas chromatography

For confirmation of unidentified compounds by HPLC organic acids, GC analysis was performed. The method included the Phenomenex Zebron 7EK-G009-22 column at temperature 50°C, increased with 10 ^{O}C/min to 150°C, injection of 1 µL at temperature 250°C with a 1:20 split, flow 4.8 ml/min and detection with FID at temperature 275°C.

HPSEC - high pressure size exclusion chromatography

To gain insight in the hydrolysis of the proposed reaction pathway, HPSEC analysis was carried out. The data set PULLULAN G shown in Appendix 5 was combined with the following method: columns TSK super AW 2500, 3000, 4000 in series, all 4 um 6,0x150 mm. It ran with eluent 0.2 M NaNO3, flow 0.6 ml/min, temperature 55°C and detection by RI Shodex.

LC-MS - liquid chromatography with mass spectrometry

To obtain information about the hydrogenation, LC-MS analysis was performed on the reaction products. First chromatography of a liquid sample is performed to see where the mass of the sample is found, then the mass spectrum is shown for a chosen retention time of the chromatography. The method was performed with the BioRAD HPX_87H 300x7.8 mm column with eluents formic acid 0.1%, flow 0.5 ml/min and at temperature 50°C. Detection was done with LCQ_Fleet and UV 210 nm.

Results

In this thesis, the catalytic hydrogenation of seaweed into the sugar alcohols sorbitol, mannitol and galactitol was investigated. Multiple experiments were performed with different feedstock and conditions. An overview of all experiments can be found in Appendix 1. Also, the raw data of HPLC analyses for all liquid phases of the hydrogenation experiments are shown in Appendix 2.

Alginate hydrogenation compared to seaweed hydrogenation

To compare seaweed hydrogenation to alginate hydrogenation, results of both experiments are given. First the alginate hydrogenation is described, followed by the results of seaweed hydrogenation. The results are stuctured in the following order: sugar alcohol yields, solid residues, HPSEC analyses and LC-MS analyses.

Alginate hydrogenation

To compare biomass hydrogenation to single component hydrogenation and to reproduce literature, alginate (major constituent of seaweed) was catalytic hydrogenated into sugar alcohols. The alginate (100 g/l) was converted for 15.5 hours in water under influence of 150°C and 50 bar hydrogen gas. The catalyst applied for this hydrogenation was Ruthenium over Carbon (Ru/C).

Sugar alcohol yields

The alginate experiment was performed twice and the liquid phase was analysed with HPLC of organic acids and with HPLC of monosaccharides. Yields of biomass into sugar alcohol according to both analysis methods of alginate hydrogenation are given in Figure 4.

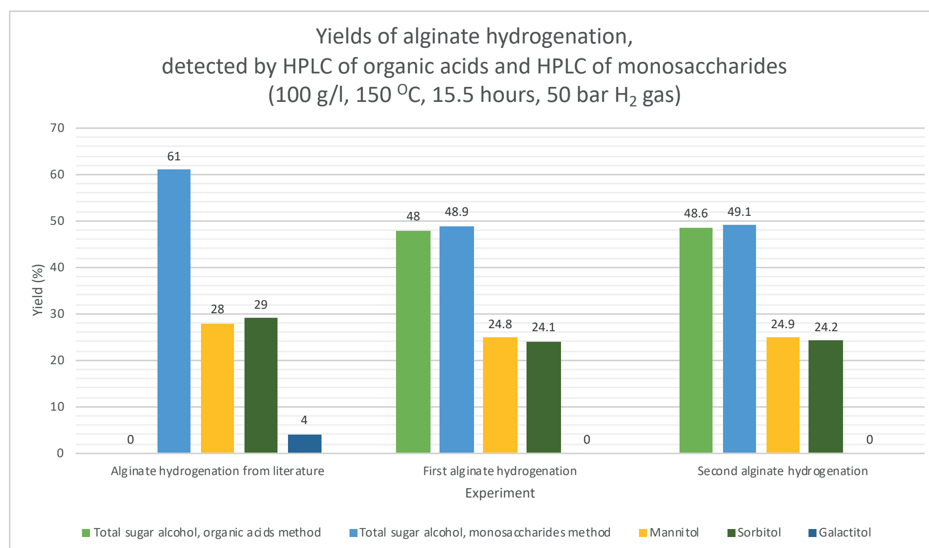


Figure 4, Sugar alcohol yields of alginate hydrogenations, detected by HPLC of organic acids and HPLC of monosaccharides

As can be seen, sugar alcohol yields on alginate of approximately 48.5% were measured for both batches and both analysis methods. Figure 4 also shows the ratio between mannitol and sorbitol is almost 1:1 and no galactitol is produced. To see the diagrams of the HPLC analysis of the alginate hydrogenations, for both the organic acid method and the monosaccharides method, Appendix 6 can be consulted.

Solid residues

Next to the liquid phase, there is also a solid phase found after hydrogenation. In Table 1 is the amount of solids is given. With the assumption that all of the catalyst is fully recovered, the amount of not hydrolysed alginate is calculated together with the consequential maximum yield calculated. As can be seen, maximum yield of 90 and 100% is found.

Table 1, Solid residue of alginate hydrogenations

	Alginate hydrogenation 1	Alginate hydrogenation 2
Solids	1.03 gram	0.47 gram
• Alginate	0.53 gram	-0.03 gram
Max yield	89.4%	100.6%

Explained in the introduction, the reaction mechanism proposed in this thesis exist of two parts, hydrolysis and hydrogenation. To examine the two steps separately, analysis of HPSEC and LC-MS were performed on the reaction product, the aqueous phase after hydrogenation.

HPSEC analysis

To examine the hydrolysis of alginate, the liquid phase was submitted to HPSEC analysis. Hereby the molecular weight of a compound is calculated with Data PULLAN set G, see Appendix 5 for the details. From this reference set, a size indication can be made. At rt = 12.5 min, molecules of 2000 Da are shown. Rt = 15.0 min, indicates the end of the analysis, meaning the molecule size is 0 Da at this point. From this the chain length can be derived and therefore the formation of small molecules: monomers, dimers and oligomers can be confirmed. In Figure 5 the HPSEC results of the liquid phase of both the alginate hydrogenations are given.

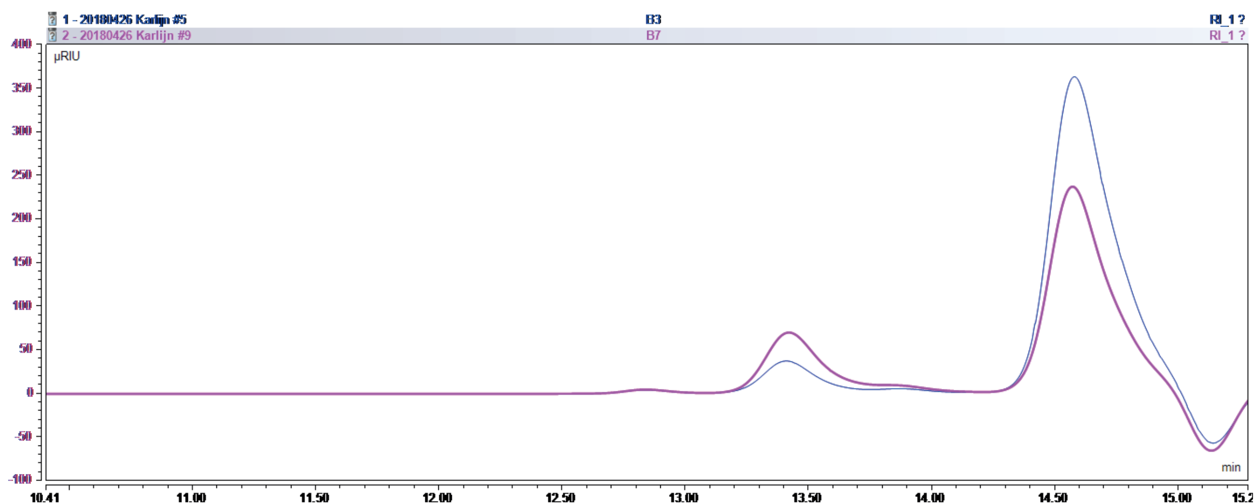


Figure 5, HPSEC results of the liquid phase for both alginate hydrogenations

As can be seen, two compounds are indicated in Figure 5. One compound is indicated at retention time 12.9 minutes and a second compounds is indicated at retention time 14.0 minutes. The chromatogram shows the second compound is higher in intensity for both experiments even though there is a different ratio for the two alginate hydrogenations.

LC-MS analysis

To confirm the hydrolysis of alginate, LC-MS analysis was performed on the liquid phase of the hydrogenation. The LC-MS analysis of sorbitol, shown in Appendix 7, has indicated sugar molecules with molecular weight of 183 D are found at a retention time 11.9 of minutes of the chromatogram. This indicates where to find the sugar molecules in the reaction samples and where to look in the other samples.

Figure 6 shows the LC-MS diagram of alginate hydrogenation. A peak is shown at retention time 11.7 minutes in accordance with the sugar peak found with sorbitol analysis. This figure shows molecules of 183 and 200 D are found at this retention time.

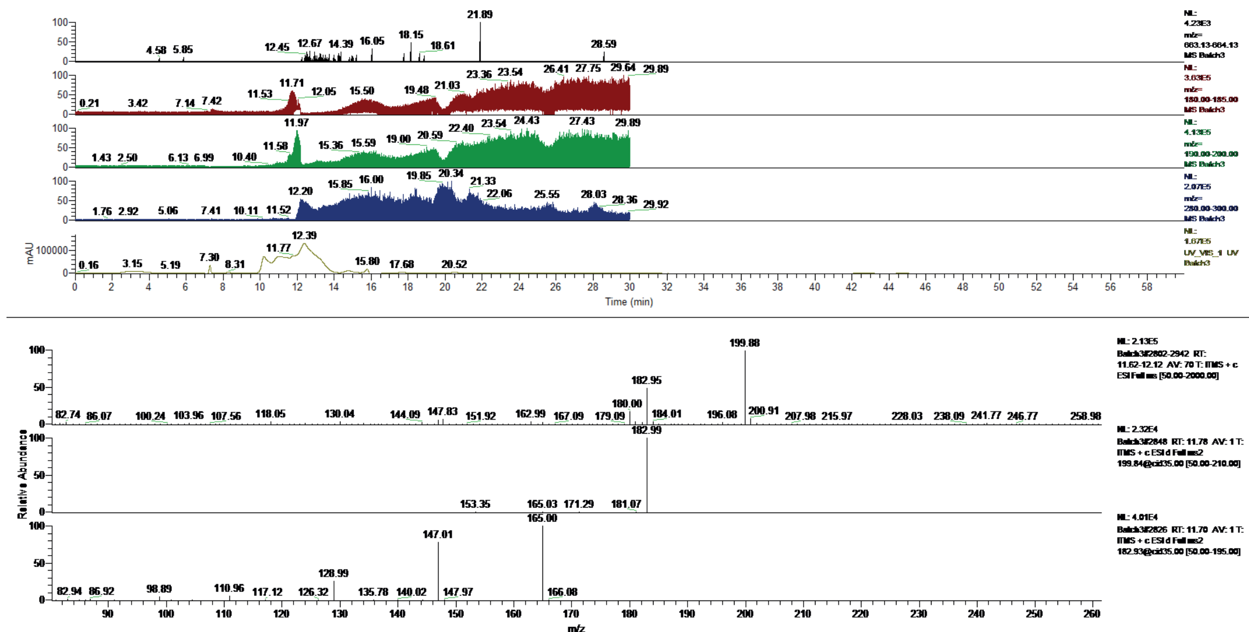


Figure 6, LC-MS diagram of alginate hydrogenation

Seaweed hydrogenation

Now the data for the alginate hydrogenation is gathered, the next step is to focus on the seaweed hydrogenation. Since the seaweed contains a lot of salts inside and outside the biomass, the influence of washing the seaweed was researched simultaneously. Hydrogenation was examined for washed and unwashed biomass together. The same conditions applied on alginate hydrogenation were applied to seaweed biomass (100 g/l) hydrogenation. Again exposure for 15.5 hours in water under influence of 150 ^{O}C and 50 bar of hydrogen gas was applied. Also with the addition of the catalyst Ru/C.

Sugar alcohol yields

For sugar alcohol yields, HPLC analysis of organic acids was performed. The yields of biomass into sugar alcohol are given in Figure 7.

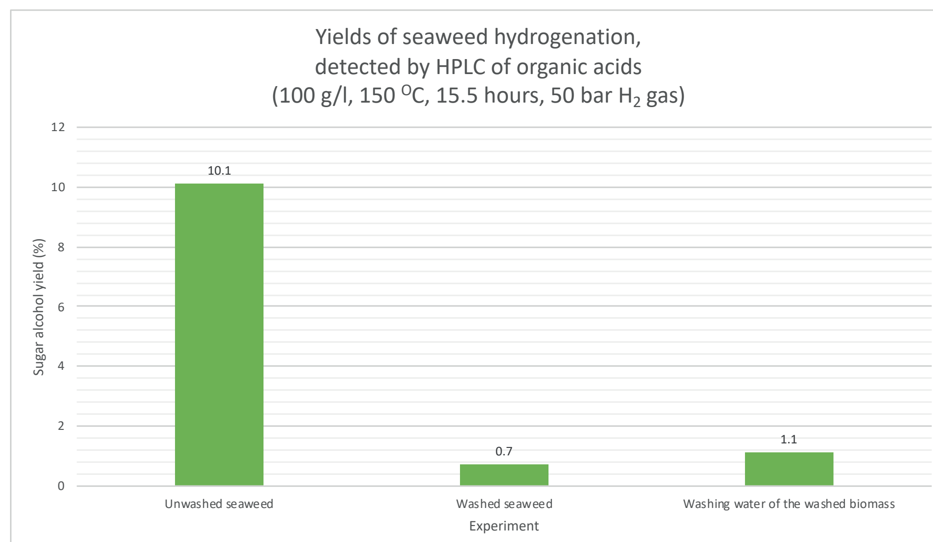


Figure 7, Sugar alcohol yields of the washed and unwashed batch and the washing water, detected by HPLC of organic acids

As can be seen, the concentration of sugar alcohols is 10 g/l from hydrogenation of unwashed seaweed. The concentration of sugar alcohols is 1% from washed seaweed. Sugar alcohol concentration found in the washing water of the washed seaweed was 1 g/l.

Solid residues

For the seaweed hydrogenation also the solid phase found was measured after hydrogenation. In Table 2 is the amount of solids given together with the consequential maximum yield calculated. As can be seen, the maximum yield for unwashed seaweed is 77% and the maximum yield of washed seaweed is 80%.

Table 2, Solid residue of (un)washed seaweed hydrogenation

	Unwashed seaweed	Washed seaweed
Solids	1.64 gram	1.48 gram
• Seaweed	1.14 gram	0.98 gram
Max yield	77.2%	80.4%

In Appendix 8 is shown how formic acid was confirmed as another product from seaweed hydrogenation, using both analysis of HPLC organic acids and GC of the aqueous phase. For all hydrogenations the formic acid yields are shown in Appendix 1.

HPSEC analysis

The hydrolysis steps were also analysed for the seaweed hydrogenation by HPSEC. In Figure 8 the HPSEC diagram of the liquid phase of unwashed seaweed (blue), washed seaweed (pink) and the washing water of the washed seaweed (black) are shown.

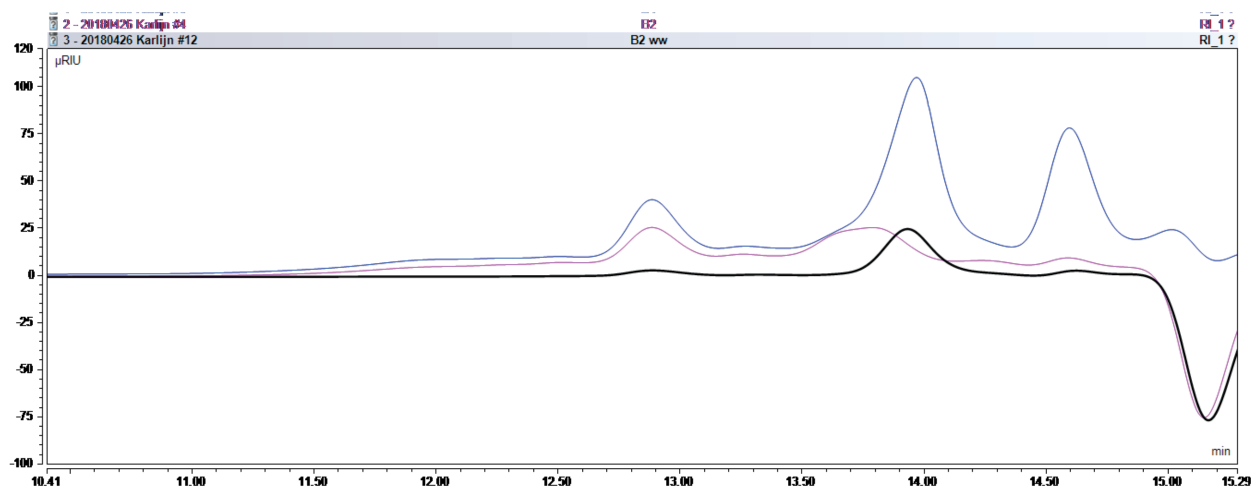


Figure 8, HPSEC diagram of liquid phase of (un)washed seaweed hydrogenation and the washing water of the washed seaweed

As can be seen, four peaks are indicated for the unwashed seaweed, rt 12.9, 13.9, 14.6 and 14.9. For washed seaweed are two peaks indicated for rt 12.9 and 13.4. The washing water shown one peak at retention time 13.9. indicated at retention time 14.0 minutes. The chromatogram shows much higher intensities for the unwashed seaweed than for both the washed biomass and washing water.

LC-MS analysis

The hydrogenation of seaweed was also examined by LC-MS analysis of the liquid phase. In Figure 9 is a peak shown at rt 11.9. The mass spectrum for rt = 11.9 is shown. Masses of 183 and 200 D are found.

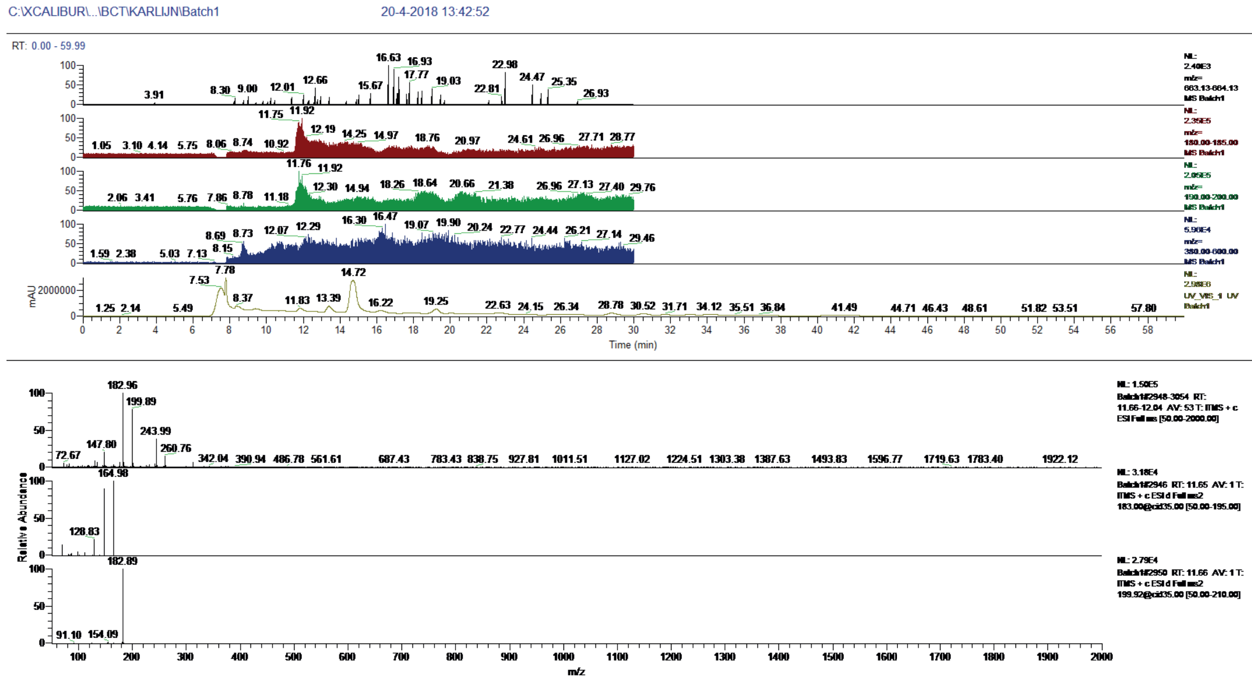


Figure 9, LC-MS diagram of hydrogenated seaweed

Effect of process parameters on seaweed hydrogenation

In order to examine the influence of reaction time and temperature on the sugar alcohol yield. The hydrogenation of seaweed was conducted between 2 and 48 hours and a temperature range between 140 and 180°C. Six seaweed hydrogenations were executed to examine the influence of reaction time on the yield.

Effect of time variation on seaweed hydrogenation

The sugar alcohol yields for the various hydrogenation times are given in Figure 10.

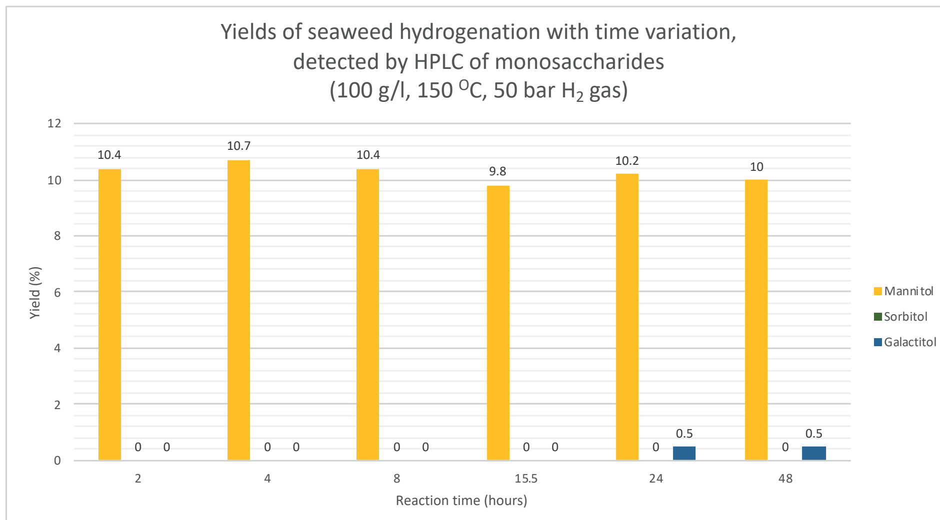


Figure 10, Yields of seaweed hydrogenation with time variation

As can be seen, in Figure 10, the sugar alcohol yields from seaweed biomass hydrogenation are around 10% for irrespective of the reaction time. Also, the sugar alcohol yield found for all the time experiments consists solely of mannitol.

Effect of temperature variation on seaweed hydrogenation

The sugar alcohol yields for the various hydrogenation temperature are given in Figure 11.

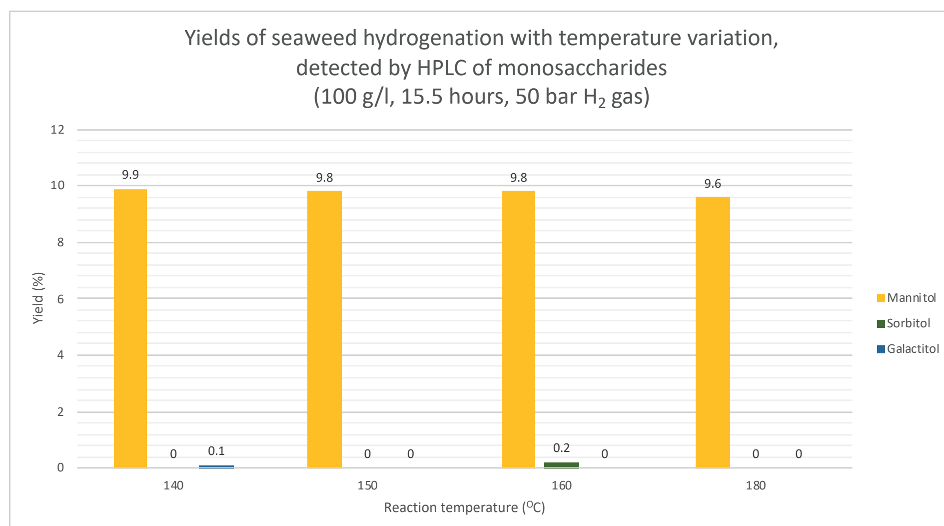
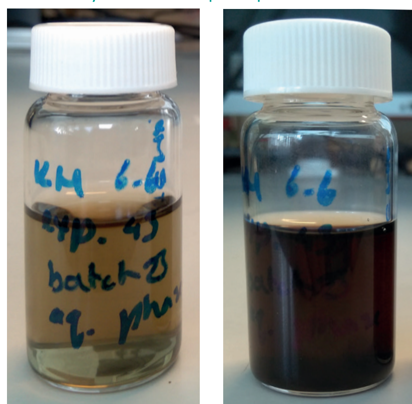


Figure 11, Yields of seaweed hydrogenation with temperature variation

As can be seen in Figure 11, the sugar alcohol yields from seaweed hydrogenation are 10% for irrespective of the reaction temperature. At lower temperatures, there is glucose and xylose found after hydrogenation. In contrast, at higher temperatures, galactitol is found.

Stability of the liquid phase



After hydrogenation, the liquid phase was stored at different temperatures. In Figure 12 the liquid phase of the seaweed hydrogenation of the unwashed with protease treated biomass is shown. On the left is the liquid phase seen directly after hydrogenation. On the right is the same liquid phase shown after storage of 24 hours at room temperature. A clear change of colour was observed from transparent green to dark brown, non-transparent. This colour change was observed by all the seaweed hydrogenations. It was also observed by the storage products in the freezer but change was notices over a longer period of time.

Figure 12, Liquid phase of unwashed with protease treated seaweed direct after hydrogenation (left) and after 24 hours of storage (right)

In depth hydrogenation experiments for a better understanding of the reaction

As explained in the introduction, after the provious experiments, a new approach was taken to take a look at specific parts of the hydrogenation of seaweed. Next, are the results given for two experiments. First, the effect of the protein content on the seaweed hydrogenation is researched. Second, the effect of seaweed presence on the hydrogenation of glucose is investigated.

Effect of protein content on the hydrogenation of seaweed

As explained in the introduction, the seaweed biomass content consist of 10% proteins. To study the effect the proteins have on the hydrogenation of the seaweed it was attempted to remove the proteins prior to the hydrogenation. The seaweed was treated with protease (4% protein loading) in order to hydrolyse the protein after which the soluble amino acids were removed by removing the protease mixture. The treatment was applied to seaweed that was both washed and unwashed seaweed prior to the protease treatment.

Sugar alcohol yields

The influence of the protease treatment before hydrogenation is validated by HPLC analysis of the liquid phase. The sugar alcohol yields are shown in Figure 13.

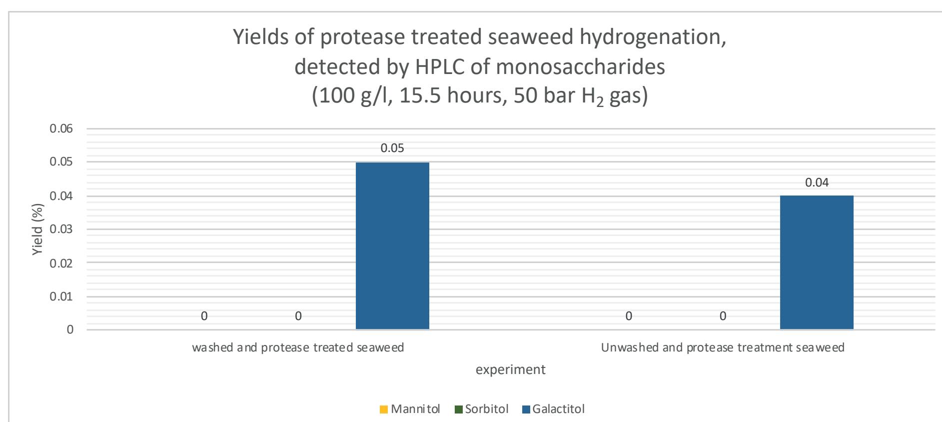


Figure 13, Yields of protease treated seaweed hydrogenation

As can be seen in Figure13, the sugar alcohol concentrations of the protease pretreated batches was found to be almost zero for both the washed and unwashed biomass and consisted solely of galactitol.

However some sugars were detected. A production of 0.30 g/l galactose was observed when the biomass was washed and pretreated with protease. When the seaweed was not washed and pretreated with protease, a production of 1.58 g/l of glucose and 0.39 g/l xylose was obtained.

HPLC analysis

The HPLC chromatogram of the untreated seaweed (blue), protease treated seaweed without washing (pink) and protease treated biomass with washing (black) is shown in Figure 14.

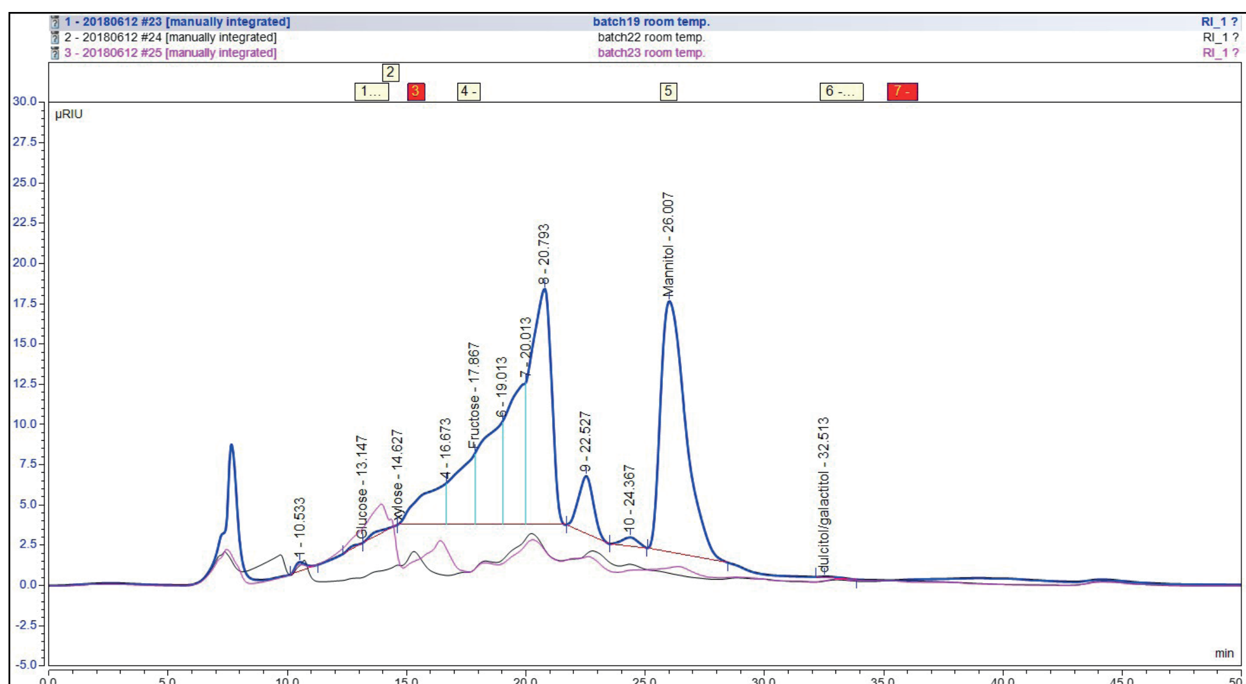


Figure 14, HPLC diagram of monosaccharides of (un)washed protease treated seaweed hydrogenation

As can be seen, the liquid phase of the protease treated have both diminished background noise in the diagram.

Effect of seaweed presence on the hydrogenation of glucose

To test the performance of the catalyst in the presence of seaweed, two glucose hydrogenation experiments were conducted. One experiment was conducted with glucose, as a reference to confirm hydrogenation of the monosaccharide. Another experiment was conducted with glucose and seaweed biomass to see what the catalyst does with the biomass.

Sugar alcohol yields

The results are shown in Figure 15.

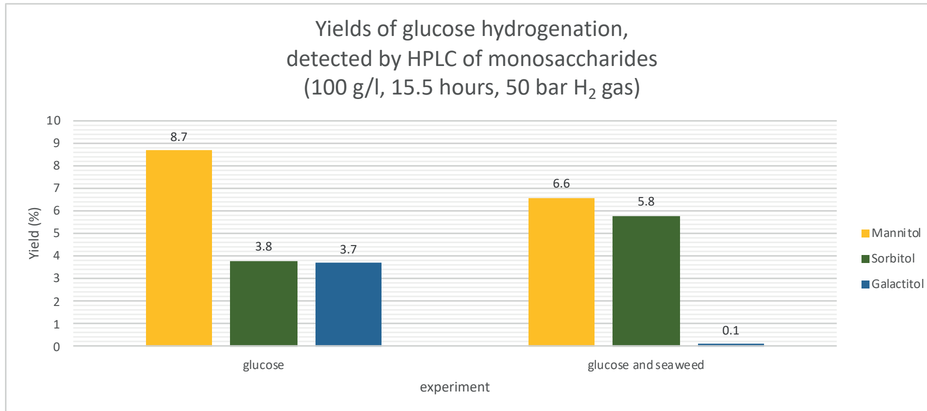


Figure 15, Yields of glucose hydrogenation

As can be seen, the sugar alcohol concentration is around 15 g/l after hydrogenation of glucose. The total sugar alcohol concentration of the glucose-seaweed batch is 11 g/l.

Meanwhile, it is interesting to see the ratio between mannitol, sorbitol and galactitol is respectively 2:1:1 by glucose hydrogenation, while combined with biomass the ratio changed to 1:1:0.

HPLC analysis

The HPLC chromatogram of the glucose hydrogenation in absence of seaweed (black) and glucose hydrogenation in presence of seaweed (blue) is shown in Figure 16.

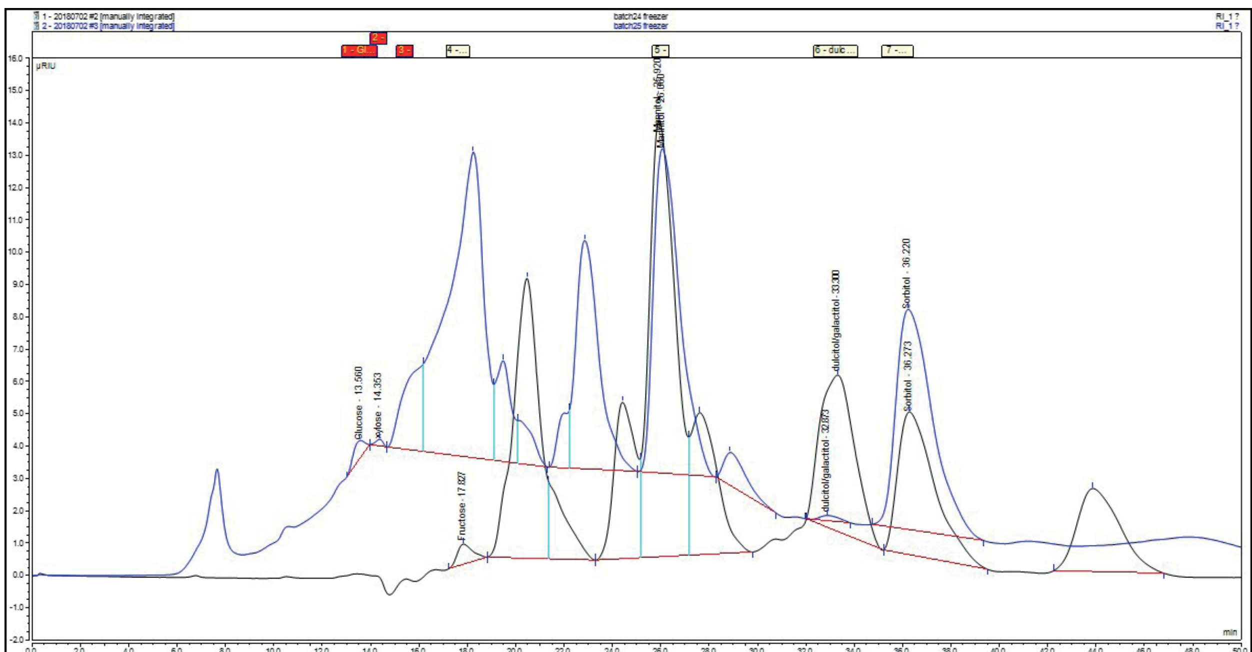


Figure 16, HPLC diagram of glucose hydrogenation in presence and absence of seaweed

In the HPLC monosaccharide chromatogram can be seen that with the glucose hydrogenation (black) gives a pretty clear image where the glucose with seaweed hydrogenation (blue) shows again a lot of bulk noise, also seen in the normal biomass batches.

Odor observation

During the execution of the catalyst experiment, a penetrating smell of burnt sugar and caramel was observed when the reactor of the glucose with seaweed batch was opened. This smell was observed before, but not identified till now because the intensity was much higher than before.

Discussion

Alginate hydrogenation compared to seaweed hydrogenation

In literature [] is already known that alginate, the major constituent of the Alaria esculenta, can be hydrogenated into the sugar alcohols sorbitol and mannitol. The hydrogenation of seaweed biomass in its entirety has not been reported yet. To explore and understand the hydrogenation of seaweed for sugar alcohol production, alginate and seaweed were both exposed to the same reaction described by Ban et al. to give the highest conversion yields. This means both reaction compounds were added to water (100 g/l) and exposed for 15.5 hours to a temperature of 150 OC and a pressure of 50 bar hydrogen gas. The catalyst applied for the hydrogenation was Ruthenium over Carbon (Ru/C), also proven efficient for alginate hydrogenation in literature.

Sugar alcohol yields

For the hydrogenation of alginate, Ban et al. found a maximum conversion yield of 61% for the optimum reaction time of 12 hours [18]. As shown in Figure 4, a conversion yield of 49% was established for the reaction time of 15.5 hours. Also indicated in Figure 4, the ratio of produced sugar alcohol mannitol: sorbitol is 1:1. This is similar to the findings of Ban et al. and can be explained by the isomerism between the two sugar alcohols. Therefore, it can be concluded the alginate hydrogenation established is reproducible from literature and the experimental set up, alginate, and catalyst are performance properly. Since both alginate hydrogenations and both HPLC analysis methods indicate the same sugar alcohol yields, it can be concluded that the reliability of the experimental execution and validating HPLC analyses are within the allowed scientific deviation.

Solid residues

It should be noted that it is unclear what happened to the other half of the alginate. The maximum yields calculated from the solid residue in Table 1 show all of the alginate compound go into reaction.

In order to compare the alginate hydrogenation to the seaweed hydrogenation, it must be known alginate takes up 35% of the biomass composition. The other seaweed saccharides laminarin, cellulose and mannitol, take up another 35% of the biomass composition. Since alginate conversion yielded 49% without optimisation a sugar alcohol yield between 17.5 and 35.0% was expected for seaweed hydrogenation. But the hydrogenation parameters chosen, result in a solid residue for seaweed hydrogenation, as seen in Table 2. A maximum conversion yield of 80% was calculated. This implies that an even lower conversion yield should be expected, one between 14.0 and 28.0% for seaweed hydrogenation.

As is shown in Figure 7, the sugar alcohol yield after hydrogenation of unwashed seaweed is 10.1%. This is lower than the 14.0% which was expected at least but is still a significant amount. Washed seaweed yielded even lower, 1.1% of sugar alcohol. This indicates a loss of 9.0% of sugar alcohol yield due to washing of the seaweed. An explanation for this could be that mannitol found present in the biomass composition (10%) was present in the unwashed seaweed reaction product and lost when the biomass was washed before hydrogenation.

An explanation for this can be that the mannitol found present in the biomass composition (10%) was present in the unwashed seaweed reaction product and lost when the biomass was washed before conversion.

Still, there is 1% sugar alcohol found when hydrogenating washed seaweed. This could be explained in two different way. First, all sugar alcohol was washed out and there was little production (1%) of sugar alcohols during conversion. Second, not all mannitol from composition was washed out. This later explanation would imply no production of sugar alcohols at all.

It should be noted that no validation can be drawn from the sugar alcohol concentration found in the washing water, measured 0.99 g/l sugar alcohols. This is not in line with the explanations, suggesting the analysis method HPLC is not useful in this situation. Either way, it is confirmed that the mannitol present in the biomass is measured in the analysis. Another compound, formic acid, was also found present after conversion. It is believed this compound was also formed during hydrogenation.

HPSEC interpretation

When comparing the HPSEC diagrams of alginate hydrogenation to the ones seaweed hydrogenation, differences are noticeable. While alginate shows two peaks, unwashed seaweed shows four and washed seaweed only two as can be seen in Figure 5 and Figure 8 respectively.

To validate the size of the molecules found in the HPSEC analysis, a reference set of Pull G data was consulted, see Appendix 5 for the details. Molecule sizes were calculated, but were not exactly in line with the expected compounds. When taking another look at the diagrams, the following connection was made. The washing water showed one peak. Based on the reference set a moll mass of 303 Da was calculated. Since it is believed mannitol was washed out in the washing experiment, the mannitol should be in the washing water. Since mannitol has a moll mass of 183 Da, this does not match each other. But, the extend of the reference set is up to 13.0 minutes. Therefore, it is believed that the reference set could not correspond with the samples analysed. As a result the peak set at rt = 14.0 min is mannitol.

When looking at the unwashed seaweed hydrogenation, four peaks are shown. As can be seen in the HPSEC diagram, the last peak occurs where a dip is expected. The dip is an indication for a reliable diagram. Also, between peaks the intensity does not reach 0 again. Nevertheless, the diagram indicated where the mass in the liquid phase can be found.

The same peak (rt = 14.0) was identified for the washing water as for the unwashed seaweed hydrogenation. This was to be expected because the unwashed seaweed hydrogenation had confirmed mannitol present. The peak on the right (rt = 14.6 min) is then believed to be a smaller compound than mannitol, possibly formic acid (46.03 Da). The peak on the right (rt = 12.9), indicates then compounds bigger then mannitol. The molecule mass should, however, be smaller than approximately 2000 Da. Therefore it is presumed the peak indicates oligomers between 4 and 7 units, taking in account both the reference set and the mannitol peak.

When looking at the washed seaweed hydrolysis, two peaks are found. A small peak again around rt = 14.0 min, believed to be the small amount of mannitol left in the liquid phase. The intensity of this peak is much lower compared to the peak shown for unwashed seaweed hydrogenation. The other peak, also found at rt = 12.9 is suggested to be oligomers. Again, this indicates washing the seaweed before conversion has a big influence on the hydrogenation results.

Alginate hydrogenation shows peaks at rt 13.4 and 14.6 as shown in Figure 5. Calculated molecular weights of respectively 643 and 142 Da are found. The rt = 13.4 peak is then believed to be a oligomer peak. The other peak (rt= 14.6) is believed to be mannitol. Calculations are more close this time but still do not fit perfectly. Since at the end of the diagram is a dip shown, it is possible this sample is much more clear and therefore has a slightly different diagram compared to the unwashed seaweed hydrogenation.

Despite the uncertainty of the molar masses of the molecules, for both alginate and seaweed hydrogenation, small molecules are found. Consequently, it was concluded the hydrolysis for both the alginate and biomass batches takes place to a great extent.

LC-MS interpretation

In order to compare LC-MS results of alginate and seaweed hydrogenation, sorbitol was analysed first to create a reference setting. As named before in the results, sorbitol indicated a strong peak at rt 11.9 for the liquid chromatography. Simultaneously, at that retention time, moll masses of 183 and 200 Da were found corresponding to the moll mass for sorbitol and mannitol. Therefore it was confirmed where the sugar alcohol content was to be found.

As shown in Figure 6, a strong peak was found for alginate hydrogenation at rt 11.7, again with moll masses of 183 and 200 Da. For seaweed hydrogenation, as shown in Figure 9, the peak was also found at rt 11.9, indicating moll masses of 183 and 200 Da. With this analysis, it was confirmed that the hydrolysis takes place.

Effect of process parameters on seaweed hydrogenation

Regarding the results above, it is concluded that the alginate can be successfully hydrolyses and hydrogenated. Seaweed can only be successfully hydrolysed. Therefore it is suggested the hydrogenation is not successful for the seaweed conversion. It is, however, possible that the reaction time is different for seaweed hydrogenation than for alginate hydrogenation. Therefore the influence of the reaction time on the sugar alcohol yield was researched and the time experiment was conducted. A time variation between 2 and 48 hours was researched in six experiments. All results in Figure 10 show 10% mannitol yield. This is similar to the primary hydrogenation yield and to the initial composition in the seaweed biomass.

It is possible that seaweed hydrogenation takes place at a different temperature than alginate hydrogenation. To see the influence of reaction temperature on the sugar alcohol yield, the temperature experiment was conducted. A temperature variation between 140 and 180 OC was researched in four experiments. The results in Figure 11 again show sugar alcohol yields of 10%.

So, at this point it is concluded that the sugar alcohol yields found in the seaweed hydrogenation experiments is not produced during hydrogenation. But the mannitol concentration originates from biomass composition. Therefore, sugar alcohol production from seaweed hydrogenation is not yet viable.

In depth hydrogenation experiments for a better understanding of the reaction

As is discussed above, the problem in the reaction mechanism cannot be found at the hydrolysis. Hydrogenation was not confirmed, therefore it is believed the problem in the conversion lies here. A few suggestions to solve this problem are made and researched.

Effect of the protein content on the hydrogenation of seaweed

First, it is possible that the protein content in biomass composition hinders hydrogenation by reacting with the sugar content, before the reaction with hydrogen gas can take place. Consequently, the protease experiment was conducted to remove the protein content before hydrogenation. As is shown in Figure 13, only a minimal amount (<0.1 g/l) of galactitol was found for both protease treated (washed and unwashed) seaweed. This implies the chosen protease pretreatment does not enhance the conversion. Moreover, the protease pretreatment results in the removal of the mannitol. Which can be explained by the method used (see materials and methods) when the protease treatment was executed in a water medium, later removed from the biomass, washing out the present mannitol.

The results of the protease experiment in Appendix 2 also show a peak of galactose from seaweed hydrogenation when washed and protease treated. Meanwhile, an increase of xylose from seaweed was shown when unwashed seaweed was protease treated and hydrogenated. This implies that other reactions are active during hydrogenation when the biomass is treated with protease.

Looking at the HPLC diagram, Figure 14, it becomes clear that the amount of compounds found has diminished. Between peaks the baseline is touched again and this is a general indication of a clear sample. On the other hand, it indicates that the original hydrogenation produces a lot of unknown compounds, which were not able to be identified. So it can be concluded that a lot of rubbish is removed from the liquid phase when treated with protease. Nevertheless the production of sugar alcohols does not simultaneously increase.

In the end, it can be concluded that the protein content of the seaweed biomass has great influence on the reaction products of the hydrogenation. The sample is proven to be much cleaner when protease treated before hydrogenation. It is possible that another enzyme would not only diminish the rubbish from the aquatic phase but also increase the sugar alcohol production. But for the scope of this research this is not further researched.

Effect of the seaweed presence on the hydrogenation of glucose

Another possible reason for the malfunctioning of the hydrogenation, is the catalyst performance. It is possible that the catalyst is inhibited by the biomass. It was confirmed that the catalytic hydrogenation is viable for alginate but not for seaweed. As already seen in the HPSEC and LC-MS results, the hydrolysis does take place but the hydrogenation steps are not demonstrated. Whether the catalyst performs correctly, by enhancing the hydrogenation, is therefore questioned.

To research the performance of the catalyst on the seaweed, a catalyst experiment was conducted. For this experiment, glucose (100 g/l) was catalytically hydrogenated by Ru/C. As shown in Figure 15, the

sugar alcohol yield found in the aqueous phase was 15%. This is less than the catalytic hydrogenation of alginate. Probably glucose reacted also into other compounds. Furthermore, a combination of glucose (100 g/l) and seaweed (100 g/l) was hydrogenated by Ru/C. The sugar alcohol concentration in the liquid phase was found 11 g/l. This indicates a decrease in sugar alcohols when biomass is added for hydrogenation.

Comparing the ratio between mannitol, sorbitol and galactitol an interesting difference was noted, as seen in Figure 15. For glucose the ratio was respectively 2:1:1, for glucose and seaweed the ratio was found 1:1:0. This means an increase in sorbitol, which is remarkable compared to the total sugar alcohol yield decrease. It is obvious that the presence of biomass in hydrogenation triggers a lot of reactions, including isomerisation reactions. This was also confirmed by the HPLC monosaccharide diagram, Figure 16 where again a lot of rubbish in the background.

From the catalyst experiment it can be concluded that the catalyst, Ruthenium over Carbon, is still able to hydrogenate glucose into sugar alcohols in the presence of the seaweed biomass. But the presence of the biomass in the reaction mixture does have a negative effect on the hydrogenation taking place. In the end, it cannot yet be confirmed or denied if the catalyst is the bottleneck of the seaweed hydrogenation.
It should be noted that colour changes of the liquid phases were observed for the liquid phase by all biomass experiments, as shown in Figure 12. Because of this observation it can be concluded that the reaction product is not stable and more reactions take place after conversion.

Also, the odor after hydrogenation is characteristic for caramelization reactions. In all biomass experiments an odor similar to burnt sugar was observed. Especially the hydrogenation of glucose with seaweed had a penetrating smell of caramel when opening the reactor. It is therefore believed that indeed other reactions, such as caramelization and the maillard reaction in combination with the protein content occur faster than hydrogenation.

Conclusion

The aim of this research was to gain a better insight in the catalytic hydrogenation of complete macro algae biomass, seaweed, into sugar alcohols.

It was found that alginate, the major constituent of seaweed, can be successfully hydrolysed and hydrogenated (with Ru/C at 150°C with 50 bar H2) into sugar alcohols with a yield of 49%, which confirms a recent report by Ban et al (2017) who obtained 61% sugar alcohol yield under similar conditions.

When seaweed was hydrogenated under the same conditions, a yield of about 10% sugar alcohol was obtained. The sugar alcohol yield dropped to 1.1% when the seaweed was washed before hydrogenation. It was established that the reaction time and the reaction temperature do not have an influence on the sugar alcohol yield. Therefore, it was concluded that nearly all sugar alcohol found after hydrogenation of the seaweed was already present in the seaweed. Thus, virtually no sugar alcohol was produced by hydrogenation of alginate, laminarin or cellulose from biomass composition. The poor performance of seaweed hydrogenation compared to alginate hydrogenation could be due to a difference in reaction path followed.

It was confirmed by the HPSEC and the LC-MS analyses of the liquid phase of the seaweed hydrogenation, hydrolysis of the polymers (alginate into oligomers and monomers) does take place under the hydrogenation conditions. Consequently, it was hypothesised that hydrogenation does not take place due to the presence of protein in the seaweed which can react with the oligomers and/or monomers or can deactivate the catalyst. This is in line with the observations that the liquid phase of the seaweed hydrogenation is not stable.

Therefore, the influence of the protein content on the seaweed hydrogenation was researched. A protease treatment to remove the proteins before the hydrogenation of seaweed, yielded 0.0% sugar alcohols. Yet it led to a much cleaner liquid phase with less unknown compounds detected. It was concluded that protease treatment as conducted in this research did not improve the production of sugar alcohols, yet stabilised the liquid phase.

Also, the performance of the catalyst (Ru/C) was researched by conducting an experiment where glucose was hydrogenated in the presence and absence of seaweed. It is known the catalyst can successfully hydrogenate alginate into sugar alcohols. But the catalyst might be deactivated by other components in the seaweed. Glucose hydrogenation yields 15% sugar alcohols. Glucose with seaweed hydrogenation yields 11% sugar alcohols. It was concluded that the catalyst is still able to hydrogenate glucose into sugar alcohols in the presence of the seaweed biomass. But the presence of the biomass in the reaction mixture does have a negative effect on the yield of the sugar alcohols. In the end, it is still suggested that (part of) the seaweed biomass inhibits the hydrogenation.

Recommendations

Based on the conducted research, several recommendations are made in order to improve the understanding of the catalytic hydrogenation of seaweed while producing sugar alcohols.

To start, it is recommended that the biomass composition is studied to a greater extend. Especially knowledge on the protein content would improve the understanding of the reactions taking place when exposed to the hydrogenation as proposed.

The production of sugar alcohols from the complete seaweed biomass is not successful at this point. Therefore, it would be interesting to expose the other saccharides from the biomass composition to the same reaction and compare laminarin and cellulose hydrogenation to alginate hydrogenation. Also, combinations of the different saccharides should be researched to understand the catalytic hydrogenation of the seaweed in total.

Since the reaction products are found to be unstable, research on the influence of the storage of the liquid phase and how to stop them could be performed. In this research already shows that an enzyme treatment, in this case protease, can improve the stability of the liquid phase. Next to protease, other enzymes can be inoculated with the biomass for a stable end product. At the same time, it can be researched if the sugar alcohol production is successful for different enzyme treatments.

It is established that the seaweed biomass has a negative influence on the hydrogenation of glucose, but the catalyst is still able to hydrogenate glucose into sugar alcohols in the presence of biomass. To what extent the seaweed biomass inhibits the catalyst is not yet known. This should be researched further and other catalysts can be nominated for the seaweed hydrogenation.

The big advantage of the proposed reaction for biomass processing, the catalytic hydrogenation, is that it avoids the energy intensive step of biomass drying. This is very beneficial for wet biomass processing and therefore fresh seaweed. In this research dried seaweed is taken as feedstock. In the future, the transition from dried seaweed to wet seaweed should be made in order to work towards a realistic process of sugar alcohol production from seaweed.

References

[1] H. Godfray, J. Beddington, I. Crute, … L. H.-, and undefined 2010, “Food security: the challenge of feeding 9 billion people,” science.sciencemag.org.

[2] J. W. van Hal, W. J. J. Huijgen, and A. M. López-Contreras, “Opportunities and challenges for seaweed in the biobased economy,” Trends Biotechnol., vol. 32, no. 5, pp. 231–233, May 2014.

[3] K. A. Jung, S.-R. Lim, Y. Kim, and J. M. Park, “Potentials of macroalgae as feedstocks for biorefinery,” Bioresour. Technol., vol. 135, pp. 182–190, May 2013.

[4] D. L. Klass, Biomass for renewable energy, fuels, and chemicals. Academic Press, 1998.

[5] E. J. Cottier-Cook et al., “Safeguarding the future of the global seaweed aquaculture industry,” no. September, p. 12, 2016.

[6] N. Wei, J. Quarterman, and Y. S. Jin, “Marine macroalgae: An untapped resource for producing fuels and chemicals,” Trends Biotechnol., vol. 31, no. 2, pp. 70–77, 2013.

[7] R. Rajkumar, Z. Yaakob, and M. S. Takriff, “Potential of the micro and macro algae for biofuel production: A brief review,” BioResources, vol. 9, no. 1, pp. 1606–1633, 2014.

[8] B. Subhadra and M. Edwards, “An integrated renewable energy park approach for algal biofuel production in United States,” Energy Policy, vol. 38, no. 9, pp. 4897–4902, Sep. 2010.

[9] K. Gao and K. R. McKinley, “Use of macroalgae for marine biomass production and CO_2 remediation: a review,” J. Appl. Phycol., vol. 6, no. 1, pp. 45–60, Feb. 1994.

[10] R. K. Greville, “Algae Britannicae.”

[11] R. P. John, G. S. Anisha, and K. M. Nampoothiri, “Micro and macroalgal biomass: A renewable source for bioethanol,” Bioresour. Technol., vol. 102, no. 1, pp. 186–193, Jan. 2011.

[12] P. Schiener, K. D. Black, M. S. Stanley, and D. H. Green, “The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta,” J. Appl. Phycol., vol. 27, no. 1, pp. 363–373, Feb. 2015.

[13] S. U. Kadam, B. K. Tiwari, and C. P. O’Donnell, “Extraction, structure and biofunctional activities of laminarin from brown algae,” Int. J. Food Sci. Technol., vol. 50, no. 1, pp. 24–31, Jan. 2015.

[14] R. M. Brown, “Cellulose structure and biosynthesis: What is in store for the 21st century?,” J. Polym. Sci. Part A Polym. Chem., vol. 42, no. 3, pp. 487–495, Feb. 2004.

[15] C. Peteiro, “Alginate Production from Marine Macroalgae, with Emphasis on Kelp Farming,” Springer, Singapore, 2018, pp. 27–66.

[16] A. Chinaza Godswill, “Sugar Alcohols: Chemistry, Production, Health Concerns and Nutritional Importance of Mannitol, Sorbitol, Xylitol, and Erythritol,” Int. J. Adv. Acad. Res. I Sci. Technol. Eng., vol. 3, no. 2, pp. 2488–9849, 2017.

[17] A. Kruse, A. Funke, and M.-M. Titirici, “Hydrothermal conversion of biomass to fuels and energetic materials,” Curr. Opin. Chem. Biol., vol. 17, no. 3, pp. 515–521, Jun. 2013.

[18] C. Ban, W. Jeon, H. C. Woo, and D. H. Kim, “Catalytic Hydrogenation of Macroalgae-Derived Alginic Acid into Sugar Alcohols,” ChemSusChem, vol. 10, no. 24, pp. 4891–4898, Dec. 2017.

Appendices

Appendix 1

Overview of the performed hydrogenations

1	A. esculenta	Ru/C	150°C	16 hours	
2	A. esculenta	Ru/C	150°C	16 hours	pre-treated: washed
3	Alginic acid	Ru/C	150°C	16 hours	
4	A. esculenta	-	150°C	16 hours	
5	A.esculenta	Ru/C	140°C	16 hours	
6	A. esculenta	Ru/C	160°C	16 hours	
7	Alginic acid	Ru/C	150°C	16 hours	
8	A. esculenta	Ru/C	180°C	16 hours	
9	S. latissima	Ru/C	150°C	16 hours	
10	D-sorbitol	Ru/C	150°C	16 hours	
11	D-mannitol	Ru/C	150°C	16 hours	
12	A. esculenta	Ru/C	150°C	24 hours	
13	A. esculenta	Ru/C	150°C	2 hours	
14	A. esculenta	Ru/C	150°C	48 hours	
15	A. esculenta	Ru/C	150°C	16 hours	pre-treated for cellulase,freeze dried
16	A. esculenta	Ru/C	150°C	4 hours	
17	A. esculenta	Ru/C	150°C	8 hours	
18	A. esculenta	Ru/C	150°C	16 hours	30.0 bar hydrogen
19	A. esculenta	Ru/C	150°C	16 hours	
20	A. esculenta	Ru/C	no heat	2 hours	original reaction mixture
21	A. esculenta	Ru/C	150°C	16 hours	liquid nitrogen pretreatment
22	A. esculenta	Ru/C	150°C	16 hours	washed and protease pretreatment
23	A. esculenta	Ru/C	150°C	16 hours	unwashed and protease pretreatment
24	Glucose	Ru/C	150°C	16 hours	
25	Glucose and A. esculenta	Ru/C	150°C	16 hours	

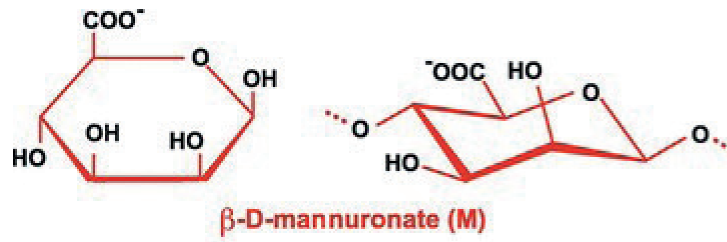
Appendix 2

Overview HPLC data

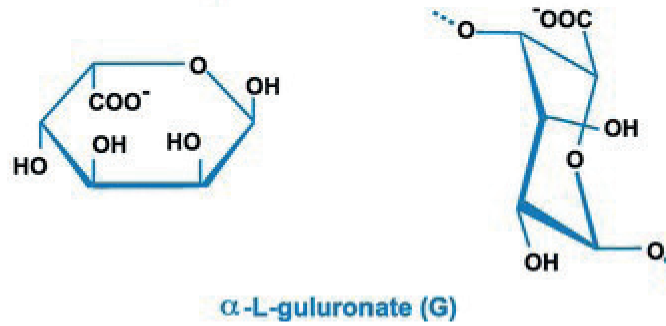
	mon	mon	mon	mon	mon	mon	mon	mon	org	org	org	org	org	
	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	
	Mannitol	Sorbitol	Galac-titol	Fructose	Galactose	Glucose	Xylose	tot. sug. Alcohol	tot. sug. Alcohol	D-mannuro-nic acid	L-guluro-nic acid	Glucose	Formic acid	
1	9.1	0.0	0.0	0.1	0.3	0.1	0.0	9.2	10.1	0.0	0.0	0.4	0.1	1
2	0.9	0.0	0.0	0.0	0.2	0.1	0.0	0.9	0.7	0.0	0.0	0.1	0.1	2
3	24.8	24.1	0.0	0.0	0.3	0.1	0.0	48.9	48.0	0.0	0.0	0.3	0.0	3
4	9.8	0.0	0.0	0.1	0.3	0.1	0.0	9.8	9.6	0.0	0.0	0.3	0.0	4
5	9.9	0.0	0.1	0.0	0.2	0.0	0.1	10.0	10.4	0.0	0.0	0.3	0.1	5
6	9.8	0.2	0.0	0.0	0.4	0.0	0.0	10.0	10.1	0.0	0.0	0.4	29.1	6
7	24.9	24.2	0.0	0.0	0.3	0.1	0.0	49.1	48.6	0.0	0.0	0.3	0.0	7
8	9.6	0.0	0.0	0.0	0.0	0.1	0.0	9.6	10.1	0.0	0.0	0.2	0.0	8
9	19.3	0.0	0.0	0.0	0.5	0.0	0.0	19.3	19.4	0.0	0.0	0.2	16.0	9
10	10.5	4.9	5.6	0.0	0.1	0.0	0.0	21.0	23.0	0.0	0.0	0.0	0.0	10
11	12.0	4.5	6.4	0.0	0.0	0.1	0.0	22.9	21.6	0.0	0.0	0.0	0.0	11
12	10.2	0.0	0.5	0.0	0.5	0.0	0.1	10.7	9.9	0.0	0.0	0.3	0.1	12
13	10.4	0.0	0.0	0.0	0.0	0.1	0.1	10.4	10.4	0.0	0.1	0.4	0.1	13
14	10.0	0.0	0.5	0.0	0.5	0.0	0.1	10.4	9.9	0.0	0.0	0.3	23.9	14
15	15.1	0.0	0.0	0.0	0.0	0.0	0.0	15.1	14.4	0.0	0.1	0.2	18.9	15
16	10.7	0.0	0.0	0.0	0.0	0.0	0.0	10.7	9.8	0.0	0.0	0.3	4.6	16
17	10.4	0.0	0.0	0.0	0.0	0.0	0.1	10.4	9.8	0.0	0.0	0.3	9.4	17
18	10.5	0.0	0.0	0.0	0.0	0.0	0.1	10.5	10.1	0.0	0.0	0.2	0.0	18
19	9.8	0.0	0.0	0.0	0.0	0.0	0.1	9.8	9.5	0.0	0.0	0.2	3.4	19
20	10.0	0.0	0.0	0.0	0.0	0.0	0.7	10.0	10.3	0.0	0.0	0.0	0.0	20
21	10.1	0.0	0.0	0.0	0.0	0.0	0.1	10.1	10.2	0.0	0.0	0.2	0.2	21
22	0.0	0.0	0.5	0.0	0.5	0.0	0.0	0.5	0.0	0.0	0.0	0.1	0.0	22
23	0.0	0.0	0.0	0.0	0.0	2.6	0.5	0.0	0.0	0.0	0.0	0.1	0.0	23
24	8.7	3.8	3.7	0.0	0.0	0.0	0.0	16.2	8.2	0.0	0.0	0.0	0.1	24
25	6.6	5.8	0.1	0.0	0.0	0.1	0.0	12.4	19.6	0.0	0.0	0.7	1.3	25
2 ww	1.0	0.0	0.0	0.4	0.0	0.0	0.0	1.0	1.1	0.0	0.0	0.0	0.1	2 ww

Appendix 3

Visual representation of uronic acids and structural saccharides of seaweed biomass

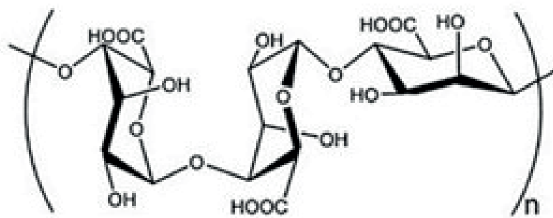


β-D-mannuronate (M)

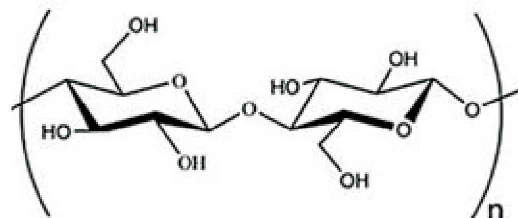


α-L-guluronate (G)

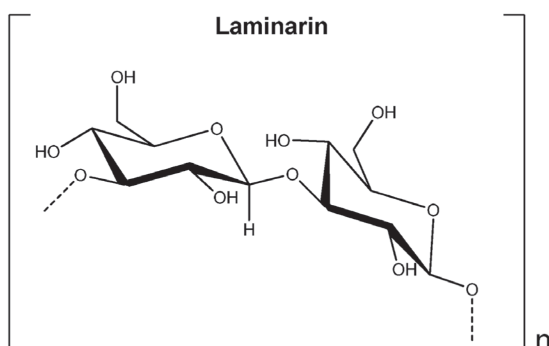
Alginic acid



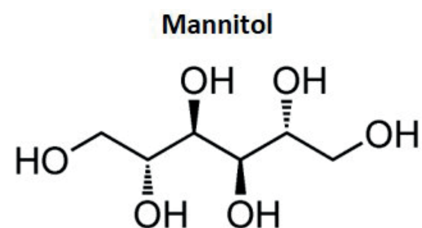
Cellulose



Laminarin



Mannitol



Appendix 4

Turning on autoclave

1. Add first the solids to the vessel, then the liquid
2. Manually close reactor by locking the side clips and tighten the screws evenly
3. Place heat block around reaction vessel
4. Check if all the sample valves are closed, if not, do so
5. Flush the reactor three times with argon gas till 20 bar. Pay attention: let the gas out slowly till 0.0 bar
6. During the last flush, check if the air seal is tight. Wait 15 minutes to be sure. If there is air leakage, check sample valves again and/or open and close the reactor again if needed. Start again at step 2 when reactor is opened again.
7. Fill the reactor with hydrogen gas till 50 bar. Check pressure again.
8. Close the gas supply
9. Turn on motor manually
10. Turn on stirrer manually
11. Turn on cooling water manually
12. Insert set point for stirrer = 1000 rpm
13. Insert set point for reactor temperature = 150°C
14. Check if temperature goes up over time

Turning off autoclave

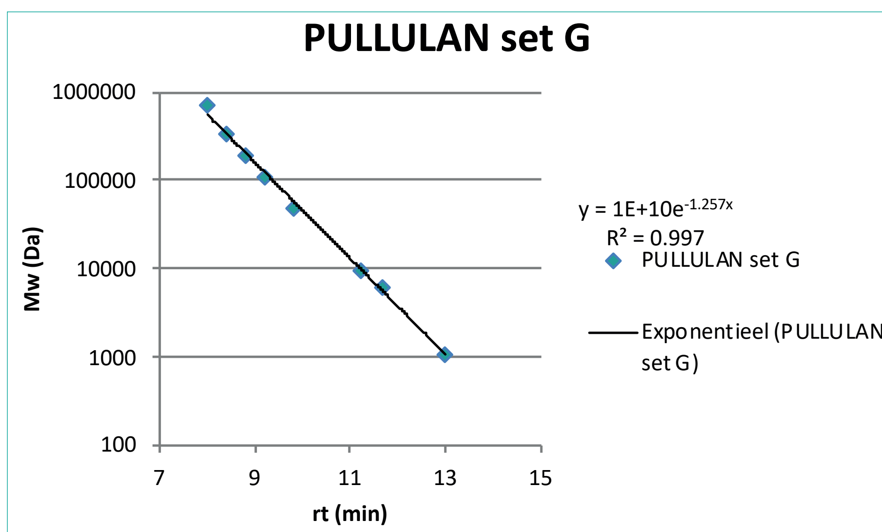
1. Insert new set point for reactor temperature = 15°C
2. Remove the heat block from the vessel by lowering it wearing heat resistant gloves
3. Wait until the reactor temperature is under 100°C, then place a beaker glass of cold water around the vessel to facilitate the cooling down
4. Wait until the reactor temperature is under 30°C, then remove the beaker glass
5. Insert new set point for stirrer = 0 rpm
6. Let out the hydrogen gas, pay attention: be super slow
7. Flush once with argon gas to 20 bar, make sure end pressure is 0.0 bar
8. Turn off motor manually
9. Turn off stirrer manually
10. Turn off cooling water manually
11. Open reactor manually by loosen the screws evenly and unlocking the side clips, be careful: the reactor can still be hot
12. Remove the reaction mixture from the vessel
13. Clean the vessel, the stirrer and the air seal ring precisely

Appendix 5

Data PULLAN set G

Given data	
rt (min)	mw (Da)
13	1080
11.7	6100
11.25	9600
9.85	47100
9.25	107000
8.8	194000
8.4	344000
8	708000

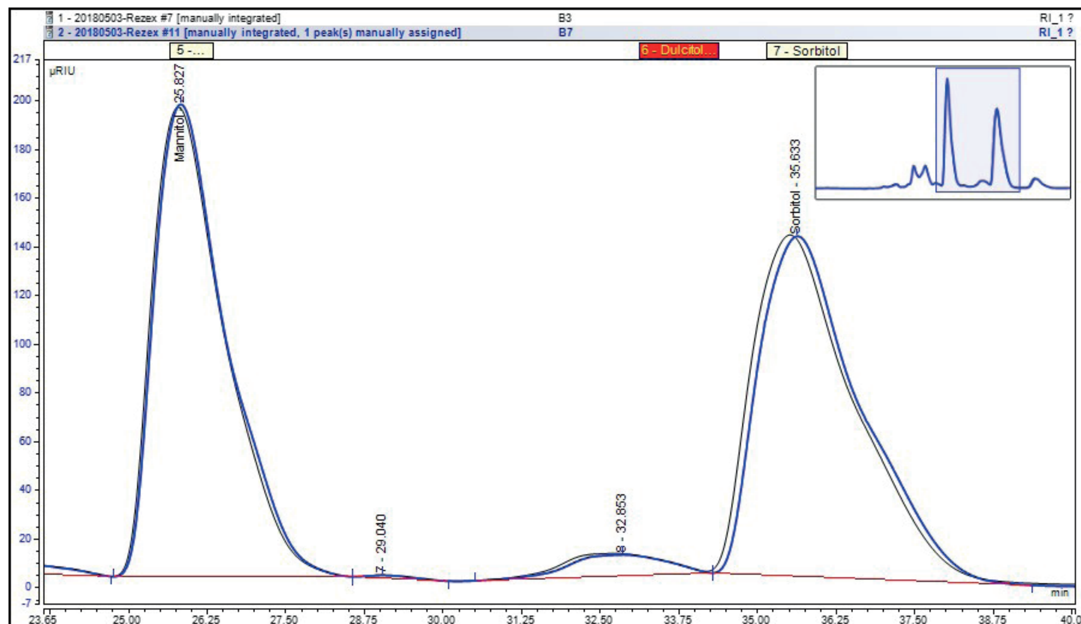
Fitting graph



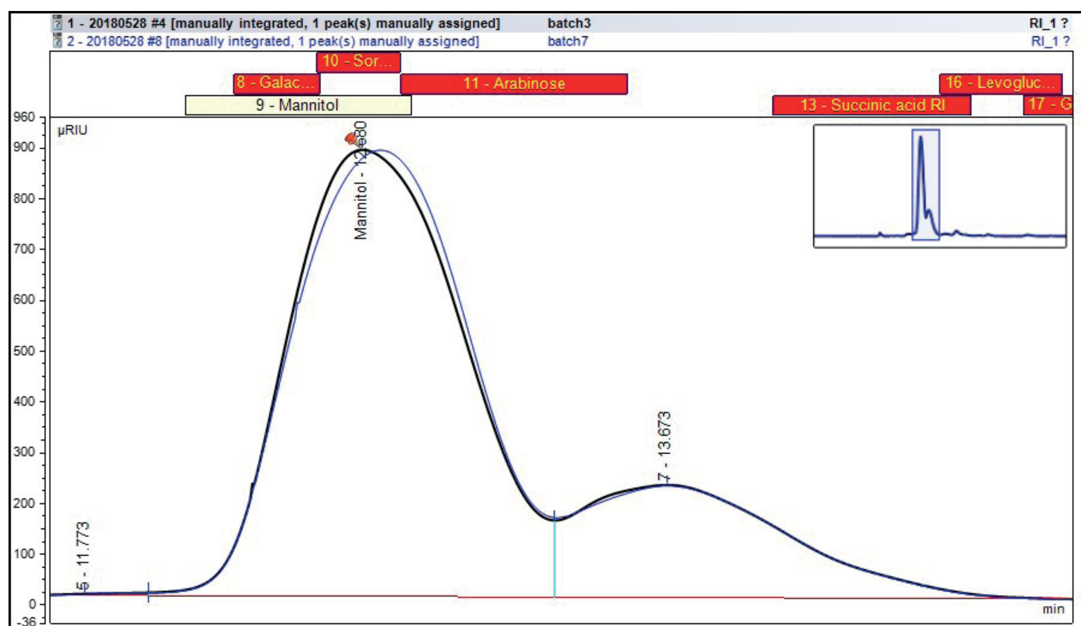
Calculated data	
rt (min)	mw (Da)
14.9	98
14.6	142
14	303
13.9	343
12.9	1206

Appendix 6

Alginate hydrogenation in duplo detected by HPLC of monosaccharides



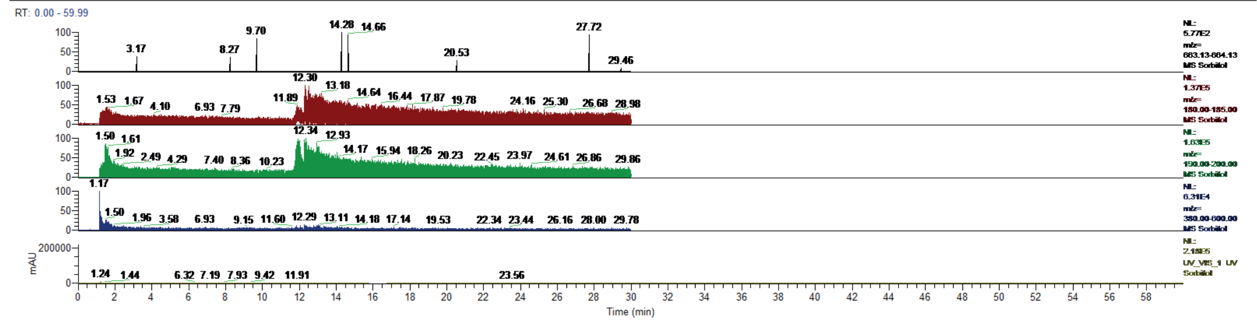
Alginate hydrogenation in duplo detected by HPLC of organic acids

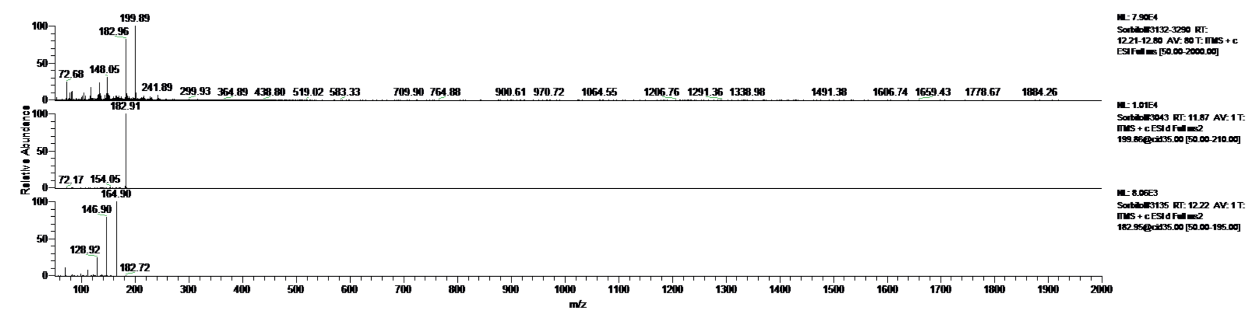


Appendix 7

LC-MS diagram of sorbitol

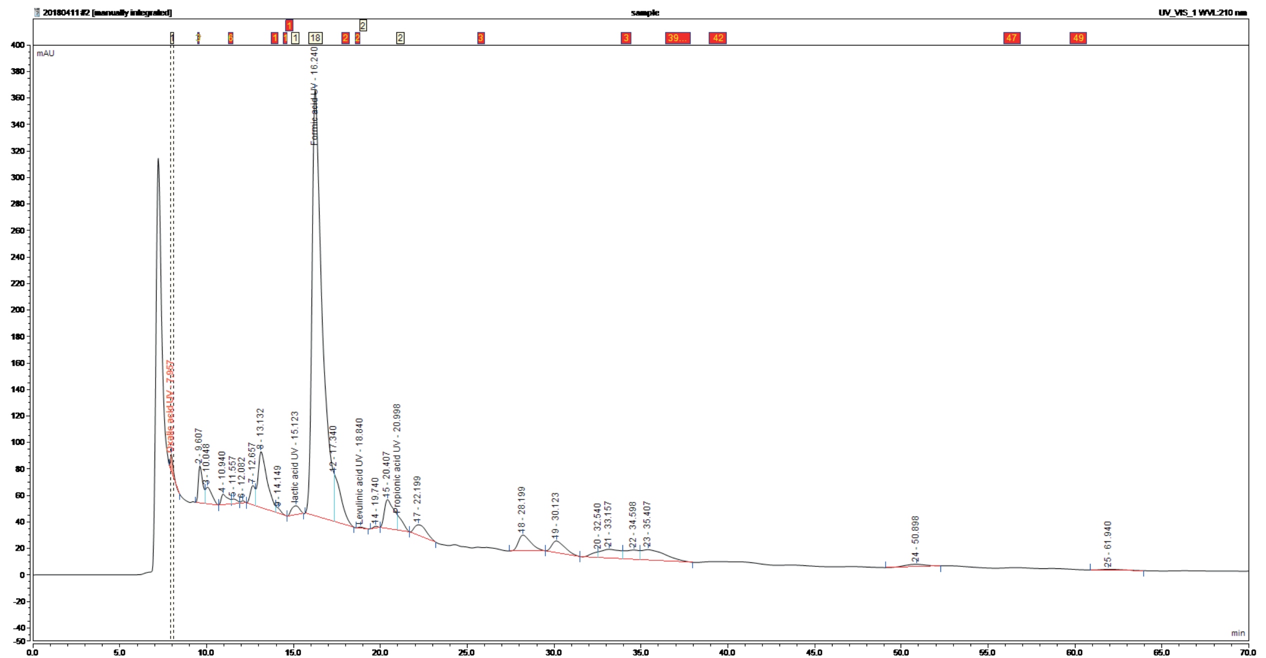
C:\XCALIBUR\...\BCT\KARLIJN\Sorbitol 20-4-2018 11:37:01



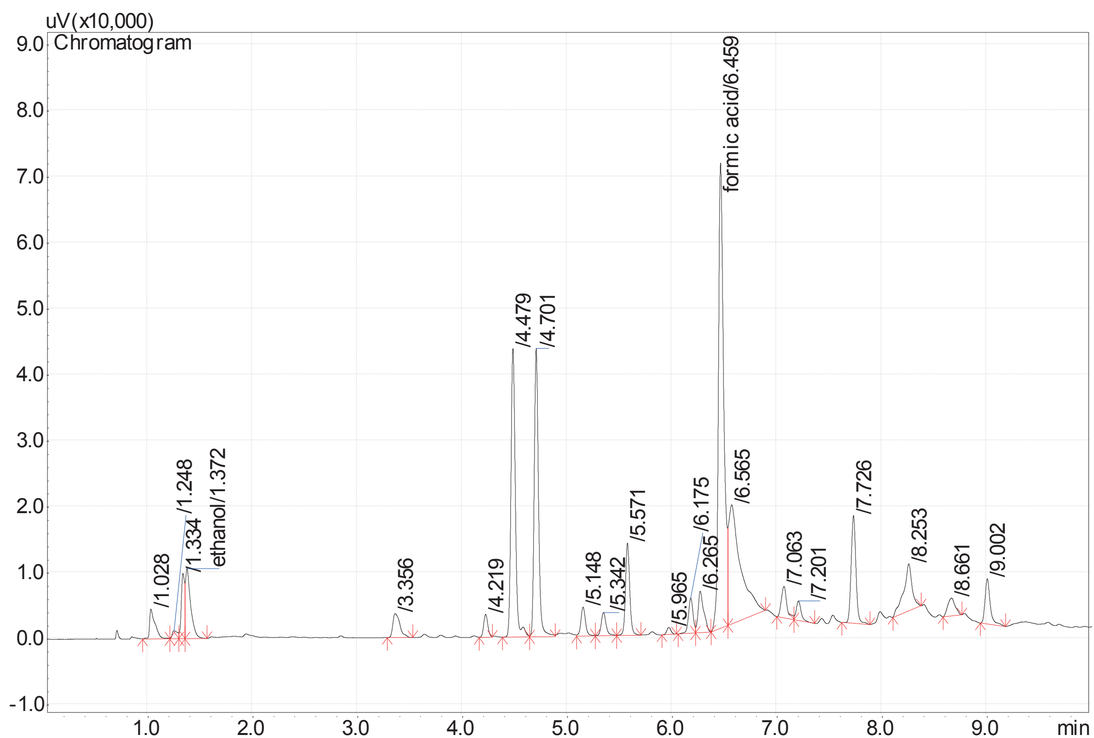


Appendix 8

Confirmation of formic acid in liquid phase



HPLC diagram UV of organic acids of seaweed hydrogenation



GC diagram of seaweed hydrogenation