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**RECOVERY OF CELLULOSE FROM GREEN ALGAE FOR TEXTILE
MANUFACTURE**

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Major in Biomass Refining

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Abstract: This thesis investigated if the cellulose from algal species *Cladophora glomerata* could be used as a feedstock for manufacturing textile fibers using the Ioncell-F process in a sustainable way. To achieve sustainability, it was investigated if the current methods for isolating algal cellulose could be replaced with more environmentally benign processes.

The current literature available on the biorefining and fractionation of algal biomass was reviewed, and it was concluded that a major issue in the field is that most of the studies cannot readily be compared with each other, making it difficult to review and compare different fractionation options. To address this issue this thesis suggests that existing knowledge on the biological categorization of algae could be used as a basis for producing more systematic information on algal fractionation.

The fractionation of the green algae, *Cladophora glomerata*, biomass was investigated by a two-stage fractionation process, and the composition of the original feedstock and the fractionation products were characterized in detail. Three different fractionation schemes were tested: 1) autohydrolysis + low-concentration alkali treatment 2) low-concentration acid treatment + low-concentration alkali treatment 3) acid treatment + alkali treatment. It was found that these treatments were not as effective as existing methods in isolating algal cellulose as the literature methods. Ultimate cellulose purity of 63 % was achieved using the methods tested in this thesis.

The cellulose produced by the fractionation study was subjected to an acid degradation to decrease its degree of polymerization (DP) to make it more suitable for textile production. It was observed to be much more resistant towards acid degradation than terrestrial celluloses, and to have leveling-off degree of polymerization of 940, which is approximately 3 times higher than that of terrestrial cellulose sources. A small amount of textile fibers was produced from this DP-adjusted material and their properties were compared with Ioncell-F fibers made from more conventional feedstocks. The produced algal fibers were observed to have slightly worse technical properties than the more conventional prehydrolysis kraft pulp-based fibers.

This thesis demonstrated the first ever production of cellulosic textile fibers made purely out of algal raw material. This result was achieved with relatively impure cellulose and it is clear that there is still much that can be done for improving this process. However, as a proof-of-concept demonstration, this thesis implies that green algae, a highly abundant and an under-utilized natural resource, can be used as a raw material for high-value cellulosic products.

Keywords: Textile fibers, algae, fractionation, Ioncell-F

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Tivistelmä: Tämä diplomityö tutki voitaisiinko *Cladophora glomerata* – viherlevän selluloosasta valmistaa tekstiilikuituja käyttämällä loncell-F teknologiaa. Lisäksi diplomityössä pyrittiin korvaamaan nykyisiä leväselluloosan talteenottometodeja ympäristöystävällisemmillä prosesseilla.

Työssä arvioitiin leväbiomassan biojalostamiseen ja fraktiointiin liittyvän tieteellisen kirjallisuuden nykytilaa ja todettiin että kyseisen alan keskeisenä ongelmana on, ettei suurin osa tehdystä tutkimuksesta ole vertailukelpoista muiden alan tutkimusten kanssa. Ratkaisuna tähän ongelmaan tämä työ ehdottaa, että olemassa olevaa tietoa levien biologisesta luokittelusta voitaisiin käyttää pohjana levien fraktiointiin liittyvän tiedon uudelleenjärjestämiseen systemaattisemmaksi kokonaisuudeksi.

Cladophora glomerata – viherlevän fraktiota tutkittiin käyttämällä kaksivaiheista fraktiointiprosessia. Sekä Leväbiomassan alkuperäinen, että fraktionaatiotuotteiden kemiallinen koostumus selvitettiin yksityiskohtaisesti. Työssä testattiin kolmea erilaista fraktionaatioskeemaa: 1) Autohydrolyysi + Keitto laimeassa emäksessä 2) Keitto laimeassa hapossa + Keitto laimeassa emäksessä 3) Keitto hapossa + Keitto emäksessä. Työssä todettiin etteivät nämä käsittelyt olleet yhtä tehokkaita kuin olemassaolevat kirjallisuudessa esitetyt menetelmät leväselluloosan talteenottamiseksi.

Työn fraktiointimetodeilla tuotettua selluloosaa käsiteltiin edelleen, jotta se soveltuisi paremmin tekstiilituotantoon loncell-F menetelmällä. Leväselluloosan polymerisaatioastetta pienennettiin happohydrolyysillä, mutta sen huomattiin olevan huomattavasti vastustuskykyisempi happohydrolyysiä vastaan kuin maa-kasveista peräisin oleva selluloosa. Lisäksi leväselluloosan polymerisaatioasteen tasaantuminen tapahtui 940 yksikön polymerisaatioasteessa, mikä on noin 3 kertaa korkeampi arvo kuin maa-kasveista peräisin olevalla selluloosalla. Työssä tuotettiin pieni määrä tekstiilikuituja tästä polymerisaatio-aste säädetystä materiaalista, ja tuotettujen tekstiilikuitujen ominaisuuksia verrattiin liukosellusta valmistettuihin loncell-F kuituihin. Tuotettujen levätekstiili-kuitujen tekniset ominaisuudet olivat hieman heikompia kuin konventionaalisten liukosellusta valmistettujen loncell-F kuitujen.

Tässä diplomityössä valmistettiin ensimmäistä kertaa koskaan selluloosapohjaisia tekstiilikuituja puhtaasti leväpohjaisesta raaka-aineesta. Nämä kuidut kyettiin valmistamaan verrattain epäpuhtaasta selluloosasta, ja on selvää, että valmistustekniikkaa kyettäisiin vielä kehittämään. Tämä työ kuitenkin kykeni osoittamaan demonstraatioluonteisesti, että viherlevät, jotka ovat erittäin yleinen, mutta alikäytetty luonnonvara, soveltuvat arvokkaiden selluloosatuotteiden raaka-aineeksi.

Avainsanat: Tekstiilikuidut, levät, fraktionaatio, loncell-F

Preface

This master's thesis was carried out at the Aalto University Department of Bioproducts and Biosystems during the year 2017 under the supervision of Prof. Herbert Sixta.

First, I would like to express my deepest gratitude to my supervisor Prof. Herbert Sixta for allowing me to have major influence on my thesis topic and providing the financial support for this thesis. The discussions we had always gave me new insights on the work, and your impeccable skill in finding the essential research problems is something I truly admire.

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Table of contents

1. Introduction	1
2. Introduction to algae as a biorefinery feedstock	2
2.1 Classification of algae	4
2.2 Main differences and similarities in chemical composition compared to woody biomass	5
2.2.1 Water content	8
2.2.2 Polysaccharides	9
2.2.3 Proteins and amino-acids	10
2.2.4 Minerals and ash	11
2.2.5 Phenolic compounds and lignin	11
2.2.6 Algal lipids and wood extractives	12
3. Fractionation of algal biomass	13
3.1 Evaluation of existing analytical fractionation methods	13
3.1.1 Industrial approach	15
3.1.2 Food science approach	16
3.1.3 Pure compounds approach	17
3.2 Evaluation of existing industrial fractionation methods	20
3.2.1 Agar	21
3.2.2 Carrageenan	22
3.2.3 Alginate	23
4 Aims of the thesis	25
5 Textile perspective on utilization of algal biomass	26
5.1 Macroalgae <i>Cladophora glomerata</i> as a raw material for cellulosic textile manufacture	26
5.2 Textile manufacture using man-made cellulosic fibers	27
6 Materials and Methods	30
6.1 Preparation of algal feedstock	30
6.1.1 Drying protocol	30
6.1.2 Milling protocol	30
6.2 Fractionation of algae	30
6.2.1 Fractionation study	30
6.2.2 DP-adjustment study	31
6.2.3 Full-scale DP-adjustment	32
6.3 Textile manufacturing trials	33
6.3.1 Preparation of algal feedstock	33
6.3.2 Preparation of the ionic liquid	34
6.3.3 Preparation of the cellulosic dope	35
6.3.4 Spinning of textile fibers	36
6.4 Analytics	38
6.4.1 Carbohydrate analysis	38
6.4.2 Oven ash	38
6.4.3 CHNS-analysis	38
6.4.4 Elemental analysis of ash	38
6.4.5 Gel permeation chromatography (GPC)-analysis	39
6.4.6 Rheological measurements	39
6.4.7 Cupri-ethylenediamine viscosity measurements	40
7 Results and discussion	41
7.1 Fractionation study	41
7.1.1 The uncharacterized compounds in the fractionation	47
7.2 Investigations on degree of polymerization (DP) of algal cellulose	48
7.2.1 GPC-Analysis	48
7.2.2 CED-viscosity analysis	51
7.3 Significance of dope rheology on the Ioncell-F process	54
7.3.1 Zero-shear viscosity	54
7.3.2 Cross-over point	55
7.3.3 Rheological measurements	56
7.4 Spinning trials	58
8. Conclusions	62
8.1 Conclusions on the literature section	62
8.2 Conclusions on the experimental section	62
Sources	65

1. Introduction

In scientific literature algae are often considered to be an extremely promising biomass source for development of future biorefineries. However, despite this, very few novel algal biorefineries can be observed to be operational in the world. This thesis aims to investigate the field of algal biorefining and to provide some understanding why such discrepancy exists.

The field of algal biorefining is investigated from several different viewpoints. Starting from very practical details of the algal industry, the discussion is taken all the way up to the fundamental issues present in scientific reporting on algae. The goal of such a broad perspective is to provide the reader with enough knowledge to assess the field of algal biorefining critically and to give some idea on how to evaluate claims made by various instances operating in the field.

As an experimental topic, this thesis will investigate the recovery of cellulose from green algae *Cladophora glomerata* and its usage as a raw material for textile fibers. This globally abundant species thrives in polluted waters and contains a significant amount of cellulose as a part of its cell wall. However, the current methods for recovering cellulose from this species of algae are mainly intended for lab-scale use and cannot be directly scaled up. The motivation is to investigate if new, more environmentally friendly ways of recovering cellulose from this green algae may be developed.

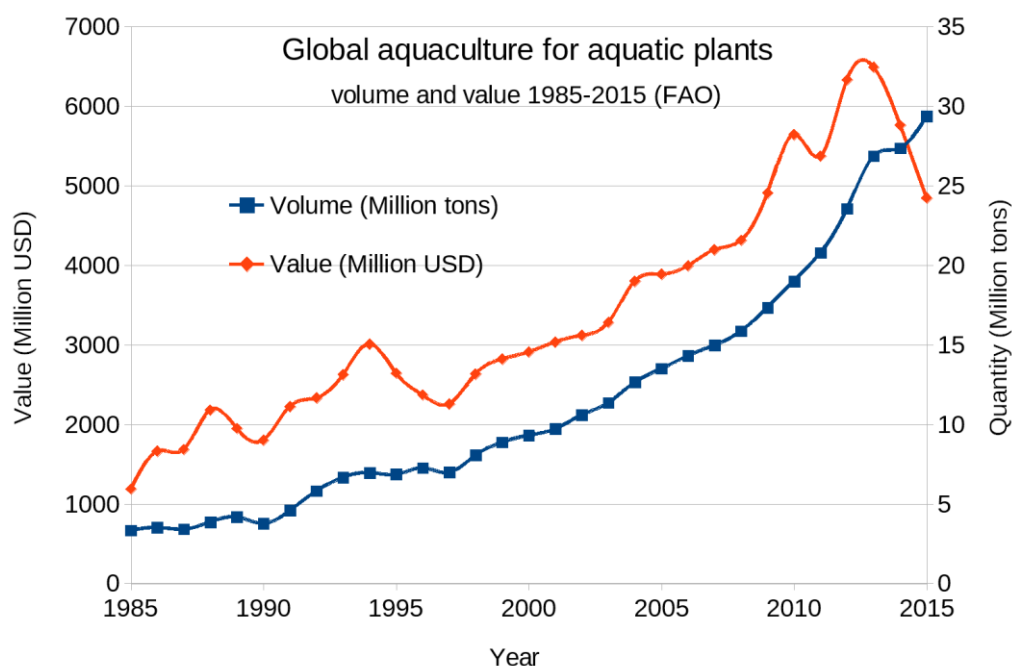
Finally, this thesis aims to test if this algal cellulose can be used for high-value applications, such as the manufacture of textile fibers. This presents a significantly more valuable product than the usage of algal cellulose as a fermentation feedstock, an application that is often reported in the scientific literature of algal biorefining as a potential usage of algal biomass. If successful, this would indicate on a *proof-of-concept* level that algae can be considered as a potential source of cellulose for textile materials.

2. Introduction to algae as a biorefinery feedstock

Algae-based industries produce a wide variety of products and are growing at a fast rate, doubling their reported production volumes in the timespan 2005-2015. FAO reports that the value of the current algal production is around 5 billion USD annually (FAO, 2016.). However, the growing disparity between the increasing production and decreasing total value of products in the recent years remains unclear in the FAO reports. The 5 billion value given by FAO also most likely excludes the algal processing industries, which have a total product value of approximately 1 billion USD annually (Naya & Bott, 2014). From other sources estimates from 10 billion (Rebours *et al.*, 2014) to 7 billion (Naya & Bott, 2014) for the total value of algal biomass based industries can be found.

However, it should be noted that official statistics concern mainly macroalgae production. FAO reports that its estimates of microalgal production are significantly understated as there are known large scale production facilities in several countries, that have not reported their production data. During the last decade, there have been several companies entering the microalgae market, but despite the great interest in biofuels, the current microalgae market is mainly focused on food with about 75% of the microalgae produced being used as food or nutritional supplements in 2012. (Sastre 2012, pg. 16) The main reason for the lack of microalgal oil refineries is, that despite the optimism around 2010, the technology was not as close to commercial viability as imagined, and many of the companies working in that field have gone bankrupt or switched focus.

Figure 1. Global aquaculture volume and value for aquatic plants in the years 1985-2015. (FAO, 2017)



The existing algae biorefining industry can be considered to be mostly isolation of carrageenan and agar from certain red algae and alginate from certain brown algae. The state of this industry at 2010 is summarized well in the article by Bixler and Porse (2010), where the value of the annual production of the hydrocolloid industry is evaluated to be slightly over one billion dollars, with the rough price per ton being around 18 000 USD for agar, 12 000 USD for alginate and 10 500 USD for carrageenan. There is further industrial production in more specific applications, such as soil additives, fertilizer, seaweed meal and pharmaceuticals and cosmetics, but this production has an estimated total annual market value of around only 50 million USD. (Naya & Bott, 2014)

As for the cost of the algal feedstock itself, a recent conference paper by Valderrama (2012) gives estimated farm gate prices from 380 USD/ton in Tanzania to 600 - 1400 USD/ton in Indonesia, Philippines and Mexico for the dry algal biomass suitable for carrageenan production. In the same paper, the sustainability of the production is discussed in the societal sense, concluding that 400 USD/ton may be considered to be the absolute minimum price level to maintain the socio-economic status of algal farmers. (Valderrama, 2012)

In the last decade, there has been a massive spike of interest towards using algae as a feedstock for novel biorefineries, particularly in projects focusing on using microalgae as a raw material for biofuel and bioenergy. This is evident from the multiple studies and investment projects launched on this topic. A reasonably comprehensive report on the work done and currently in progress can be found from the IEA report; "State of technology review - Algal bioenergy" (2017).

However, despite the extreme effort, no commercially viable biofuel production schemes have been developed as the production costs of the algal biofuels remain too high. For microalgal biomass, several calculations and simulated LCA and cost analysis studies have been made by various organisations, such as VTT (Arnold *et al.*, 2013) IEA (Laurens *et al.*, 2017) and similar results can be found in several journal articles (Barlow *et al.*, 2016; Zhang *et al.*, 2017). An important factor in this lack of economic viability is the recent fluctuation in crude oil prices. (Laurens *et al.*, 2017) The prices have peaked in 2008 at 140 \$ per barrel, then stayed relatively stable in the 2010-2014 interval, slowly rising from 80 \$ per barrel to 100 \$ per barrel, finally in the later half of 2014 collapsing to the current price level of approximately 50\$ per barrel. (NASDAQ, 2017) Obviously, this price fluctuation has had a tremendous effect on the commercial viability of algal fuels. For using macroalgal biomass, even in just energy applications, the IEA report (Laurens *et al.*, 2017) simply concludes: "*Numerous parameters, including species, method of cultivation, harvest method,*

suitability of various feedstock storage methods, cost of the harvested seaweed, cost of the produced biofuel, etc., have not yet been adequately assessed and much additional research is required.”

The most critical expenditures to create sustainable algal biofuels come currently from the costs related to growing and harvesting of microalgal biomass. (Darzins *et al.*, 2010; Laurens *et al.*, 2017) As a recent solution to this problem, it has been suggested that an extra focus would be given to the other products that could be extracted and produced simultaneously with biofuels, improving the financial viability of algal biorefineries, as the production of liquid fuels or energy from algae is not foreseen to be viable in the near future. (Laurens *et al.*, 2017) In this context, the topic of this thesis seems particularly relevant.

2.1 Classification of algae

The three main groups of algae most commonly discussed in an industrial context are green algae (*Chlorophyte* & *Charophyte*), red algae (*Rhodophyta*) and brown algae (*Phaeophyceae*). However, these categorizations represent only a fraction of the total biological diversity found in algae. Furthermore, the species even within these common categorizations might have enough diversity to make generalizations of this category level insufficiently accurate for technical applications.

Another commonly used division is to classify the algae into multicellular macroalgae and unicellular microalgae. As both of these groups contain extremely diverse set of organisms, a great care should be taken, not to over- or under generalize any findings using this terminology.

The way of addressing the inherent diversity present in algal biomass in articles related to biorefining of algal biomass is to include a multitude of species in the analytical studies. This approach has been used for several studies found in literature (Knoshaug *et al.*, 2013; Garcia *et al.*, 2011; Tran *et al.*, 2010; Laurens *et al.*, 2017). However, in most of these studies, the categorization is based on the content of the desired products with little weight placed on the biological systematics of the chosen species. This raises the question of how valid is the information provided by these kind of comparisons.

For example, the study by Knoshaug *et al.* (2013) comparing algae and some aquatic plants as sources of cellulose, lists *Cladophora*, *Rhizocolonium*, *Chaetomorpha* and *Ulva* (*Ulva* contains also species formerly known as *Enteromorpha*) as green seaweeds containing cellulosic fibres, but fails to mention that the first three species (*Cladophora*, *Rhizocolonium* and *Chaetomorpha*) belong all to the order *Cladophorales*, while *Ulva* belongs to the order *Ulvales*.

A major effect of this lack of distinction is that these species in the order *Cladophorales* contain highly crystalline cellulose, with crystallinities up to 95.2% (Nicolai & Preston, 1959; Atalla *et al.*, 1985; Mihranyan *et al.*, 2004), while the *Ulvales* species contain cellulose with crystallinity as low as 36% (Jmel *et al.*, 2016). Furthermore, the hydrolysate of the cellulose-rich microfibrils, originating from *Cladophora rupestris* (*Cladophorales*-family) contain only glucose, while in *Ulva lactuca*, (*Ulvales*) the hydrolysate contains a mixture of glucose and xylose. (Cronshaw *et al.*, 1958) It is obvious that the sugar composition of the polysaccharide fraction(s) affects the biorefinery concepts. Therefore, it is very important to characterize the different algae species in detail.

The study of Cronshaw *et al.* (1958) also serves as a beautiful example on how the data provided by categorization of algae could be combined with fractionation studies in biorefining. In their study they fractionated various algal species and provided ample information on the effects of this fractionation both on the morphology of the cell wall, and the fractions cleaved by each fractionation stage. This allowed to make quite profound categorisations dividing the algae into three distinct groups based on their fractionation behaviour and changes in the cell wall structure during fractionation. Furthermore, the species used in the study were selected to provide as high morphological diversity as possible, making the results as generalizable as possible.

The lack of biological understanding might be partly to blame for the fragmentary situation of the current field of algal biorefining. The variance in algal biomass, both within and between species is an issue left unaddressed in many articles of the field, and has ultimately resulted in a situation where results of various studies cannot be compared with each other in a meaningful way. There are of course some positive examples in modern literature as well, such as the study by Siddhanta *et al.* (2011), which in addition to investigating the cellulose content of various algae also reports the relative amounts of α - and β -cellulose present and the biological systematics on the level of class and order. Of course, this categorization is not without its problems, but at least it acknowledges that all algal cellulose is not chemically identical and reports this fact instead of ignoring it.

2.2 Main differences and similarities in chemical composition compared to woody biomass

This chapter focuses on the differences in chemical composition of the algae and woody biomasses. Differences and similarities in regards to other traits, such as cell structure, biology, cultivation and ecology will not be discussed in this section of the thesis. In addition, a comparison of algal biomass to other terrestrial biomasses, such as legumes (e.g. soybean) or annual grasses will be left outside the scope of this thesis. Since it can be assumed that the reader is familiar with the compositional

structure of wood, the focus will be led on the composition and properties of algal biomass. First, some issues found in the scientific literature regarding the compositional analysis of algae will be addressed. Then a short overview of the main differences between algal and woody biomasses will be given. Finally, the major components of these biomasses are compared with each other.

As algal biomass is analysed for various purposes, such as food, biorefining and biology, the studies in these fields often use different analytics to analyse the composition, some of which might not be completely accurate for algae. When reading the literature, the reader should take into account that this can lead to some discrepancies in the reported data. This phenomenon had been observed by Angell *et al.* (2015) in respect to the determination of protein content of different algal biomasses, who observed that the two main methodologies used currently for algal protein analysis over- or underestimate the real protein content depending on the methodology used.

However, some studies also exhibit a lack of proper understanding of algal biomass, claiming for example that the green algae *ulva lactuca* contains lignin. (Yaich *et al.*, 2011) This is a major claim as even though some green algae may contain primitive “lignin-like” - compounds, no green algae containing true lignin has yet been discovered. (Martone *et al.*, 2009) The existence of the claimed lignin content can most likely be attributed to the analytical method Yaich *et al.* have applied to analyse the composition of the *ulva lactuca*. The analytical method used was originally developed to analyse cattle feed (Van Soest *et al.*, 1991), which was implicitly assumed to be terrestrial biomass. As this kind of error was made in an article published at the relatively prestigious journal of *Food Chemistry* (1976-), the journal itself having an impact factor of 4.529, it is likely that more examples of this kind of incorrect analytics can be found in the literature.

The chemical composition of algae differs significantly from the woody biomass, not only purely in a chemical way. Furthermore, the compositional variation both between and within algal species is immense, especially in microalgae. This is further aggravated by the fact that algae can undergo massive changes in their chemical composition depending on the growth environment. For example, the microalgae *Vovocales* can contain, on dry weight basis 80 % lipids or 80 % neutral hydrocarbons or alternatively 40 % glycerol, depending on the cultivation conditions. (Hu, 2013, pg. 114) Another example of an algae exhibiting extreme compositional plasticity is the *Chlorella pyrenoidosa*, with compositional details available in Table 1.

As an example of this, many algal species show distinct seasonal variation in their composition. This phenomenon is apparent for example in species *Saccharina* and *Laminaria* that belong to brown

algae. (Haug & Jensen, 1954; Jensen, 1956) *Kappaphycus alvarezii*, a member of red algae (Kumar, 2015) and *Ulva fasciata* and *Caulrepa racemosa* can be mentioned as examples of green algae (Rao *et al.*, 2015). It is however good to notice that this seasonal variation is another term one must be careful with regards to understanding algal composition. The studies referred here for brown algae have been done in Europe, with 4 seasons (spring, summer, autumn, winter), while the studies on red and green algae have been done in India, with the seasonal situation described best as 3 seasons (summer, monsoon, post-monsoon).

To give a rough estimate of the differences in the qualitative and quantitative composition between wood and algae, compositional data from wood, some macroalgae and some microalgae are presented in Table 1. The selection of these macro and microalgae is based on the availability of literature data, and should not be taken as a comprehensive compositional estimation of all algae. Furthermore, when possible, apparent variations (season, growth location etc.) have been included in data, demonstrating the plasticity of algal biomass.

Table 1, Rough mass-composition of spruce and some algae

Component / Species	Spruce (<i>Picea abies</i> , terrestrial wood)	<i>Chlorella pyrenoidosa</i> (green microalgae)	<i>Ulva lactuca</i> (green macroalgae)	<i>Laminaria digitata</i> (brown macroalgae)	<i>Gracilaria cervicornis</i> (red macroalgae)	<i>Cladophora glomerata</i> (green macroalgae this thesis uses in its experimental section)
Water content on harvesting	56%	99.93%	80 – 90%	79 – 90 %	88 % ^a	No literature data found
Composition of dry solids						
Hemicelluloses	28.3%	6 – 46%	50 – 55 %	59 – 70 %	55 – 61 %	3 – 49 %
celluloses / fibrous carbohydrates	41.7 – 49 %	less than 16 %	9 %	11 – 13 %	4 – 6 %	12 – 39 %
Proteins	0.2 %	7 – 88 %	9 – 40 %	6 – 10%	17 – 23 %	11 – 32%
Ash	0.8 %	5 %	14 – 35 %	35 – 39 %	9 – 12 %	2 – 46 %
Algal lipids / Wood extractives	0.5 – 1.7 %	5 – 86 %	0.3 – 8 %	5 – 6 %	4 – 5 %	0.8 – 5 %
Lignin / Phenolics	27.2-29 %	1 – 2 %	0.4 %	0.1 – 0.2 %	0.2 %	0.005 %

^awater content of fresh *Gracilaria lichenoides* used, as no such data for *Gracilaria cervicornis* was found

For this table, data has been compiled from: (Frédérique & Holmbom, 2004; Dermibas, 2002; Nurmi, 1999; Harod, 1976; Northcote *et al.*, 1958; Yaich *et al.*, 2011; Ortiz *et al.*, 2006,; Schiener *et al.*, 2015; Ito & Hori, 1989; Westermark *et al.*, 1986; Li *et al.*, 2007; Bruhn *et al.*, 2011; El-Baky *et al.*, 2008; Schaal *et al.*, 2010; Marinho-Soriano *et al.*, 2006; Martins *et al.*, 2013; Hasan & Rina, 2009; Akküz *et al.*, 2011; Applier & Jauncey, 1983; Khuantrairong *et al.*, 2011; Thiamdao *et al.*, 2012; Muztar *et al.*, 1978; Koch, 2006, pg. 23)

2.2.1 Water content

The first obvious difference between the terrestrial and algal biomass is the water content. The water content of typical fresh woody substrates varies between 40 and 60% on total weight (Kozhin *et al.* 2011, pg. 3). For algae the water content is considerably higher, with fresh harvests having the water content between 79 – 96 % (Hasan & Rina, 2009; Schiener *et al.*, 2015). Finally, it is worth noticing that microalgal growth suspensions may have a dry matter content as low as 0.02-0.05 %. (Milledge *et al.* 2013)

2.2.2 Polysaccharides

Analogous to wood, algae may contain significant percentage of their dry weight as polysaccharides. Typical 65 – 82 % polysaccharide content can be found in woods, such as Spruce and Aspen. (Koch, 2006, pg. 4). In algae this polysaccharide content is usually lower, but according to the data compiled by Holdt & Kraan (2010), macroalgal species found in the Atlantic Europe can contain polysaccharides from 4 – 76 mass-% on dry weight basis, so universally valid generalisations cannot be made. However, when discussing individual algal species, the variance in polysaccharide content is usually much more moderate (see Table 1).

The polysaccharides in algae can be divided into two categories, structural and storage polysaccharides. Usually structural polysaccharides make up the algal cell wall. These structural polysaccharides are divided in two parts; a skeletal support structure, usually made of cellulose, but xylan and mannan supports can also be found and an amorphous matrix filling the framework made by the skeletal supports. (Lee, 2008, pg. 8-10) However sometimes other components, such as proteins or inorganic compounds may form a significant part of the cell wall.

The most common amorphous components of the structural polysaccharides are compounds such as alginic acid, fucoidin, agar and carrageenan, (Lee, 2008, pg. 9-10) but there are several other types of polysaccharides fulfilling this role as well, such as ulvans (Lahaye & Robic, 2007). Many of these amorphous polysaccharides are however species-specific, and can only be found in specific species or families of species.

The storage polysaccharides are polysaccharides that algal organisms consume for energy. The most common three polysaccharides used for this purpose are: starch (green algae), floridean starch (red algae) and laminaran (brown algae). Of these, algal starch is similar to the starch found in terrestrial plants and floridean starch has similar structure to starch, but contains no amylose. Both of these starches contain α -D-glucose as their main unit. However, the division, especially between red and green algae is not very strict and red algal species using starch as their storage polysaccharide can be found. The Laminaran is structurally significantly different, containing (1,3)- β -D-glucose and (1,6)- β -D-glucose and mannitol as the main units. (Misurcova, 2011, pg. 183-186)

An in-depth investigation of different polysaccharides found in red, green and brown algae can be found in the article published by Elizabeth Percival (1979), compiling the results of almost 25 years of research. She reports about the division of different polysaccharides along different families of

algae and furthermore about some properties, such as gelling behaviour, stability, structure and biosynthesis of these polysaccharides.

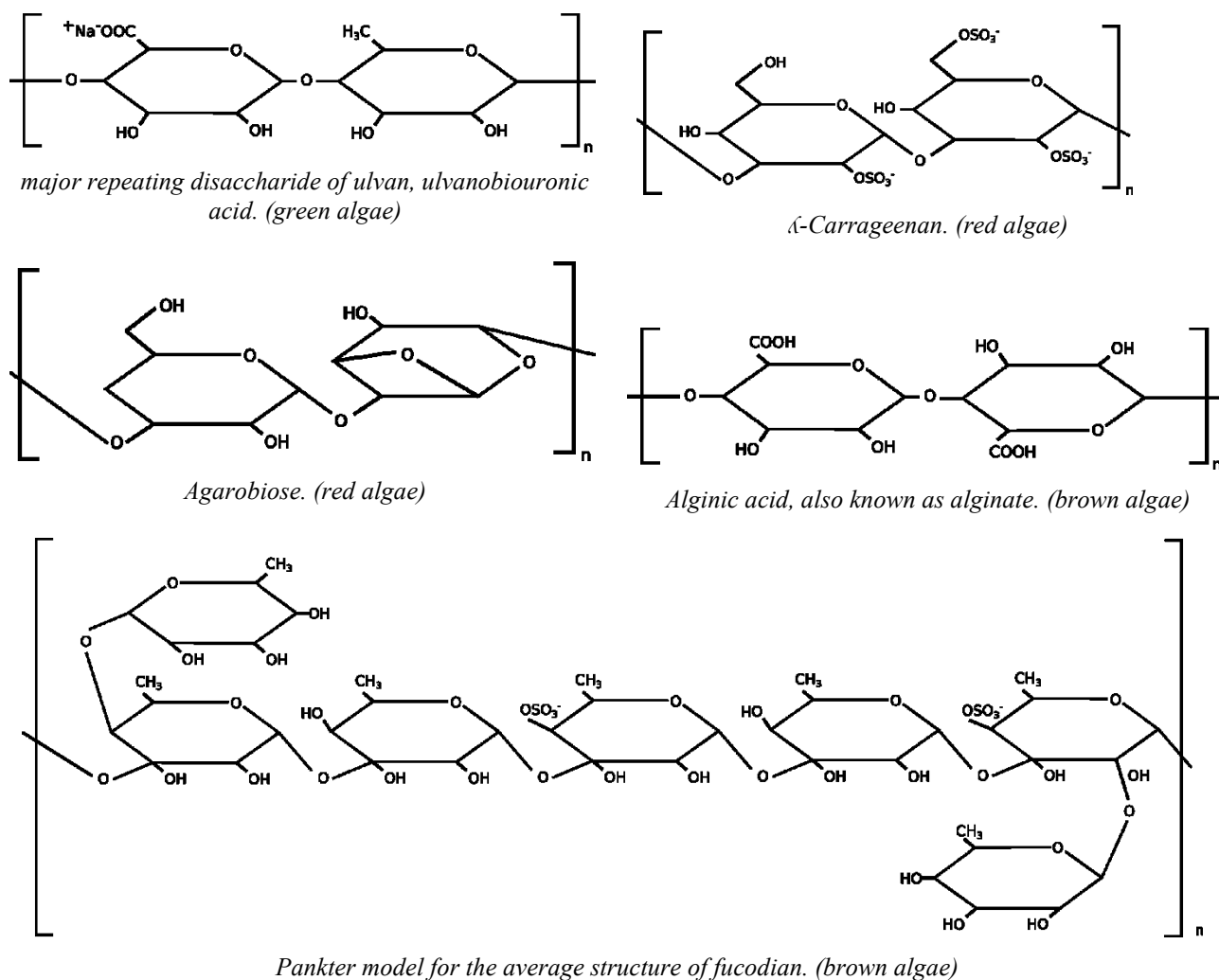


Figure 2, Structural representation of some common amorphous algal polysaccharides.

Structures presented in figure 2 based on the work of; (Lahaye & Robic, 2007), (Campo *et al.*, 2009), (Lahaye *et al.*, 1991), (Lee, 2008, pg.10) and (Li *et al.*, 2008).

2.2.3 Proteins and amino-acids.

The protein contents of wood can be generally considered to be extremely low in comparison to algae. For example, when estimating the protein content of the whole spruce wood, a value of 0,2 % is reported in the work of Westermarck *et al.* (1986). However, it is worth mentioning that some fractions of the wood may show higher concentrations of protein, as protein content of 15 – 17.2 % in spruce needles was reported in the same article. (Westermarck *et al.*, 1986)

The protein contents of algae differs according to the species. Generally brown macroalgae have lower protein contents (3-15% of dry weight), when compared to green and red macroalgae (10-47% of dry weight). (Fleurence, 1999) Typically algal proteins have been studied mostly in the field of nutrition and compared with leguminous plants, such as the soybean. When comparing the amino acid composition of algal biomass with leguminous plants there are some differences depending on the choice of algal species, but when taking a rough overview of the amino-acid contents, they share more similarities than differences, according to the data compiled by Fleurence (1999).

2.2.4 Minerals and ash

In wood, minerals and ash form a relatively small part of the total weight. However, the differentiation of ash content between tissues is apparent, as wood bark has significantly higher ash content (~ 6 %) than wood trunk (~ 0.8 %). (Dermibas, 2002). For algae, which by definition (Lee, 2008, pg. 3) are thallophytes (e.g. lacking roots, stems and leaves) such differentiation does not exist. However, on a cellular level, the distribution of non-organic tissue in algae may be very specific. For example, in the case of diatoms (Bacillariophyceae), the organisms are able to secrete an external cell wall composed of silica. (Lee, 2008, pg. 369)

The ash content of algae is heavily dependent on the growing conditions, and as a study by Schiener *et al.* (2015) shows, the ash content of brown algae can vary between 21 – 41 %, depending on the season. Obviously there are also species for which the change in ash and mineral content is not as dramatic. As a rough generalization, it may be said, that brown algae contain higher amounts of ash than red and green algae. It should be kept in mind however that exceptions caused by specific species and growth environments apply.

2.2.5 Phenolic compounds and lignin

The major difference between terrestrial plants and algae is that as a general rule, algae do not contain lignin at all. The presence of lignin in algae is extremely rare, and typically limited to lignin-like compounds found in primitive green algae. (Martone *et al.*, 2009) However, in a recent study, Martone *et al.* (2009) found that a certain red algal species has secondary cell walls containing lignin, raising fundamental questions about the evolutionary histories of terrestrial plants and algae.

However, some algae can contain other phenolic compounds in significant amounts. Some families of brown algae can have up to 30 % of phenolic compounds of their dry weight. (Bourgougnon *et al.*

2011) These compounds form a variety of different chemicals that may appear as a structural part of the cell wall, or as a part of the chemical defence system of algae.

2.2.6 Algal lipids and wood extractives

As algal lipids and wood extractives, especially tall oil has been a topic of interest in the research area of liquid biofuels, a short comparison of the similarities and differences of these two groups of compounds is given here. The discussion focuses on the extractives of softwood, and the topic of hardwood extractives, such as tannins is left outside the scope of this thesis.

Extractives is a common name for secondary wood metabolites that can be extracted from wood using organic solvents or hot water. In softwoods, the mass percentages of these compounds on dry softwood is in the range of 0.2 – 0.8 % for terpenes, 0.3 – 0.4 % fats and 0.08 – 0.09 % waxes. (Koch, 2006, pg. 35-36.) These compounds undergo some chemical changes during sulfate pulping, but do not degrade in significant amounts, and can ultimately be extracted as tall oil. (Holblom & Rainer, 1978)

In algae, the lipid content and composition depends on the growth conditions. Under ideal growth conditions algae produce moderate amounts of fatty acids (5 – 20 % of dry weight of some microalgae), which have good nutritional properties, but are less ideal for biofuel production. However, in stressed conditions many microalgal species begin to produce neutral lipids that are considered to be a good feedstock for biofuels. (Sharma *et al.* 2012)

The main difference between these two groups of compound is that the wood extractives in the tall oil contain a much more diverse group of compounds than the algal lipids. As documented by Holblom & Rainer (1978) tall oil contains a diverse mixture of fatty acids, resin acids and terpene alcohols. However, when algal oil extract have been studied for energy purposes, a much narrower distribution of compounds is observed.

A typical study by Yoo *et al.* (2010) shows that for three microalgal species almost 100% of the algal fatty acid composition is explained by two 16-carbon fatty acids and three 18 carbon fatty acids. Of course, these algae had been grown under stress conditions, and are species selected for optimal lipid production. A more comprehensive review on the lipid production systems and the variety of lipids produced by algae is provided by Schuchmann *et al.* (2012) and is perhaps more reflective of the real diversity of algal lipids. However, when comparing algal lipid extracts and the tall oil-extracts on a large scale, it is clear that algal lipids are a more homogenous group of compounds.

3. Fractionation of algal biomass

This section will familiarize the reader with the current technologies and methodologies used in fractionating algal biomass, both in the research world and in current industrial applications. A large amount of novel fractionation methods and fractionation schemes have been proposed for algal biomass in the recent years. However, it is difficult to make comprehensive comparisons based on this data to estimate the best species or refining concepts of algal biomass. (de Jong & Jungmeier, 2015) as different studies use different assumptions and methodologies as their basis.

In some aspects these different methodologies might be guided by the industries they try to serve. Different fields of research have different topics of interest and these interests get reflected in the scientific data produced, causing an unintentional omission of data from less interesting perspectives. For example, the same species of algae may appear different, whether one wants to use it as a feedstock in chemical, medical, food or energy industry. The green algal species *Ulva lactuca* can be considered as an example, as it is considered to be a valuable feedstock for producing fermentation media and 1,2-propanediol, (van der Wal *et al.*, 2013), healthy as human nutrition (Tabrasa, 2012), and to be the principal source of a physiochemically, rheologically and biologically interesting compound: ulvan (Lahaye & Rovic, 2007).

Ultimately, there is no straightforward fractionation concept for algal biomass. It all depends on the goals and the applications of the study. Of course it can be argued that some studies and some fractionation schemes contain more economic, societal or ecological significance than others, but it is hard to argue that their approach would be the only correct one. This chapter aims to introduce the reader to some analytical fractionation methods used in the scientific studies of algae and to describe the most important industrial-scale fractionation processes used for algal biomass.

3.1 Evaluation of existing analytical fractionation methods

The existing literature on algal fractionation in the laboratory scale could be roughly divided into three categories based on the topics they discuss and the methodologies they use. These categories could be referred to as the industrial, the food science and the pure compounds approach. This categorization is presented for the first time in this thesis, and its validity is still an open question. However, as the following sections aim to prove, this type of categorization may be helpful in organizing and evaluating the information provided by current scientific literature.

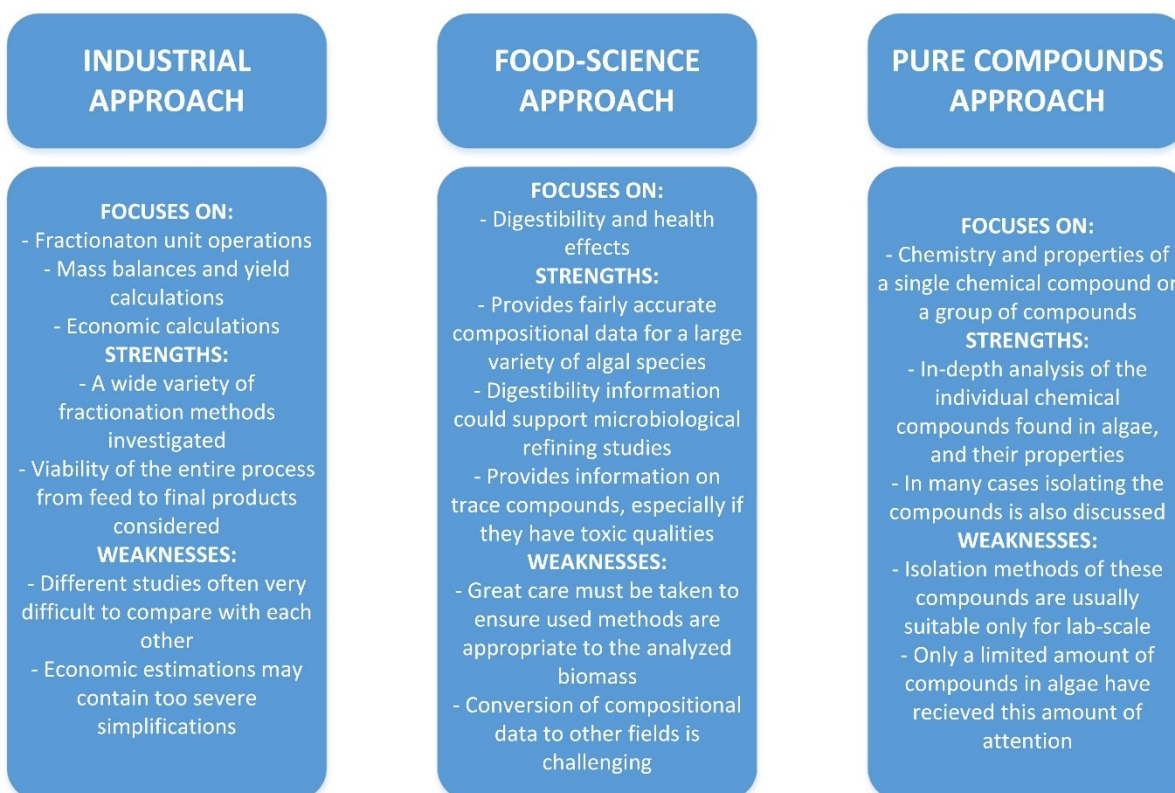


Figure 3, Main categories of scientific literature regarding fractionation of algae

Combining the results of two or more approaches a synergistical benefit may be obtained. A good example of this is the usage of the Kjeldahl-method, which ties together the total protein and nitrogen content of a given sample. Based on the elemental analysis results, the nitrogen contents determined in the field of industrial research can be converted to total protein content and vice versa using the appropriate conversion factors. An extensive study on the proper conversion factor for various algal biomasses and algal biomass in general was recently conducted by Angell *et al.* (2015) and can be considered a significant improvement to the reliability of this approach and an obvious analytical benefit to all future studies.

Unfortunately, there are also many analytical results that cannot be directly transferred between different fields this way, not at least without extensive background work. For example the insoluble dietary fiber content of a given algae, analysed in the food sciences, may be comparable to the cellulose content of the algal biomass analysed in the value-chemicals field. However, this is not always the case, and great care must be taken if these values are used. An unfortunate example in this regard is the study by Knoshaug *et al.* 2013, where some of the original sources do not necessarily properly describe the difference between fiber and cellulose content of the biomass. Furthermore, one of the source articles used by Knoshaug (2013): “Laksitoresmi *et al.*, 2010”, had even suffered reaction (Notice of redaction DOI: 10.1109/ICAMS.2010.5553284). The specific

reason for redaction has not been published, but the redacted article had used terms like pulp, cellulose and fiber rather ambiguously and thus highlights the problem in comparing “identical” materials prepared by different methodologies

3.1.1 Industrial approach

In scientific literature describing the fractionation of algae, a common approach is to present the fractionation process in the context of biorefinery. Usually this means that less emphasis is put on the chemistry of the fractionation and it is more the products and yields of the entire process that are stressed. Typically, these studies are based on lab- or pilot-scale trials, and the results are scaled up to provide an estimate of the viability of the fractionation as an industrial process.

The best-studied sub-field in this area is the production of fuels from algae. Conversion concepts such as: gasification to syngas, thermochemical liquefaction to bio-oil, pyrolysis to bio-oil and syngas and charcoal, anaerobic digestion to methane and hydrogen and alcoholic fermentation to ethanol exist, and each of these conversion concepts has several options on how the fractionation of algal biomass is technically conducted and what fractions are considered to be valuable products. (Brennan and Owende, 2010) In the scope of this thesis it is impossible to investigate all these fractionation methods in detail, but some general conclusions on the quality of the information available can be made.

A distinct weakness in the literature is that there is no textbook-level knowledge on the fractionation chemistry of algae. Such literature exists for woody biomass, with the most common reactions of carbohydrates and lignin being well investigated and reported for kraft pulping (Sixta et al., 2008), sulfite pulping, prehydrolysis and manufacture of organosolv pulp (Gustafsson et al., 2011). Furthermore, there is textbook-level knowledge available on terrestrial biomasses also on the fractionation processes commonly applied to algal biomass to produce liquid fuels, such as pyrolysis, hydrothermal liquefaction and gasification (Basu, 2011).

The lack of proper literature and poor understanding of the fractionation reaction mechanisms is also reflected in the articles. In work by Alba *et al.* (2011), the chemistry of hydrothermal liquefaction of algal biomass is explained in three stages: “1. *Hydrolysis of biomass macromolecules (lipids, proteins and carbohydrates in the case of algal biomass) into smaller fragments.* 2. *Conversion of these fragments by, for example dehydration, into other smaller compounds.* 3. *Rearrangement via condensation, cyclization and polymerization producing new components.*” However, Alba *et al.* (2011) acknowledge that these three reaction mechanisms are

taken from literature investigating the liquefaction of woody biomass and might not be suitable for algae.

The approach taken by Alba *et al.* (2011) is apparent in many articles discussing the fractionation of algal biomass. Others, such as Trivedi *et al.* (2013), address the problem by an simple omission of the reaction chemistry involved, focusing only on the product yields, operating conditions and chemical loads of the specific process they are investigating. As Trivedi *et al.* (2013) investigate the production of ethanol from the algal species *Ulva fasciata* they compare their results with other studies that have investigated production of algal bioethanol. However, when taking into account the plasticity of algal biomass, the variance in experimental protocols and fractionation procedures (such as the choice of enzymes used during saccharification), the comparisons they draw between other studies are not as conclusive as one might hope.

Algal biorefining has been a popular topic in the last decade, the amount of data available is immense. This means that initial feasibility studies can be found for many species of algae and a wide variety of fractionation methods, making studies of this type a good source of raw information if a detailed understanding of the mechanisms of the fractionation is not needed. However, as the data is difficult to compare and evaluate, great care should be taken not to make inaccurately detailed conclusions on issues like the financial viability of a given algal biorefining process.

3.1.2 Food science approach

In food sciences algal biomass is treated as a source of nutrition for humans or animals. This means that the composition of digestible chemical components is usually investigated to a detailed degree. The fractionation schemes used in isolating components are based on background knowledge and standards used in food sciences. Instead of fractionation the focus is on the isolation, recovery and analysis of the components of interest. From the viewpoint of industrial fractionation, this might be a problem, as components outside the scope of interests are often outright ignored and the effects of the fractionation technique on them is not reported. However, as many algal species have been studied for human or animal consumption, these studies may provide fairly accurate initial compositional data for species what have not been yet analyzed by other fields.

As the food sciences may study the digestability of the algal feedstock from many perspectives, great care must be taken when interpreting the data available. Plenty of different fractionation techniques can be found in the literature and the evaluation if the used fractionation technique is appropriate for the given context may be difficult. An example of this is the most likely incorrect identification of the presence of lignin in *ulva lactuca* by Yaich *et al.* (2011), discussed in the

section "Main differences and similarities in chemical composition compared to woody biomass" of this thesis. Furthermore, the analytical techniques may show even greater variance if the nutritional value is studied for multiple species with different digestion systems, such as pigs, cows and fish. A recent study "investigating the challenges in measuring insoluble fibers" (Mertens, 2003), gives a good idea of the difficulties related to the relationship between the dietary fractionation and the actual chemical composition of a biomass from the perspective of a single component used in food analysis. It can be expected that in other food-analytic methods similar challenges exist.

However, if one pays less attention to the chemical specificity of the results, and instead investigates the techniques used in the various standards of the food-science field, much valuable information may be recovered. Even though a given isolation technique of a chemical compound might be unsuitable for development of a fully fledged fractionation process, it might give some idea on the general principles that should be applied, and the steps needed to remove impurities. This is especially true for fractionation processes based on usage of enzymes or microbial organisms, as enzymatic treatment studies are often conducted in food-science literature and combined with the usage of standard sample isolation methods.

Another advantage of the food science studies is that they often contain also information on the observed health effects of consuming the algal biomass, and make suggestions on which compounds may be responsible for these effects. This means that the presence and composition of trace components is very well documented, and complements the information available from other fields of study. Furthermore, from the viewpoint of biological fractionation, the identification of potential inhibitors and antimicrobial agents may be particularly valuable. As many of the health-affecting compounds present in algae are trace compounds, it may be valuable knowledge in itself to know which compounds are present in which species of algae, and are these compounds found in trace amounts or larger quantities.

3.1.3 Pure compounds approach

The publications using this approach are perhaps the most useful in regards to studying fractionation of algae. Even though these studies usually focus on one or a few chemical compounds, the chemistry of these compounds is discussed in extensive detail. An example of this approach is a study on ulvan, an amorphous polysaccharide present in Ulvaes, by Lahaye & Robic (2007). In their article, Lahaye & Robic discuss the differences in ulvans recovered from different species and the effects of the different fractionation processes, such as acid hydrolysis and enzymatic hydrolysis on

the ulvan structure are reported. Furthermore, the disparities in other studies on ulvan are explained by the physicochemical properties of ulvan, and it is explained how these properties may affect the isolation of this compound.

Unfortunately, studies like these are less common than the studies found in the two previous categories. An obvious reason for this is that this approach is much more challenging to conduct successfully. Answering the question why certain chemical compounds behave in a certain way requires a large amount of background work, experience, and a well-planned and executed experimental setup. Many of these studies have almost a review-article type of nature, as they often compile data from multiple sources to complement the investigations made in the study. Finally, the choice of chemical components that these studies focus on is slightly arbitrary, as different fields of science have different sets of properties which they might consider to be of particular interest and worth reporting. As the compounds being isolated and studied vary, the extraction methods show also a large degree of variance. However, even for extraction of a single component several different extraction method may exist as shown in Table 2.

Table 2, various methods for isolating algal cellulose found in the scientific literature

Method source	Fractionation scheme used (temperature, reaction time, reaction medium)
Cronshaw <i>et al.</i> (1958)	<u>Hot water extraction</u> 100 °C , 12h, water <u>Alkali treatment</u> 25 °C, 4h, 4N NaOH-solution <u>Chlorite treatment</u> 75 – 80 °C, 4h, dilute NaClO ₂ solution
Wada <i>et al.</i> (1993)	<u>These two treatments repeated until sample achieves complete whiteness</u> <u>1. Bleaching treatment.</u> 80 °C, 4h, 0.3 % NaClO ₂ solution <u>2. KOH-immersion</u> Temperature not reported, overnight, 5% KOH
Mihranyan <i>et al.</i> (2004, [1])	<u>Chlorite treatment</u> 60 °C, 3h, 0.32% NaClO ₂ solution <u>Alkali treatment</u> 60 °C, overnight, 0.5M NaOH-solution <u>Acid treatment</u> Momentarily heated to 100 °C and let to cool freely, overnight, 5% HCL-solution

Perhaps one of the most extensive of these studies in respect to fractionation is the study done by Cronshaw *et al.* (1958) titled "A chemical and physical investigation of the cell walls of some marine algae". In this study, chemical fractionation and an in-depth study of the fractionated compounds is combined with electron microscopy for 15 species of algae. Furthermore, this study openly reviews

its own fractionation techniques and discusses the difficulties encountered during various fractionation stages, and how these issues were resolved in the study.

In Cronshaw's study the algal cell wall is solubilized stage-by-stage with a three-stage extraction process; a hot water extraction stage, an alkali-treatment stage and finally a chlorite-treatment stage. From each stage, both the solubilized fraction sugars and the solid extract sugars are analyzed, and SEM-images and x-ray diffractions from the solid residues are taken. In the end, the result is an massive systematic study on the fractionation behaviour of various species of algae. Furthermore, as a result of the study, the species used in the study could be reclassified into three categories according to the structure, composition and fractionation behaviour of their cell walls.

This article is quite well cited, having only 153 citations according to the Google Scholar service. It was also one of the oldest published articles, where a method for extracting pure cellulose from algae was described. However, the more recent authors that describe recovery methods for pure algal cellulose (Wada et al., 1993; Mihranyan et al. 2003) do not seem to be aware of this article. Also, the modern writers fail to report the background of their extraction method, or the reasons why they consider their method to be suitable for recovering pure cellulose from algal biomass. As a comparison, in Cronshaw's (1958) work the chlorite-treatment is said to facilitate the removal of the proteins from the algal biomass.

However, despite their merits in purifying algal cellulose and investigating its properties, these studies do not solve the practical problems of algal fractionation and cellulose recovery. The methods described in these articles might be suitable for recovery and purification of algal cellulose on lab-scale, but they contain extremely high chemical loads, that would cause severe problems in processing if a scale-up using these methods would be done. However, these studies, and other studies like these, might serve as the initial starting point to designing new, more environmentally friendly processes for fractionation of algal biomass.

3.2 Evaluation of existing industrial fractionation methods

The hydrocolloid industry is most likely the best currently available example of an industrial-scale fractionation operation that uses algae as a feedstock. As a well-established, mature industry, it will most likely have already encountered and developed solutions to many of the issues related to industrial scale-use of algal biomass. Unfortunately, in scientific literature regarding algal fractionation, the hydrocolloid industry is often mentioned, but rarely discussed in sufficient detail to benefit the development of novel fractionation methods. This thesis aims to alleviate this

problem by giving a short overview of the main unit operations of the fractionation processes used in hydrocolloid industries.

An excellent review on this topic has been provided by Dennis (2003) in FAO technical paper "A guide to the seaweed industry". This paper discusses in detail the production of main algal hydrocolloids from cultivation of algal biomass to the purification of the final hydrocolloid product. From Dennis's work, the details regarding to the fractionation of agar, alginate and carrageenan are summarized in the following paragraphs. However, it should be taken into account that even Dennis's work does not give all details of the extraction processes. Dennis explains in his paper that in many cases the details of the fractionation steps are trade secrets of the hydrocolloid manufacturers. The extraction processes used are summarized in flowcharts in Figures 4,5 and 6.

3.2.1 Agar



Figure 4, Flowchart of agar production

Agar is recovered from the red algae, with the most common species used industrially being *Gelidium* and *Gracilaria*. These species are not very closely related, showing different lineages already at the class level, belonging to the classes Rhodophyceae (*Gelidium*) and Florideophyceae (*Gracilaria*). The extraction procedures for these two families differ slightly, with *Gracilaria* requiring an alkali pretreatment in 2-5% sodium hydroxide and a subsequent washing step to produce agar of acceptable strength for commercial purposes. The main extraction process is a hot-water extraction with temperatures of 95 – 110 °C and a reaction time of 2 – 4 hours. This extract is kept hot to avoid gelling of the extracted fluid and filtered through a fine filter cloth to remove the solid residues. After filtering the extract is cooled to form an agar gel with about 1 percent agar and 99 percent of water and water-soluble impurities.

Two different solutions exist for increasing the gel concentration. In the freeze-thaw process the gel is frozen and during thawing the water leaves the gel, removing about 90% of the water and increasing the agar-content of the gel to 10 – 12 %. In the synacresis-process the gel is surrounded by a filter cloth and pressed between two grooved metal plates. The pressure used is increased very slowly over the duration of 24 hours. With this technique, gels with agar content of about 20 % are achieved. After the concentration increase the gel is dried with hot air and milled to the required particle size.

3.2.2 Carrageenan

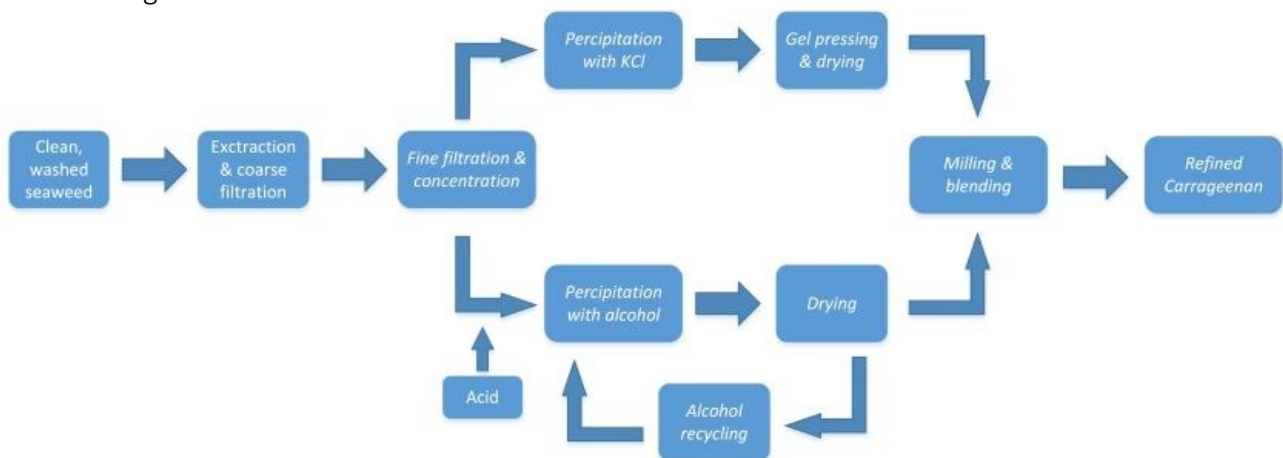


Figure 5, Flowchart of carrageenan production

Carrageenan is produced from a wide variety of red algae, with the current main production organisms being *Kappaphycus alvarezii* and *Euchema denticulatum*. In carrageenan several different types of gel exist, all with distinctly different properties. These gels are classified as Iota- (ι), Kappa- (κ) and Lambda- (λ) carrageenan.

For carrageenan two different production methods exist. In the original method the carrageenan produced is referred to as "refined carrageenan", the newer method produces "semi-refined carrageenan" (SRC). In refined carrageenan production the washed seaweed is heated in an alkaline solution for several hours. The undissolved seaweed residue is filtered out, followed by a secondary filtration, vacuum distillation and ultrafiltration. The resulting extract has the carrageenan content of about 2 – 3 %.

The solid carrageenan can be recovered from this extract using two methods. The alcohol-method and the gel-method. Of these the alcohol method is more universal, as the gel method is suitable only for κ-carrageenan. In the alcohol method, isopropanol is added to the extract until the carrageenan precipitates as a fibrous coagulum. This coagulum is then pressed to remove solvent

and washed with further alcohol. This coagulum is then dried and milled to make the commercial product. The gel method is based on the κ -carrageenans ability to form gels with potassium salts. The carrageenan extract is extruded into a potassium salt solution, forming gel-filaments. From these filaments dry κ -carrageenan can be recovered using either freeze-thaw or synacresis process similar with agar-manufacture.

In production of SRC, the algal biomass is treated with potassium hydroxide. This results in the removal of soluble protein, carbohydrate and salts when the seaweed is washed. However, the main mechanical structure of the seaweed is retained. The potassium causes the formation of a gel in ι and κ -carrageenan, and thus preserve in the undissolved fraction of seaweed. After the alkali treatment the seaweed is washed and laid out to dry. The dried seaweed is then sold as SRC or seaweed flour.

3.2.3 Alginate

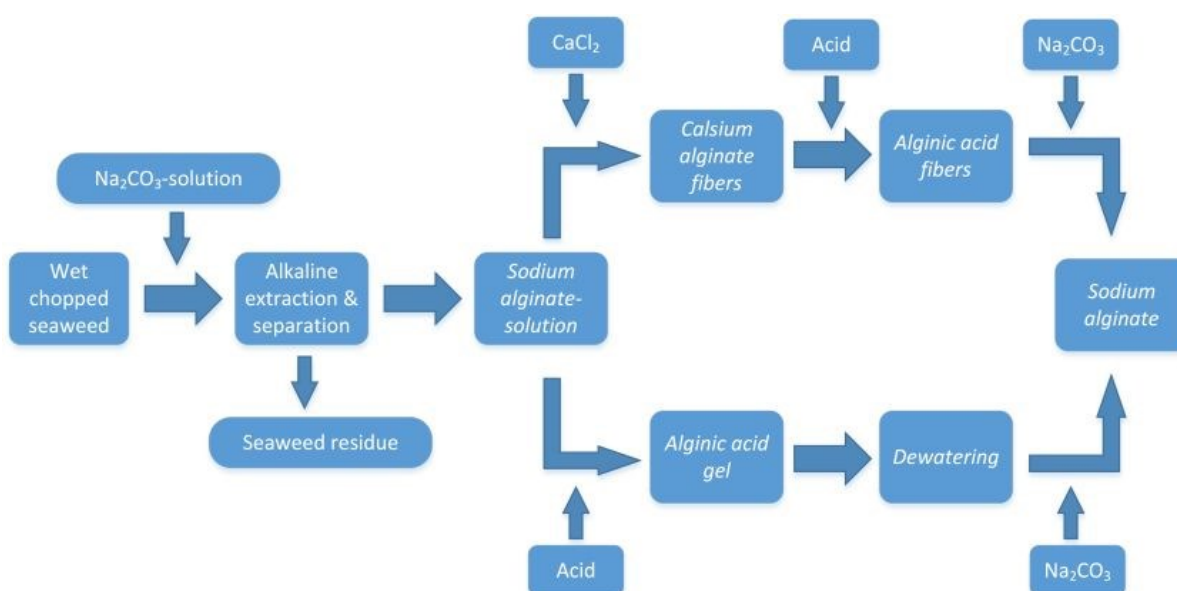


Figure 6, Flowchart of alginate production

Alginate is manufactured from brown seaweed and is naturally present there as sodium, calcium and magnesium salts. In alginate production, the target is to produce the sodium salt of the alginate, the only water-soluble compound of these three. The first step of alginate production is to convert produce a dilute suspension of sodium alginate by subjecting the raw algal feed to sodium carbonate and alkaline extraction. This suspension is then filtered to produce the feed for subsequent processing steps. This suspension typically has the alginic acid content of 1 – 2 % with rest of the mass being water.

For further purification of alginate, two processes exist; The alginic acid method and the calcium alginate method. In alginic acid method, acid is added to the filtered suspension to induce gelling. This gel is centrifuged with filter cloths to the consistency of 7 – 8 %. Next, alcohol (ethanol or isopropanol) is added to produce a 50:50 mixture of water and alcohol. Then solid sodium carbonate is added until the product reaches the desired pH. Finally this paste is extruded as pellets, dried and milled.

In the calcium alginate method a soluble calcium salt is added to the filtered extract. This induces the formation of solid calcium alginate. Depending on the mixing, calcium alginate may form solid fibers or a gelatinous solid. In this method the target is to produce solid fibers, sieve them out and then wash the excess calcium out. Then dilute acid will be added to convert calcium alginate into alginic acid, which retains the fibrous characteristics of the calcium alginate. These alginic acid fibers are screw pressed to 20 – 25 % alginic acid concentration. The resulting paste is mixed with sodium carbonate until correct pH is achieved, extruded to pellets and finally dried and milled.

4 Aims of the thesis

This thesis aims to investigate if the cellulose from algal species *Cladophora glomerata* could be used as a feedstock for manufacturing textile fibers using the Ioncell-F process in a sustainable way. To achieve sustainability, it is investigated if the current methods for isolating algal cellulose could be replaced with more environmentally benign processes.

For this purpose, a fractionation study will be conducted, with an in-depth analysis of the isolates to investigate the removal of unwanted components and the purity of the cellulose achieved by the fractionation.

The cellulosic raw material for textile fibre production will be prepared using the material produced by the fractionation study as a feedstock. The objective of the thesis is to achieve a proof-of-concept level demonstration of the suitability of *Cladophora glomerata* as an option for sustainable production of textile fibers.

5 Textile perspective on utilization of algal biomass

The production of non-synthetic textiles worldwide is facing a difficult situation. The global consumption of textile fibers is growing yearly at fast rate (~4 % in 2013/2014), but the natural resources available for production of natural fibers, such as cotton, bast, silk and wool cannot be increased sustainably at the same rate. Availability of traditional textile feedstocks, such as cotton, forming 80% of the natural fiber market, has been stagnating and annual production between the years 2010 -2015 has decreased by 1.7%. This stagnation or even decrease in production is expected to continue as water availability in cotton-growing countries becomes more limited and loss of arable land used for cotton farming in favor of food crops. (Michud, 2016)

Overall, this means that alternative methods and raw materials must be considered to satisfy the global need of cellulosic fibers. To answer this problem the most promising solution seems to be development of man-made fibers, such as viscose, Tencel® and Ioncell-F, that can utilize other cellulose sources, such as dissolving pulp, as raw material for manufacturing textiles (Michud, 2016).

5.1 Macroalgae *Cladophora glomerata* as a raw material for cellulosic textile manufacture

The selection of an algal species suitable for manufacturing textiles is not trivial. For example, cellulose can be found in species of cyanobacteria (Nobles *et al.*, 2001), green algae (Mihrianyan *et al.*, 2003), red algae (Siddhanta *et al.* 2009) and brown algae (Kloareg & Quatrano, 1988). Furthermore, the morphology and structure of these celluloses may vary, making species selection a non-trivial matter for any work that aims to use algae as a feedstock. From an engineering viewpoint, this means that when designing biorefineries using algae as a feedstock, great care must be placed upon the selection of species to find one that has enough desirable qualities to warrant processing.

The choice of *Cladophora glomerata* as the main species being studied in this thesis was based mainly on the comparatively high reported cellulose content (12 – 39 %). This was considered to be the most important parameter affecting the yield of the final textile from the raw algal biomass and thus the viability of the textile manufacture in a sustainable way. A further motivation for the study was, that the cellulose from this algae has already received extensive attention by Mihrianyan *et al.*(2004[1],2004[2], 2007, 2011), and thus, some preliminary properties of the cellulose were already known.

Finally, *Cladophora glomerata* is an extremely widespread species, considered to be ubiquitous of all river algae in the northern hemisphere (Blum, 1956). It benefits greatly from eutrophication of waters and grows rapidly in nutrient-rich waters. For example, Gubelit & Berezina (2010) report that *Cladophora glomerata* was observed to be responsible for 90% of the total primary production of a habitat in the Neva estuary, one of the most eutrophic areas of the Baltic Sea. From the sustainability perspective, this particular species of algae represents an abundant and excessively available feedstock, and the usage of it might even help to alleviate the extent of eutrophication in polluted waters.

However, to achieve sustainability in the textile manufacture, in addition to the raw feedstock, also pre-processing of the material must be sustainable. As current literature methods for isolating cellulose from *Cladophora glomerata* have fairly high chemical loads, and use toxic chemicals, such as NaClO₂ (Cronshaw *et al.*, 1958; Wada *et al.*, 1993; Mihranyan *et al.*, 2004), there is a distinct opportunity for developing more benign cellulose isolation methods.

5.2 Textile manufacture using man-made cellulosic fibers

Man-made cellulosic textile fibers are cellulose-based fibers for textile applications that are produced by a two-stage process. In the first stage, cellulose is dissolved into a solvent either by direct dissolution, or by derivatization followed by dissolution. Subsequently, the solvent and the possible derivatives are removed through regeneration or coagulation in an antisolvent, producing pure cellulosic textile fibers. There are also some cellulosic fibers, that are converted into fibers in their derivatized form, such as cellulose acetate and cellulose triacetate, but they will not be discussed in the scope of this thesis.

The most prevalent man-made cellulosic fiber currently is viscose, with the global annual production being 5.3 million tons in 2016, covering 87% of all man-made cellulosic fibers. (The Fiber Year 2017) The viscose production uses as a feedstock dissolving pulp. Typically, the viscose producers optimize their production to one or two types of pulp, as different pulps have different reaction rates in the derivatization reactions of the viscose process. In the viscose process, the pulp is steeped in alkali to convert it to its alkoxide derivative (alkcell) and lower its degree of polymerization. This alkcell-intermediate is then made to react with CS₂ in the process of xanthation, where the cellulose is derivatized to sodium cellulose xanthate forming a cellulosic dope. This dope is then injected through small holes into a bath of sulphuric acid, where the acid hydrolyzes the xanthate and

releases the CS₂, regenerating the cellulose as fibers. (Wilkes and Woodings, 2001) However, despite its prevalence, this process has some disadvantages that cannot be easily remedied, such as the acute toxicity of CS₂, large consumption of various process chemicals and the consumption of energy during the process. (Rana *et al.*, 2014)

Cuprammonium rayon process uses exclusively cotton linters as a feedstock. Production of textile fibers using wood pulp has been attempted in the 1940s but poor processability and fibre quality resulted in abandonment of this development. The process begins with the purification of cotton linters with sodium hydroxide and sodium hypochlorite, subsequently the purified cellulose is dehydrated to a water content of ~ 50 %. This cellulose is then dissolved in cuprammonium solution and this solution is extruded to produce thin filaments. These filaments are then stretched, coagulated and regenerated with water and dilute acid. However, the cuprammonium rayon process struggles with high production cost and is being produced at a scale of 20 000 – 25 000 tons per year only. (Kamide and Nishiyama, 2001)

Tencel-fibers made using the Lyocell process had an annual production in 2016 of approximately 220 000 tons. (The Fiber Year 2017) In the Lyocell-process, the cellulosic pulp is mixed with an aqueous solution *N*-Methylmorpholine *N*-oxide (NMMO). Subsequently the excess water is evaporated to produce a highly viscous dope. This dope is then extruded through an air-gap into a water/NMMO-bath for the regeneration of cellulose. The Lyocell-process benefits from an excellent rate of solvent recovery, with over 99% of the solvent being recycled in the process. However, the high processing temperatures, and the tendency of NMMO to degrade exothermically require meticulous process control to avoid runaway reactions. Furthermore, NMMO suffers from a loss of degree of polymerization in the cellulosic feedstock during processing if no stabilizers are added. (White, 2011) The Lyocell-process is reported to have some flexibility towards the raw material of the dope with successful solution of feedstocks such as multiple different dissolving pulps, sugarcane, recycled newsprint and thermomechanical pulp achieved by Dever *et al.* (2003), but unfortunately, no information regarding the spin-stability of such novel dopes is currently available.

The Ioncell-F process is a recent development in the field of manufacturing man-made cellulosic fibers. The Ioncell-F technology takes advantage of the dry-wet spinning technology to produce textile fibers of comparable quality, but instead of NMMO, the solvent used for dissolving cellulose is an ionic liquid. This change of solvent addresses the shortcomings of NMMO, such as the poor chemical and thermal stability, while producing textile fibers of comparable quality. In a recent

Doctoral dissertation by Anne Michud (2016) the development of the Ioncell-F process is reported in great detail, and the detailed explanation of the process, its challenges and opportunities that it provides can be found there.

Table 3, Basic information on man-made cellulosic fiber manufacturing processes

Name of the process	Name of the fiber	Primary solvent	Derivatization of cellulose	Primary antisolvent	Fiber spinning	Fiber cross-section	Fiber wet tenacity [cN/dtex]
Viscose	Viscose	Carbon disulphide- NaOH	Yes	Sulfuric acid	Wet-spinning	Warped oval	12,5
Modal (modified viscose)	Modal	Carbon disulphide- NaOH	Yes	Sulfuric acid	Wet-spinning	Warped oval	18,4
Cuprammonium rayon	Bemberg/cupro	Tetraaminecopper dihydroxide	No	Water	Wet-spinning	Round	17,6
Lyocell	Tencel	N-Methylmorpholine N-oxide	No	Water	Dry-wet spinning	Round	37,5
Ioncell-F	Ioncell	Ionic liquid (1,5-diazabicyclo[4.3.0]nonenium acetate)	No	Water	Dry-wet spinning	Round	42,6

Data for the table compiled from Röder et al. (2009), Sixta et al. (2015), Toshi et al. (1994)

Of these technologies, the Ioncell-F shows significant robustness towards the raw material, as it has been successfully used to produce textile fibers from waste paper and cardboard (Ma *et al.*, 2016), cellulose-lignin blends (Ma *et al.*, 2015), cotton waste (Asaadi, 2016) and textile waste (Smirnova, 2017). This robustness makes it the ideal technology to investigate if it would be possible to manufacture textile fibers from algal biomass. In this thesis, instead of developing Ioncell-F process further, the existing knowledge on Ioncell-F process is used to investigate the behavior of a novel raw material in it.

6 Materials and Methods

6.1 Preparation of algal feedstock

The algal feedstock was obtained from a Czech company: *Rawat Consulting*. The algae used in this thesis was cultivated in open pond cultivation between May 1 and August 30 in an open pond cultivation system situated in an outdoor facility at Brno (49°12'00.0"N 16°37'00.0"E). After harvesting, the algae was sun-dried at the harvesting site on metal wire mesh screens by the personell at *Rawat Consulting*. The algae was delivered to Finland in this air-dried state.

The species of the algal feedstock was checked in collaboration with Dr. Jaanika Blomster, an university lecturer in limnology at Viikki and one of the instructors of this thesis. The dry algal biomass from Czech was stored in a cardboard box for 1-4 months before use. Before using the algae the experimental work, the algal feedstock was washed with water at 20 – 30 °C to remove most of impurities, such as pine needles, leaves and insects. Following the washing the samples were dried according to the drying protocol and milled according to the milling protocol. This milled, washed sample will be referred in this thesis as raw algal powder.

6.1.1 Drying protocol

The drying of the samples was done in a heat cabinet set to 60 °C and the samples were kept there for at least 24 hours before being analyzed, with the maximum storage time being 8 weeks.

6.1.2 Milling protocol

Samples were milled in this thesis using a Wiley mill unit (Arthur H. Thomas Co. Scientific apparatus, Philadelphia USA) using a 20 mesh screen. For carbohydrate analysis, GPC-analysis, dope preparation and the IR-spectroscopy the samples were milled by the same wiley mill using a 60 mesh-screen.

6.2 Fractionation of algae

6.2.1 Fractionation study

For the main fractionation study, a monowave microwave reactor (Microwave synthesis reactor, Monowave 300, Anton Paar) was used with a ruby thermometer tip. For each trial run, a small magnetic mixer, approximately of 2 g dried and milled sample and 20 g of the reaction solution was inserted into the sample vial. The heat ramping program was set to heat up as fast as possible, and

the mixer speed was set to 600 RPM. After the reaction was complete, the reaction solids were recovered using a Büchner funnel and filter paper (VWR, 415, ret. 12 – 15µm) , from the initial filtrate the pH was measured before washing the sample. Then the solid residue that remained on top of the filter paper was washed with deionized water until the washing solution reached a pH between 6.5 and 7.5. The solid residue was then dried according to the protocol used in this thesis. The details of the reaction solutions used in the study and the operative conditions of the monowave reactor can be found in the Table 4 below.

Table 4, Compositions of reaction liquids and operating conditions of the Monowave reactor-system for the fractionation study.

Reaction	Reaction abbreviation	Sample used in reaction	Reaction solution composition	Reaction temperature	Reaction time	Rotation speed of the mixer
Autohydrolysis	AH	raw algal powder	Deionized water	170 °C	60 min	600 RPM
Soda cook	AH+SD	AH-reaction solids	0.05M NaOH	150 °C	60 min	600 RPM
Acid cook	AC	raw algal powder	0.05M H ₂ SO ₄	150 °C	60 min	600 RPM
Soda cook	AC+SD	AC- reaction solids	0.05M NaOH	150 °C	60 min	600 RPM
Acid cook - Severe	AC-SEVERE	raw algal powder	0.1M H ₂ SO ₄	150 °C	60 min	600 RPM
Soda cook -Severe	AC+SD-SEVERE	AC-SEVERE- reaction solids	0.4M NaOH	150 °C	60 min	600 RPM

NaOH and H₂SO₄ Solutions were prepared from Emsure-brand 95-97% sulfuric acid and 50% sodium hydroxide solution (EMD Millipore corp.)

6.2.2 DP-adjustment study

For the DP-adjustment study, a monowave microwave reactor was used with a ruby thermometer tip. For each trial run, a small magnetic mixer, approximately of 0,5 g dried and milled sample and 10 g of the reaction solution was inserted into the sample vial. The heat ramping program was set to heat up as fast as possible, and the mixer speed was set to 100 RPM. After the reaction was complete, the reaction solids were recovered using a Büchner funnel and filter paper, the solid residue that remained on top of the filter paper was washed with deionized water until the washing solution reached a pH between 6.5 and 7.5. The solid residue was then dried according to the

protocol used in this thesis. The details of the reaction liquids and the operative conditions of the monowave reactor can be found in the Table 5 below.

Table 5, Compositions of reaction liquids and operating conditions of the Monowave reactor-system for the DP-adjustment study.

Reaction	Reaction abbreviation	Sample used in reaction	Reaction solution composition	Reaction temperature	Reaction time	Rotation speed of the mixer
20% Acid	20%30MIN_DP	AC+SD-SEVERE - reaction solids	20 mass-% H ₂ SO ₄	80 °C	30 min	100 RPM
10% Acid	10%30MIN_DP	AC+SD-SEVERE - reaction solids	10 mass-% H ₂ SO ₄	80 °C	30 min	100 RPM
5% Acid	5%30MIN_DP	AC+SD-SEVERE - reaction solids	5 mass-% H ₂ SO ₄	80 °C	30 min	100 RPM
3% Acid	3%30MIN_DP	AC+SD-SEVERE - reaction solids	3 mass-% H ₂ SO ₄	80 °C	30 min	100 RPM
1% Acid	1%30MIN_DP	AC+SD-SEVERE - reaction solids	1 mass-% H ₂ SO ₄	80 °C	30 min	100 RPM
0.5% Acid	0.5%30MIN_DP	AC+SD-SEVERE - reaction solids	0.5 mass-% H ₂ SO ₄	80 °C	30 min	100 RPM
20 % Acid, 45 min	20%30MIN_DP	AC+SD-SEVERE - reaction solids	20 mass-% H ₂ SO ₄	80 °C	45 min	100 RPM
20 % Acid, 60 min	20%45MIN_DP	AC+SD-SEVERE - reaction solids	20 mass-% H ₂ SO ₄	80 °C	60 min	100 RPM
20 % Acid, 90 min	20%90MIN_DP	AC+SD-SEVERE - reaction solids	20 mass-% H ₂ SO ₄	80 °C	90 min	100 RPM
20 % Acid, 150 min	20%150MIN_DP	AC+SD-SEVERE - reaction solids	20 mass-% H ₂ SO ₄	80 °C	150 min	100 RPM
20 % Acid, 210 min	20%210MIN_DP	AC+SD-SEVERE - reaction solids	20 mass-% H ₂ SO ₄	80 °C	210 min	100 RPM

H₂SO₄ Solution was prepared from Emsure-brand 95-97% sulfuric acid (EMD Millipore corp.)

6.2.3 Full-scale DP-adjustment.

According to the results of the DP-adjustment study a larger batch of algae was subjected to DP-adjustment on a larger scale using a glass reactor with a water heated jacket. A magnetic mixer was placed in the reactor vessel, and subsequently 47.4 g of algae and 948.1 g of 80 °C H₂SO₄ was added. The reactor was then sealed with a plastic lid. It was noticed during operation that 100 RPM was

insufficient to achieve turbulent mixing, so the mixer speed was increased to 130 RPM. After the reaction was complete, the reaction solids were recovered using a Büchner funnel and filter paper, the solid residue that remained on top of the filter paper was washed with deionized water until the washing solution reached a pH between 6.5 and 7.5. The solid residue was then dried according to the protocol used in this thesis. The main operational parameters of the system can be seen in table 6 and the reactor-system can be seen in Image 1.

Table 6, Compositions of reaction liquids and operating conditions of the Monowave reactor-system for the DP-adjustment study.

Reaction	Reaction abbreviation	Sample used in reaction	Reaction solution	Reaction temperature	Reaction time	Rotation speed of the mixer
20% Acid	AC+SD-SEVERE_Full_DP-adjusted	AC+SD-SEVERE - reaction solids	20 mass-% H ₂ SO ₄	80 °C	210 min	130 RPM

H₂SO₄ Solution was prepared from Emsure-brand 95-97% sulfuric acid (EMD Millipore corp.)



Image 1, Full-scale DP-adjustment reactor system.

6.3 Textile manufacturing trials

6.3.1 Preparation of algal feedstock

For the textile manufacturing trials the raw algal powder was subjected to chemical treatments similar to the treatments conducted with the monowave microwave reactor^(a3). These reactions took place in an air-bath digester using teflon-coated metal reactor bombs for the reactions in acidic conditions and stainless steel metal reactor bombs for all others. The detailed operating conditions of the air-bath digester can be found in Table 7.

Table 7, Reaction liquids and operating conditions of the air-bath digester system.

Reaction	Reaction abbreviation	Sample used in reaction	Reaction solution composition	Reaction temperature	Reaction time
Autohydrolysis	AH_Full	raw algal powder	Deionized water	170 °C	60 min
Acid cook-Severe	AC-Severe_Full	raw algal powder	0.1M H ₂ SO ₄	150 °C	60 min
Soda cook-Severe	AC+SD-Severe_Full	AC-SEVERE-reaction solids	0.4M NaOH	150 °C	60 min

NaOH and H₂SO₄ Solutions were prepared from Emsure-brand 95-97% sulfuric acid and 50% sodium hydroxide solution (EMD Millipore corp.)

The air-bath digester was adjusted to 30 °C over the target reaction temperature during the heat up-period to achieve faster heating. When the temperature inside the reactor bombs was within 10 °C of the target temperature, the digester temperature was adjusted to target temperature and the reaction chamber was vented to cool it to the appropriate temperature. The reaction time was considered to start from the moment the temperature inside the reactor bomb reached the target temperature. After the reaction was complete, the reaction bombs were removed from the digester and sunk into a water bath for cooling.

After the bomb had cooled to a temperature less than 50 °C, the pH of the reaction solution was measured using pH-paper and the contents of the bomb emptied into a wire mesh centrifugation bag. The pulp was washed in the centrifugation bag until the spent washing water became clear. The washed pulp was then homogenized with a pulp homogenizer using an anchor-type mixing blade, and dried according to the drying protocol

6.3.2 Preparation of the ionic liquid

The ionic liquid used in this thesis was 1:1 molar ratio solution of 1,5-diazabicyclo[4.3.0]non-5-ene acetate ([DBNH]oAc). This solution was prepared using the reactor-system depicted in image 1, with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (Fluorochem) and glacial acetic acid (VWR). Both components were utilized as received, the reactor was loaded first with DBN and acetic acid was gradually added under external cooling due to the exothermic nature of the reaction until an equimolar ratio was reached. The solution was stirred for 1 h at 80 °C after complete addition of the required amount of acetic acid.

6.3.3 Preparation of the cellulosic dope.

Ionic liquid cellulose solutions (dopes) were prepared from algal feedstocks prepared with the air-bath digester. The cellulosic raw material used for dope manufacture were AH_Full and AC+SD-Severe_Full, prepared according to the methodologies discussed earlier. Additionally a reference dope was used to produce comparable data on rheological measurements of the dope. This reference dope was made using dissolving birch (*betula pendula*) prehydrolysis kraft pulp (B-PHK) provided by Stora Enso Enocell pulp mill Ionic liquid used in dope manufacture was equimolar [DBNH]OAc, prepared according to the methodology discussed in previous chapter. The technical specifications regarding the dope manufacture can be seen in Table 8 below.

Table 8, Technical specifications of ionic liquid dopes used in this thesis.

Dope name	Cellulosic material	Cellulosic material content (mass-%)	Ionic liquid	Kneading time	Kneading temperature
Enocell 13%	Birch prehydrolysis kraft pulp	13 %	Equimolar [DBNH]OAc	90 min	80 °C
AH 10%	AH_Full - solids	10 %	Equimolar [DBNH]OAc	180 min	80 °C
AC+SD-Severe 13%	AC+SD-SEVERE_Full_DP-adjusted – solids	13 %	Equimolar [DBNH]OAc	180 min	80 °C

Two kneaders of different sizes were used to manufacture the dopes used in this thesis. Both kneaders were maintained at the temperature of 80 °C during the entire kneading process. Liquid [DBNH]OAc was added to the mixing chamber, then an appropriate amount of milled solids was added to produce a dope at the desired consistency. Before sealing the mixing chamber, the solids and the [DBNH]OAc were hand mixed until the milled solids were wetted. After wetting the mixing chamber was sealed and a vacuum of under 20 mbar was introduced to remove entrained air bubbles and moist air. After desired vacuum level had been reached, mixer was started at 30 rpm and the dope was kept in the mixing chamber for a duration of 3 hours. For small dopes (under 30 g), a small vertical kneader with anchor blades was used (Image 2). For larger dopes a larger kneader unit with an anchor blade was used (Image 3).



Image 2, small kneader unit



Image 3, large kneader unit



Image 4, disassembled filtering unit

After dissolving the solids in [DBHN][OAc], the resulting dope was filtered to remove impurities. The filtering system (Image 4) was kept at 80 °C during the filtering. The filtering system was used with a 5 μm (Ymax2, GKD, Germany) metal filter mesh. The filtered dopes were stored protected from moisture.

6.3.4 Spinning of textile fibers.

Spinning of textile fibers was done using two machines depicted in Images 5 and 6. Both machines have been provided by Fourne. The drawing ratio (e.g. the ratio of extrusion speed to the take-up velocity of the fibers) was adjusted by controlling the extrusion speed and the godet speed. A simplified overview of the spinning unit from the work of Michud (2016) is re-presented here in figure 7.

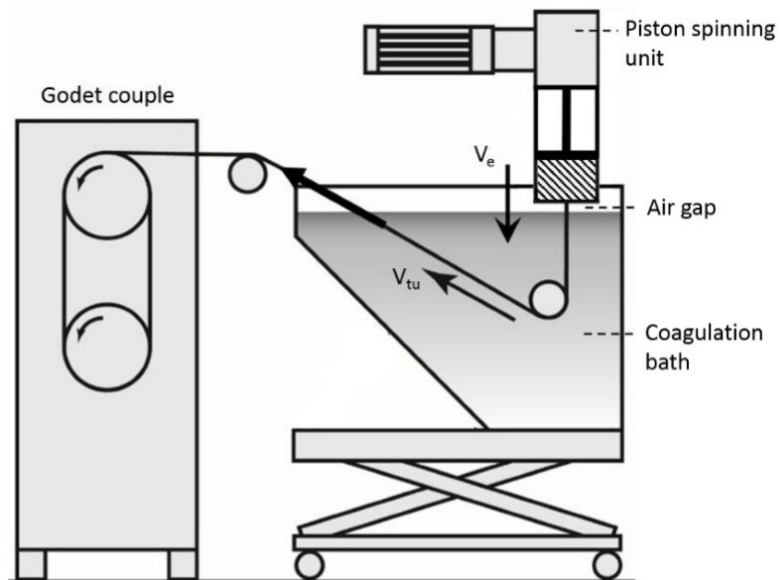


Figure 7, simplified re-representation of the dry-jet spinning unit. V_e signifies extrusion velocity and V_{tu} the take-up velocity. Image taken directly from Michud (2016).

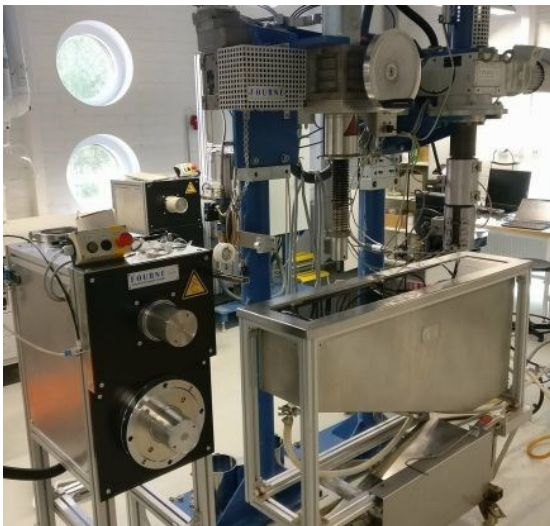


Image 5, KS-42 spinning unit



Image 6, KS-16 spinning unit

6.4 Analytics

6.4.1 Carbohydrate analysis

The carbohydrate content of the samples was determined according to the method specified in the NREL-LAP “Determination of structural carbohydrates and lignin in biomass” (Sluiter *et al.*, 2008 [1]). The method was modified by omitting all lignin, acetate and acetyl-related analytics from the sample preparation procedures.

6.4.2 Oven ash

For determination of ash content of the samples, the standard method specified in the NREL-LAP “Determination of Ash in biomass” was used. (Sluiter *et al.*, 2008 [2]) Of the two procedures introduced in the laboratory protocol, the procedure 10.4.2 with the ramped furnace temperature was used. The sample was held at 105 °C for 12 minutes, at 250 °C for 30 minutes and at 575 °C for 180 minutes.

6.4.3 CHNS-analysis

CHNS (Carbon,Hydrogen,Nitrogen,Sulphur) - analysis was ordered as an external work by the Universität Wien Mikroanalytisches Laboratorium. The device they used was EA 1108 CHNS-O elemental analyzer by Carlo Erba Instruments. Samples sent to CHNS-analysis were dried and milled beforehand according to the methods presented in this thesis, and subsequently dried overnight at 105 °C at the analysis location. Using the results from the CHNS-analysis the total protein content of the sample was calculated by using the method introduced by Angell *et al.* (2015), multiplying the measured Nitrogen content of the sample by 5 to estimate the total protein content. All sulfur in the samples was assumed to be in the form of sulphates, and the measured sulphur content of the samples was multiplied by a factor of 2.99626, which is the molar mass of SO₄ divided by molar mass of S, to estimate the total mass-% of sulphate present in the samples.

6.4.4 Elemental analysis of ash

Before starting the elemental analysis, the oven ash from the samples was dried overnight in an oven set to 105 °C. Amount of 200 +/- 30 mg of this dry ash was weighed into Teflon cups, then concentrated acids were added to each cup in the following order: 7,5ml of HCL, 2,5ml of HNO₃, and finally 0,5 ml of HF. After adding the acids, the Teflon cups were sealed and heated for 50 min at 200 °C, in a Milestone, Ethos-microwave oven, and after the treatment cooled down to room temperature. After cooling, 5 ml of saturated boric acid (H₃BO₃) was added to each cup, and the

heat treatment was repeated with the same parameters. After the second heat treatment, samples were diluted with 50ml of MilliQ-water. These samples were then stored and used for analytics.

Sodium and Potassium contents of the samples were determined by Varian 240 AA-atomic absorption device, using an air-acetylene-flame. The wavelengths used were 589,0 nm for Sodium and 765,5 nm for Potassium.

Calcium, Magnesium, Aluminium and Silica contents of the samples were determined by using Perkin Elmer DV 7100 ICP-OES-device. The following wavelengths were used: Calcium (317,933 nm), Magnesium (285,213 nm), Aluminium (394,401 nm), Silica (251,611 nm & 288,158 nm). These compounds were assumed to be present as their most common oxides, calcium as CaCO_3 , magnesium as MgO , aluminium as Al_2O_3 , and silica as SiO_2 . The total mass-% of these compounds was estimated by the equation:

$$\text{mass} - \% \text{ of compound} = \frac{\text{molar mass of the compound } (\text{CaCO}_3, \text{MgO}, \text{Al}_2\text{O}_3, \text{SiO}_2)}{\text{molar mass of the element in the compound} (\text{Ca}, \text{Mg}, 2 \cdot \text{Al}, \text{Si})}$$

6.4.5 Gel permeation chromatography (GPC)-analysis

First 50 ± 5 mg of each sample was weighed into a tube. Then a solvent exchange series was conducted with MilliQ-water, acetone and pure N,N-dimethylacetamide (DMAc). In each step of the series 4ml of solvent was added, and after 8-12 hours removed by vacuum and replaced by the next solvent. After the vacuum removal of DMAc, the solid residue from each sample was transferred into glass bottles and 5.0 mL of 90 g/l LiCl/DMAc was added. Subsequently, the samples were dissolved at room temperature under a constant slow speed magnetic stirring for 24 hours. After dissolution 0.50 ml of each sample was taken to make diluted samples. The concentrated samples were stored at 6 °C for two weeks, afterwards 0.50 ml of each sample was taken to make diluted samples. To prepare the diluted samples, 0.50ml of the concentrated sample was diluted with 4.5ml of pure DMAc. These diluted samples were then filtered into GPC-measurement vials through 0.2 μm syringe filters and measured using the UltiMate 3000-system.

6.4.6 Rheological measurements

Rheology measurements on the produced dopes were conducted using two rheometers (Physica MCR 300 & Modular compact rheometer MCR302) with an identical measurement setup, using an peltier-plate and PP25-shear plate. The temperatures tested ranged from 55 to 100 °C. Viscosities were measured using 30 point angular frequency sweeps ranging from 0,001/s to 100/s at each

temperature. From the measured data, the zero-shear viscosity, and the magnitude and the angular frequency of the cross-over point were determined

6.4.7 Cupri-ethylenediamine viscosity measurements

Viscosities in cupri-ethylenediamine solution (CED) were measured in this thesis according to the standard SCAN-CM 15:99. The DP of the original sample was estimated from this sample according to the methodology of Michud (2015). The equation used in this thesis for determining the DP was

(η = intrinsic viscosity determined by SCAN-CM 15:99). This equation is suitable for samples if $\eta > 400$ ml/g, and all samples measured in this thesis using SCAN-CM 15:99 fell within that range.

7 Results and discussion

7.1 Fractionation study

This fractionation study aimed to investigate if cellulose from *Cladophora glomerata* could be recovered using less intensive and more sustainable chemical treatments than the ones used in literature so far. An overview of the fractionation schemes investigated in this study can be seen in Figure 8 and their chemical consumption is compared with fractionation methods used in literature in Table 9.

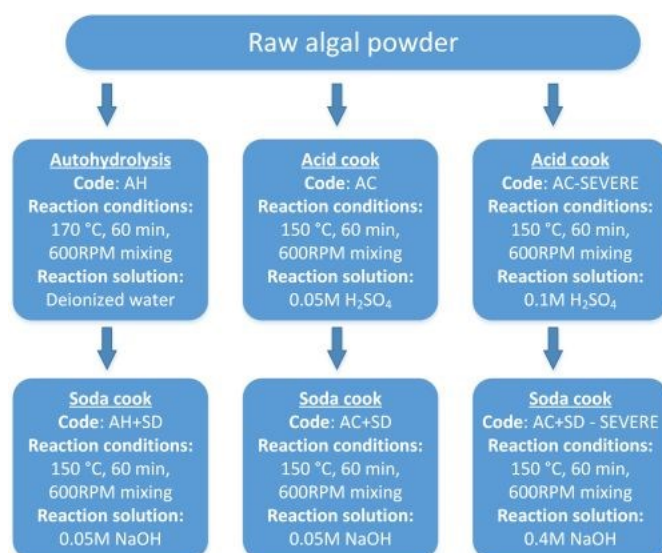


Figure 8, Overview of the chemical treatments used in the fractionation study

Originally this study was supposed to include only the AH, AH+SD; and AC, AC+SD – treatments, but as it became apparent during the study, that these two treatments had almost identical effects with each other, the higher-chemical load treatments AC-SEVERE and AC+SD-SEVERE were added to the study.

Table 9, comparison of chemical usage and product yields between this study and literature values assuming 1000 kg of raw feedstock.

Method	Mihranyan <i>et al.</i> (2004)	Cronshaw <i>et al.</i> (1958)	AH+SD	AC+SD	AC+SD-SEVERE
Water usage (washing not included)	32 000 kg	185 000 kg	20 000 kg	20 000 kg	20 000 kg
NaClO ₂ usage	400 kg	2 000 kg	none	none	none
Acid usage (calculated as H ₂ SO ₄)	450 kg	none	none	27 kg	53 kg
Alkali usage (calculated as NaOH)	240 kg	4000 kg	7 kg	8 kg	53 kg
Cellulose yield	216 kg	285 kg	460 kg	460 kg	310 kg
cellulose purity	pure	pure	50%	54%	63%

To estimate the chemical expenditures of the Mihranyan method, data from (Xiang et al., 2016) and (Guo et al., 2017) was used to provide the stage-by stage yield data and the chemical dosages were adjusted accordingly. The cellulose isolation method used by Wada et al. (1993) was excluded from this table as the literature data available was not specific enough to calculate the chemical consumptions.

The original raw algal powder and the solid residues from each reaction stage were analyzed in detail. The results of these analytics are presented in Figure 9 and Figure 10. From these figures it is obvious, that the treatments used in this thesis were not enough to purify cellulose. However, it was clear that the treatments used in this thesis were strong enough to induce partial fractionation. Silica was separated from other ash, as special focus was assigned to tracking of the silica content, as it was expected to cause problems in the rheological properties of the dope.

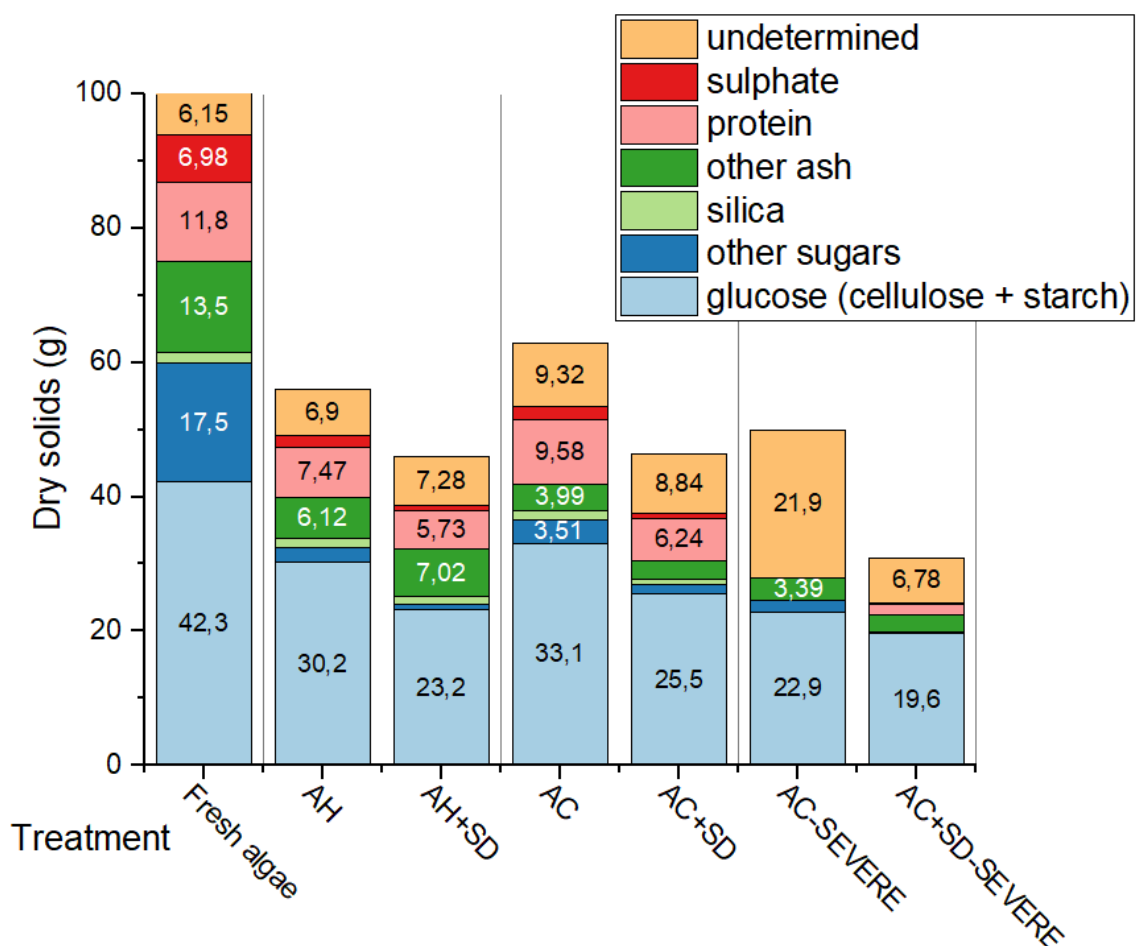


Figure 9. Combination of yield-calculations and mass composition data. This graphs shows the mass composition of the algal biomass as a function of different treatments, starting from 100g of dry solid feedstock. The term other sugars means the combined amount of arabinose, rhamnose, galactose, xylose and mannose in the sample. Mass contents of under 3 g not shown on the graph.

Note: for the AC-Severe, the protein content and the silica content were not determined. For AC+SD-Severe the silica content was not determined.

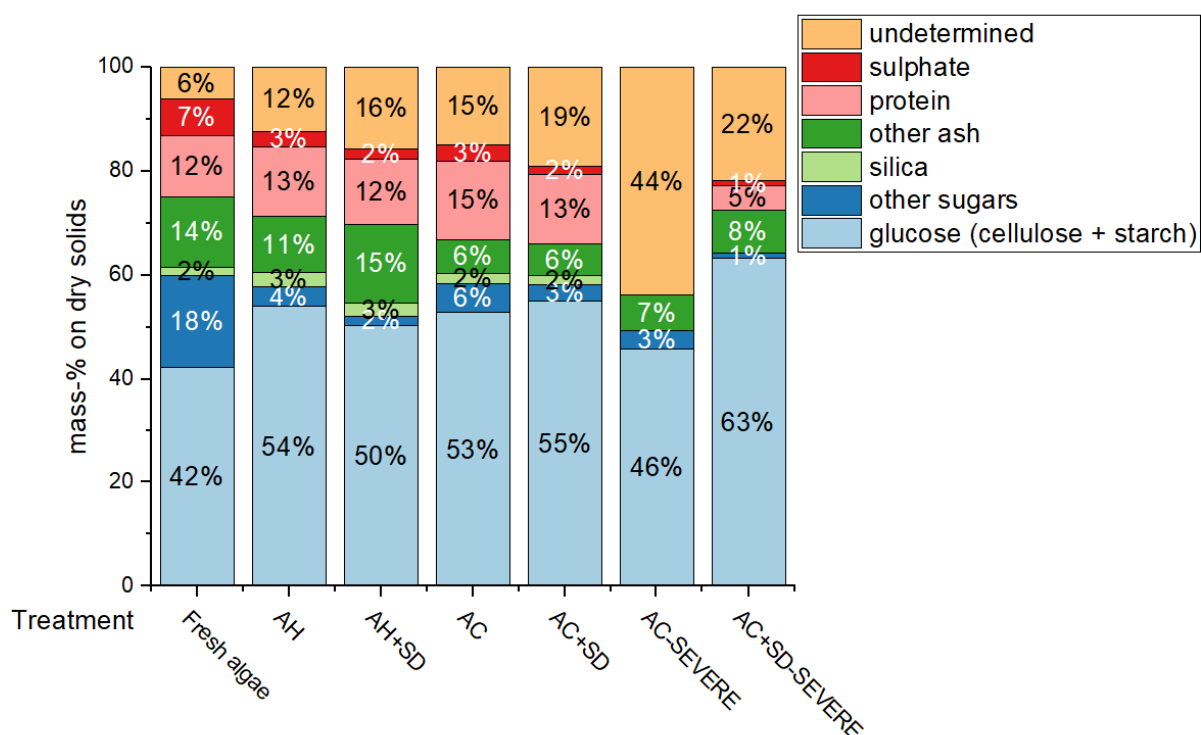


Figure 10. Percent-wise mass composition of the algal biomass after each chemical treatment. The term other sugars means the combined amount of arabinose, rhamnose, galactose, xylose and mannose in the sample.

Note: for the AC-Severe, the protein content and the silica content were not determined. For AC+SD-Severe the silica content was not determined.

The AH, AC and AC-Severe treatments used in this thesis were particularly effective in selectively removing hemicelluloses, and most likely starch from the raw algal biomass. However, unfortunately the separation and isolation of the relative amounts of cellulose and starch was not conducted in this thesis. However, as starch solubility increases rapidly as a function of temperature, and the degradation of polymeric starch is already clearly observable at 120 °C (Crochet *et al.*, 2004), it can be approximated with some confidence that the treatments AH, AC and AC-Severe are all capable of removing almost all starch in the raw algal biomass. Thus it can be assumed that the decrease in absolute glucose content of the samples as a result of treatments AH+SD, AC+SD and AC+SD-Severe are mostly result of cellulose degradation.

However, the AH – treatment differs from AC and AC-severe treatments in one critical aspect. The treated biomass in acidic solutions shows much lower ash contents than the reactions AH, AH+SD reaction path. This is mainly explained by the decrease in CaCO₃ – content of the samples. (Table 10). This can be explained by the well known reaction of CaCO₃ with acids.



The silica remained relatively inert throughout the different fractionation treatments, and was thus concentrated in the biomass. As a minor decrease of silica was observed in the alkali stage (AH+SD and AC+SD), it may be possible that further decrease of silica could have been achieved with higher alkali loads. This hypothesis was however not tested, as it was not possible to analyze the AC-Severe and AC+SD-Severe in the scope of this thesis. This is unfortunate, as there is evidence in literature, that for other high silica content biomass, (rice husks), the silica content could be at decreased with alkaline treatment. (Ludueña *et al.*, 2011)

Table 10. Results of the ash elemental analysis given. Ash components given as mass-% on the total oven ash measured. Samples from AC-Severe and AC+SD-Severe were not analyzed.

Ash component	Raw algal powder	AH	AH+SD	AC	AC+SD
Ash (K)	0.6 %	0.3 %	0.3 %	0.6 %	0.7 %
Ash (Na)	0.2 %	0.2 %	0.6 %	0.3 %	0.3 %
Ash (MgO)	2.7 %	1.4 %	1.1 %	0.7 %	1.0 %
Ash (CaCO ₃)	66.7 %	63.3 %	69.7 %	29.1 %	35.5 %
Ash (SiO ₂)	11.3 %	19.2 %	14.2 %	24.4 %	20.9 %
Ash (Al ₂ O ₃)	1.0 %	1.9 %	2.0 %	1.9 %	3.0 %
Ash (residue)	17.5 %	12.8 %	12.1 %	42.9 %	38.4 %
Total ash amount of 100g of solid product of the treatment	15.3 g	13.5 g	17.7 g	8.4 g	7.7 g

As the treatments AH+SD and AC+SD, do not cause any major changes on the biomass composition (Figure 13), yet decrease the total yield, it seems that these treatments provide very little additional value into isolating cellulose out of algal biomass. However, the AC-SD-Severe treatment seems to cause a substantial increase in the observed cellulose content of the sample, thus justifying the usage of the treatment. A possible explanation for this difference can be inferred from the results in Table 11. With AH+SD and AC+SD, the reaction solution becomes neutralized to pH 7 during the reaction, essentially converting the treatments into autohydrolysis.

For AC+SD-Severe treatment, the pH of the reaction solution changes from the initial 13 to final pH of 9, implying that there is significant consumption of alkali, but also that not all available alkali was consumed as was the case with reactions AH+SD and AC+SD. Thus, the AC-SD-Severe is only one of the alkali treatments used in this thesis, that bears resemblance to the alkali purification steps used by Mihranyan *et al.* (2004) and Cronshaw *et al.* (1958). It is also more than likely that the alkali dosage in AC+SD-Severe treatment was still too low for effective cellulose purification, as the chemical reactivity of compounds in biomass undergoes significant changes as the pH changes from 13 to 9.

Table 11. pH and colour of the reaction solutions after the reactions.

Reaction solution	PH before reaction	PH after reaction	Colour of the solution after reaction
AH	6	7	light brown
AH+SD	12	7	Dark brown
AC	1	4	Light green
AC+SD	12	7	Green
AC-SEVERE	1	1	Light green
AC+SD-SEVERE	13	9	Dark green

The main results of this fractionation study could be inferred from Figures 9 and 10. The chemical loads used in the fractionation study of this thesis were not enough to produce a pure cellulosic material comparable to the cellulosic materials reported in literature. Considering that the chemical loads used by this study were one or two orders of magnitudes smaller than the chemical loads in the literature methods, this result was not completely unexpected. However, the fractionation study demonstrated, that in the best case 69 % of the non-cellulosic material could be removed using the AC+SD-Severe presented in this thesis.

7.1.1 The uncharacterized compounds in the fractionation

Perhaps the most curious feature of this fractionation study is the increase in the undetermined fraction as a function of the treatments. This phenomena is especially apparent in Figure 11. In the figure it can be seen that the lowest amount of undetermined compounds was in the raw algal powder (6.15 g). As all other fractionations contain more undetermined compounds than this, it is obvious that some of the compounds present in the raw algal powder are reclassified after fractionation as unidentified compounds.

The relative amount of the uncharacterized compounds seemed to increase with the number of fractionation stages as seen in in Table 12. An exception on this trend was the AC-Severe treatment, with an extremely high content of undetermined solids (44%). For the AC-Severe treatment part of the uncharacterized components can be explained by the lack of protein content determination, but it does not offer sufficient explanation to explain the entire phenomena.

Table 12. Comparison of the fractionation yield and the amount of uncharacterized compounds

Fractionation stage	Total dry solids yield of the fractionation stage	Amount of undetermined compounds in the fractionation stage
Raw algal biomass	100 %	6.2 %
AH	56.0 %	13.1%
AH+SD	46.1 %	16%
AC	62.8 %	16%
AC+SD	46.3 %	19,5%
AC-Severe	49.9 %	44,6%
AC+SD-Severe	62.0 %	22%

This phenomena is most likely explained by the Maillard-reactions occurring during the fractionation treatments. The Maillard reactions are known mainly from the food industry, and they explain the appearance of coloration and the flavor-changes that appear during processing of food, such as

roasting coffee, baking of bread and cooking of meat. They start when a reducing sugar condenses with an free amino-group. This leads into a complex pathway of further reactions, with a wide range of reaction products. Further information on Maillard-reactions can be found in an article by Martins *et al.* (2001). In the context of this thesis the Maillard reactions create an ever-increasing amount of compounds that are difficult to characterize. This hypothesis further supported by the fact that phenomena typical to the Maillard-reactions e.g. the browning of the biomass and the appearance of new aromatic-compounds (scent of the algal material changed during fractionation) was observed during the fractionation study.

7.2 Investigations on degree of polymerization (DP) of algal cellulose.

In this thesis, the degree of polymerization of algal cellulose was studied using two different methods. First GPC was used to investigate the molar mass distribution (MMD) of the algal fractionation products. Two identical GPC-analysis were conducted, one according to the standard measurement protocol, and another after two weeks later with the sample being stored at 6 °C inbetween measurements. Later, CED-viscosity was used to conduct a DP-adjustment study on AC+SD-Severe treated algal biomass.

7.2.1 GPC-Analysis

The compilation of the GPC-analysis results can be found in Table 13. The reason for repeating the GPC-analysis is that the samples containing algal cellulose showed erroneous results when analyzed with short dissolution time. Similar erroneous results were encountered in a by Arnold *et al.* (2013). The issue is that standard GPC-sample preparation protocols produce a sample which underrepresents the high molecular weight fractions of algal cellulose.

In this study it was observed that if the Li/DMAc sample was stored in a refrigerator for two weeks, dissolution was significantly improved and the MMD could be measured more accurately. This behaviour was similar to the behaviour of high-molecular weight bacterial cellulose investigated by Shen *et al.* (2010). However, the work of Potthast *et al.* (2003) suggest that cellulose undergoes degradation in the Li/DMAc system. To investigate the effect of this two weeks of storage, a sample made out of dissolving birch (*betula pendula*) prehydrolysis kraft pulp (B-PHK) provided by Stora Enso Enocell pulp mill was analyzed together with the algal samples. It was observed for the Enocell-sample that the additional dissolution time caused some loss of DP, but the shape of MMD remained almost identical.

Unfortunately, it must be mentioned that repeatability of results algal cellulose samples using the GPC was fairly low. For the normal dissolution time samples, the GPC-signal was extremely weak, implying that the amount of dissolved sugars was quite low. As a similar observation was made by Arnold *et al.* (2013).

For the GPC samples with two week dissolution time, on the other hand the problem was that the DP of the cellulose most likely was too high to be analyzed with good repeatability this technique. It was observed that when measuring two high-DP samples in a row, that the signal response used to measure sample quantity in GPC did not fully stabilize between measurements. The results were considered accurate enough to be reported in this thesis, but it should be mentioned that high DP-algal cellulose might require adjustment of the GPC measurement protocol. The nature of this adjustment however is considered to be outside the scope of this thesis. To give the reader a better idea of the actual shape of the MMD measured and the phenomena discussed here, the MMD-graphs of all measured samples are included in Figures 11-15.

Table 13. GPC-results with normal dissolution time (24 h) and extended dissolution time (2 weeks). The Mn and Mw were converted to DPn and DPw using the molar mass of cellulose (162.64 g/mol).

Fractionation process	DPn (24h)	DPw (24h)	DPn (2 weeks)	DPw (2 weeks)
AC+SD	149	1492	2939	12131
AC-Severe	136	2751	7509	15493
AC+SD-Severe	430	7348	3110	10932
AH	131	3999	5309	13212
Enocell	419	2417	385	2024

In discussing the GPC-results and their significance, the results in Table 13 with the short dissolution time are considered to be inaccurate and are not included in the discussion. The ultimate value of the results from Table 1 is clear. Even though Mihranyan has studied the cellulose of *Cladophora glomerata*, to a great detail, the neither DPw or the MMD of this cellulose has not been reported before. The only available DPw from a species belonging even to the same order (*cladophorales*), is from *Aegagropila linnaei*, for which a DPw of 3647 is reported (Guo *et al.*, 2017) when cellulose from it was purified using the Mihranyan *et al.* (2004) method. Thus, this work presents a novel reporting on the DPw and MMD of algal cellulose in *Cladophora glomerata*.

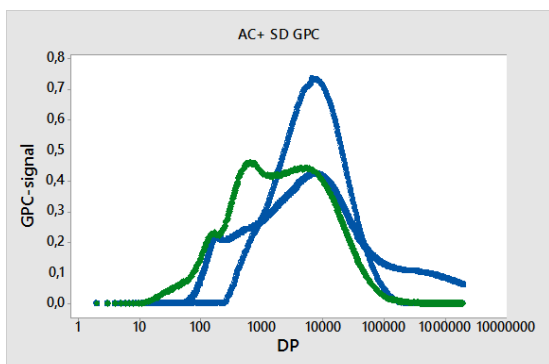


Figure 11, MMD of AC+SD,

green = sample with normal dissolution time

blue = sample with long dissolution time

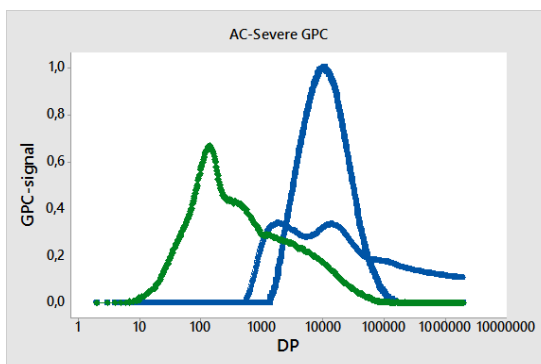


Figure 12, MMD of AC-Severe

green = sample with normal dissolution time

blue = sample with long dissolution time

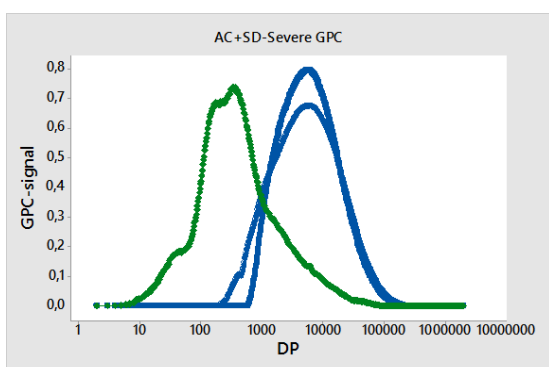


Figure 13, MMD of AC+SD-Severe

green = sample with normal dissolution time

blue = sample with long dissolution time

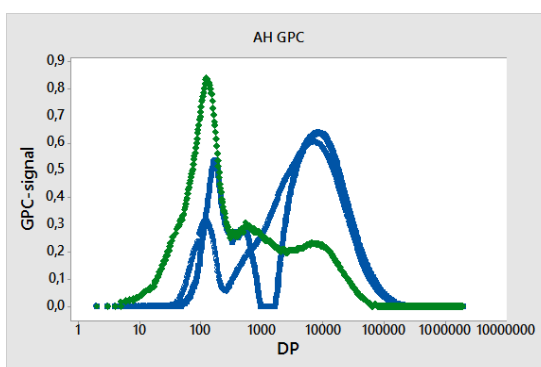


Figure 14, MMD of AH

green = sample with normal dissolution time

blue = sample with long dissolution time

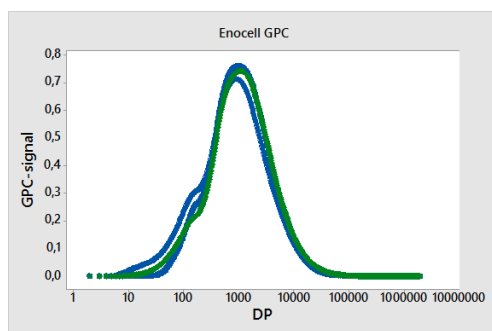


Figure 15, MMD of Enocell

green = sample with normal dissolution time

blue = sample with long dissolution time

7.2.2 CED-viscosity analysis.

Of the two spinning trials made in this thesis, the initial spinning trial with autohydrolyzed dope ended unsuccessfully. After this a decision was made to adjust the DP the samples prepared by the AC+SD-Severe-treatment, to a comparable level with Enocell-pulp, a material that can be easily spun into textile fibers using the Ioncell-F method. The reason for choosing the AC+SD-severe as a starting material, was that it had the highest cellulose concentration of all prepared samples. A viscosity of 450-470 ml/g in cupri-ethyldiamine solution prepared according to the standard SCAN-CM 15:99, corresponding to an average DP of 1097 - 1162 was set as the target. Viscosity values were converted to DP-values according to Michud (2015)

It should however be noted, that the AC+SD-Severe is not pure cellulose. This means that for a given viscosity value, the DP of the cellulose is underestimated as in the calculations of this section the standard SCAN-CM 15:99 is followed, a standard that was developed for pure cellulose samples, without any additional correction factors. As the goal of this study was to produce a material with the desired CED-viscosity, this inaccuracy was accepted. However, the DP-values reported in this study are most likely systematically too low for algal cellulose in general. This is expected to explain the discrepancy in the estimated DP for AC+SD-severe material between GPC-analysis and the CED-viscosity analysis.

Two sets of studies were made by using the Monowave-microwave reactor system. The initial reaction time and conditions were adapted from the in-lab protocol used to prepare cotton. First the effect of concentration on DP was tested by treating the original sample in solutions of sulfuric acid (ranging from 0,5 mass-% to 20 mass-%) The results of the initial study are presented in Table 14.

Table 14. results of the initial DP adjustment study.

Treatment	Viscosity (ml/g)	DP
Untreated AC+SD-Severe	1344	4632
80 °C, 30 min, 20 % H ₂ SO ₄	725	2056
80 °C, 30 min, 10 % H ₂ SO ₄	>> 861	2578
80 °C, 30 min, 5 % H ₂ SO ₄	Out of standard range	Not measured
80 °C, 30 min, 3 % H ₂ SO ₄	Out of standard range	Not measured
80 °C, 30 min, 1 % H ₂ SO ₄	Out of standard range	Not measured
80 °C, 30 min, 0.5 % H ₂ SO ₄	Out of standard range	Not measured

The results of the initial study were quite surprising, as the algal cellulose proved extremely resistant towards acid degradation. For comparison, an earlier study, conducted by Leena Katajainen at Aalto-University (unpublished data), showed that cotton, was much more sensitive to sulfuric acid degradation. The viscosity of cotton was before DP adjustment 1986 ml/g, corresponding to a DP of 7743. After an DP-adjustment reaction (82 °C, 2.6 mass-% H₂SO₄ reaction solution, 9 min reaction time) the cotton viscosity achieved a value of 458 ml/g corresponding to a DP of 1124. Because of this, most of the sample points planned for the study were outside of the measuring range of standard SCAN-CM 15:99

Based on the results of the first study a secondary DP-adjustment study was conducted using 20 mass-% H₂SO₄ as the reaction solution. The results of this second study are presented in Figure 16.

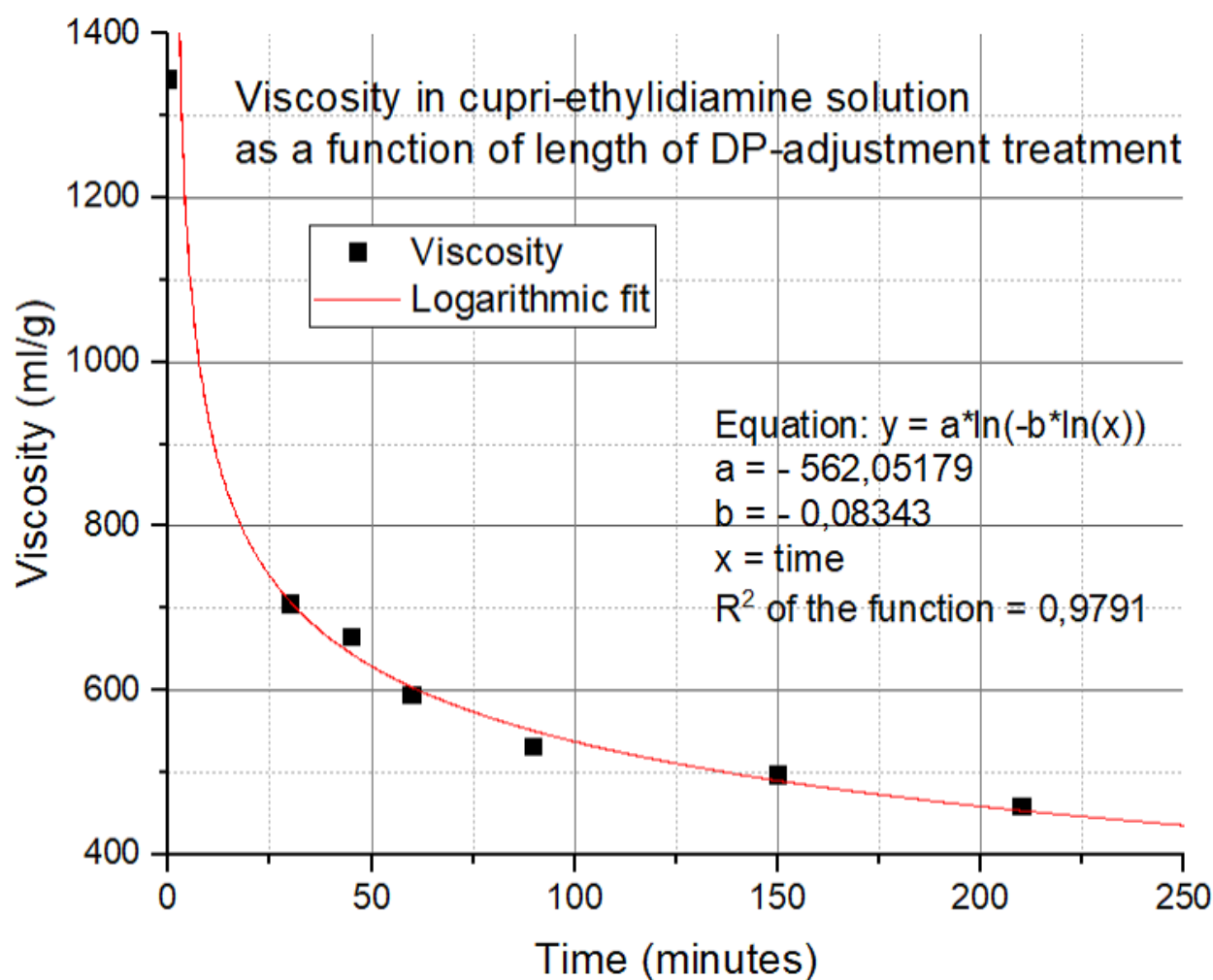


Figure 16, Viscosity of AC+SD-Severe algal cellulose as a function of reaction time. (80 °C, 20 mass-% H₂SO₄)

From this second study, a reaction time of 210 min was deemed appropriate for adjusting the DP of the AC+SD-Severe biomass into a perfect range (viscosity of 450-470 ml/g, 210 min reaction time corresponds to a viscosity of 460 ml/g) in respect to spinning. However, the most interesting finding of this study is not this purely technical detail, but the observation of an unusually high leveling of degree of polymerization (LODP) value for algal cellulose. As the viscosity of CED-solution containing algal cellulose seems to level off at around 400 ml/g, this corresponds with the cellulose DP of around 940. For comparison, the LODP of most celluloses is considerably lower, usually around corresponding to a DP of 200 – 300 units. (Nishiyama et al., 2003). Furthermore, because the feedstock used in the DP-adjustment study was not pure cellulose, this study most likely underestimates the DP of cellulose systematically, which means that the actual LODP of the pure cellulose in the algal biomass is higher than 940 units. This unique LODP value has not been

investigated or reported before in the literature on algal cellulose, and represents a novel finding of this thesis.

7.3 Significance of dope rheology on the loncell-F process

The success of the dry-jet wet spinning of cellulose/[DBNH]OAC – dopes displays a tight connection to the viscoelastic behaviour of the dope in question. In the doctoral dissertation of Michud (2016) it was concluded that a matrix of viscoelastic properties that were favorable for spinning could be identified, with the critical properties being the zero-shear viscosity, and the modulus and frequency of the cross-over point between the storage- and loss-moduli of the dope. These two critical measures of spinnability will be explained in detail in this section and their effects on spinnability and characterization of [DBNH]OAC – dopes will be discussed.

7.3.1 Zero-shear viscosity

Shear viscosity is the viscosity of a visco-elastic material at a given shear rate. Shear stress can be defined by the equation: $\tau = \mu \frac{\delta v}{\delta y}$, where τ = shear stress, δv = velocity differential, δy = position differential and μ = dynamic stress viscosity. For clarification, see Figure 17.

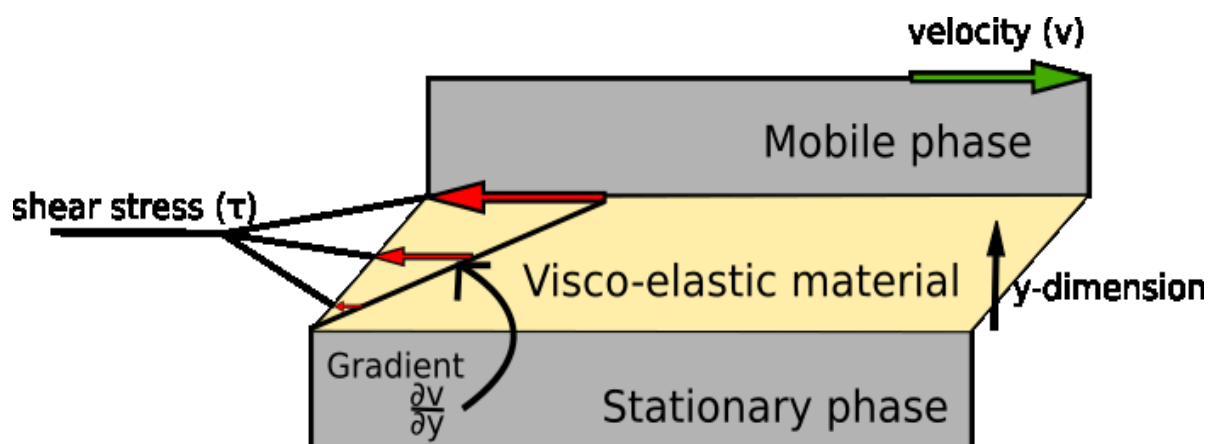


Figure 17, Graphical example of some basic viscosity related terms in a 2D-plate system: τ = shear stress, δv = velocity differential, δy = position differential. As the mobile plate moves with velocity v , it deforms the visco-elastic material. However, the visco-elastic material is fixed at the stationary phase. This results in a situation, where the magnitude of the deformation depends on the y -dimensional position of the visco-elastic material. The viscosity of a substance is a value that relates this deformation to the shear stress that opposes the movement of the material.

In this system the zero-shear viscosity can be understood as the viscosity of the material when an infinitesimally small velocity is enforced infinitesimally close to the stationary phase. This zero shear viscosity is related to the temperature and molecular weight (Bytner & Smith, 2001), which essentially allows the determination of the DP of cellulose at a given temperature using only the zero-shear viscosity as a measure. Of course such DP-determination requires in-depth knowledge

of the cellulosic material in question, as the apparent DP depends on the total molecular mass distribution of the material (Michud *et al.*, 2014).

From the purely technical standpoint, to achieve successful spinning using Ioncell-F, it has been reported that the zero-shear viscosity of the polymer melt should be between 27 000 and 40 000 Pas, with the optimum zero-shear viscosity in respect to spinning properties being around 30 000 Pas. (Michud, 2016)

7.3.2 Cross-over point

To understand the concept of the cross-over point, two central properties of visco-elastic material must be understood first. These are the shear storage modulus (G') and the shear storage modulus (G''). The shear storage modulus (G') expresses the energy stored in the material as a result of a shear deformation (comparable to v in Figure 8.). If this deformation is suddenly removed the stored energy will be released and act against the deformation in a springlike manner. The shear loss modulus (G'') expresses the energy spent deforming the material in shear deformation dissipated as heat.

The crossover point is defined as the point where the numerical values for these two parameters overlap. This also signifies the transition point from a mostly gel-like material ($G' > G''$) to a mostly liquid-like ($G'' > G'$) and vice versa. The exact location of this crossover point varies depending on the visco-elastic profile of the material that is being analyzed, and for some materials this crossover point cannot be found at all. However, for polymer-melt like materials, such as ionic liquid dope, the location of the cross-over point can help to predict the polymer structure. (Figure 18.)

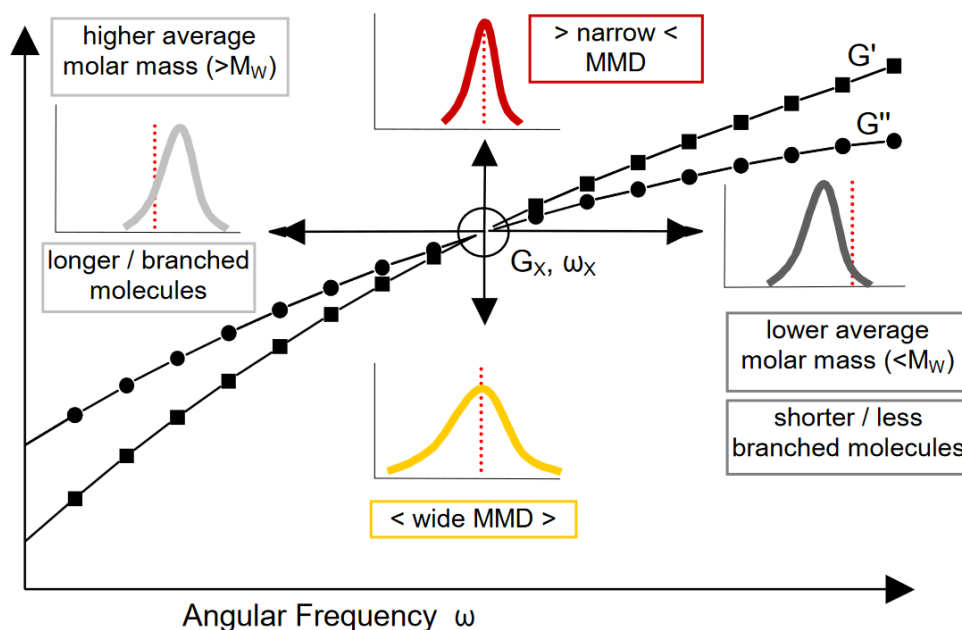


Figure 18, the location of the cross-over point as a function of molar mass (M_w) and molar mass distribution (MMD) Image taken from Goodyer (Anton-Paar)

In respect to spinning, in her thesis Michud (2016), mentions that for succesful dry-jet wet spinning of an ionic liquid dope, the magnitude and the angular frequency of the dope must be in the correct range. This range is $0.8 - 1.5 \text{ s}^{-1}$ for the angular frequency of the crossover point and the magnitude of the cross over point should be between 3000 and 6000 Pa.

7.3.3 Rheological measurements

For the rheological study three dopes were investigated. Dopes in Figures 19, 20 and 21 are reported in the following format: (Cellulose source in dope, mass-% of the cellulosic material in dope, temperature of the dope.) It should be noted however, that the AC+SD-severe dope was manufactured from the DP-adjusted AC+SD-severe dope. The DP-adjusted AC+SD-Severe dope raw material had the CED-viscosity value of 450ml/g.

From the rheological measurements, an initial estimate was made if the dope could be spun into textile fibers. The data regarding the cross-over point can be seen in Figure 19 and it can be compared to the theoretical knowledge given in Figure 9. From these results it is obvious that the algal, both AH and AC+SD-Severe dopes have too wide MMD and too long average DP to qualify as optimal spinning dopes. For the AC+SD-Severe dope this is an unexpected result, as the DP of the material was adjusted to the Enocell range. An possible explanation for this phenomena is that as the AC+SD-Severe material contained only 63% cellulose, the CED-measurement gave a lower

viscosity value for the AC+SD-Severe material than what would have reflected the real properties of the pure cellulose in the material. However, it is hard to imagine what could be done to remedy the situation, as the DP-adjustment produced a material where the DP of cellulose was fairly close to the LODP of the cellulose. (Figure 16)

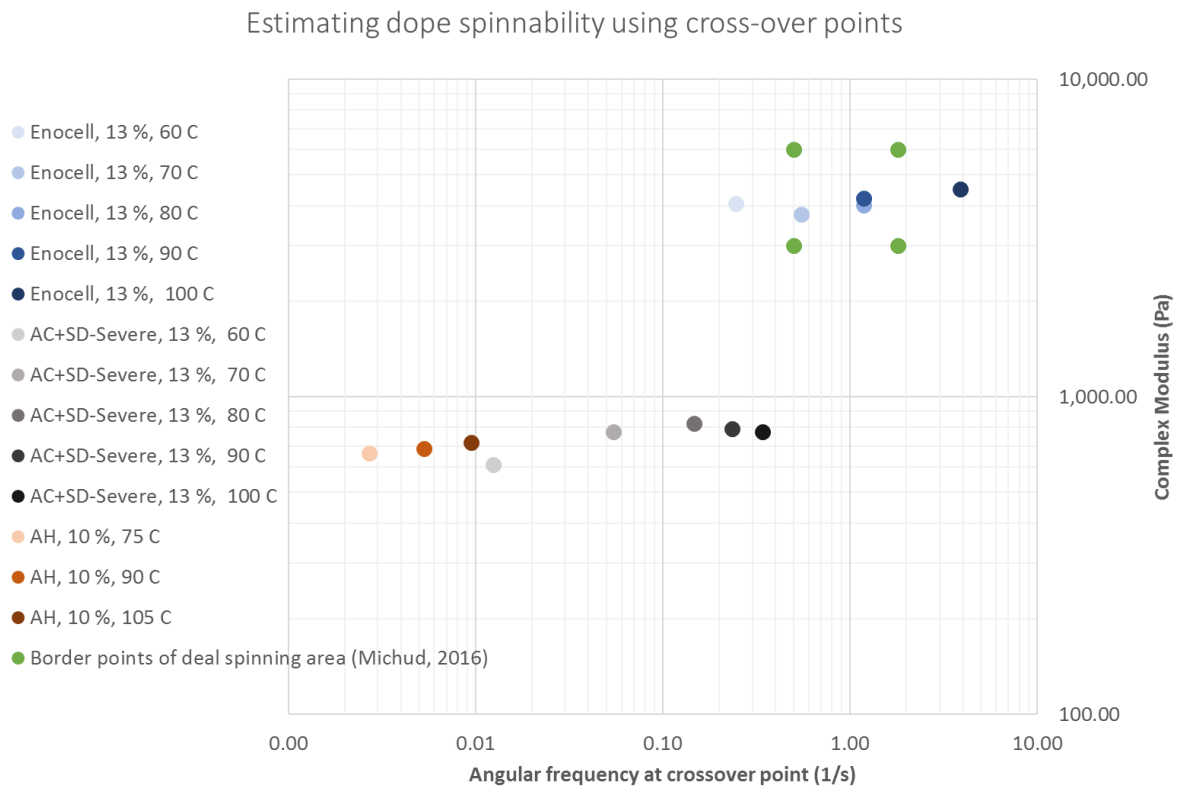


Figure 19, investigation on the cross-over points of the dopes used in this thesis on a double-logarithmic plot

The investigation of complex viscosities (Figure 20) reveals that AH dopes are most likely unspinnable, while the AC+SD-Severe dope should be spinnable in the range between temperatures 70 °C and 80 °C. This corresponded well with the results observed during the spinning trials. In figure 21 the raw data from the oscillatory complex viscosity measurement is shown to demonstrate the visco-elastic profile of the Enocell and AH+SD Severe dopes.

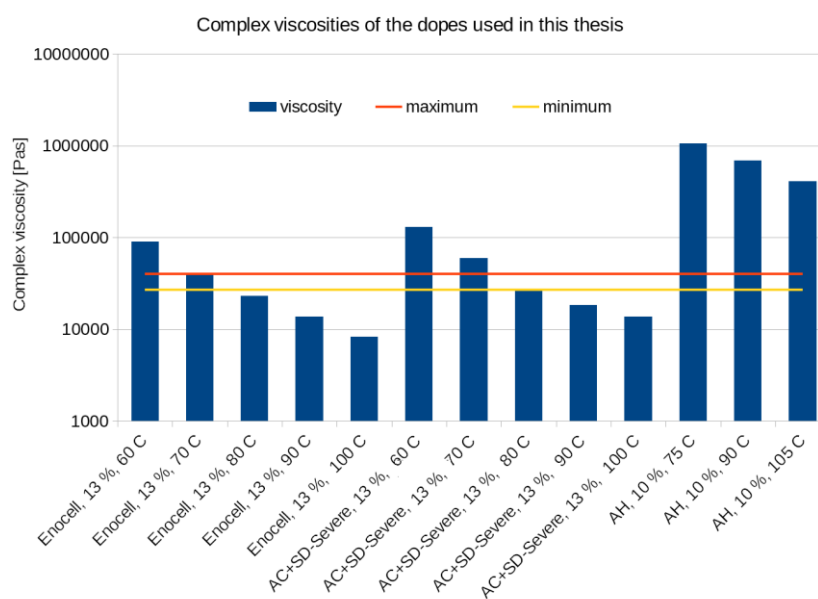


Figure 20, investigation on the complex viscosities of the dopes used in this thesis

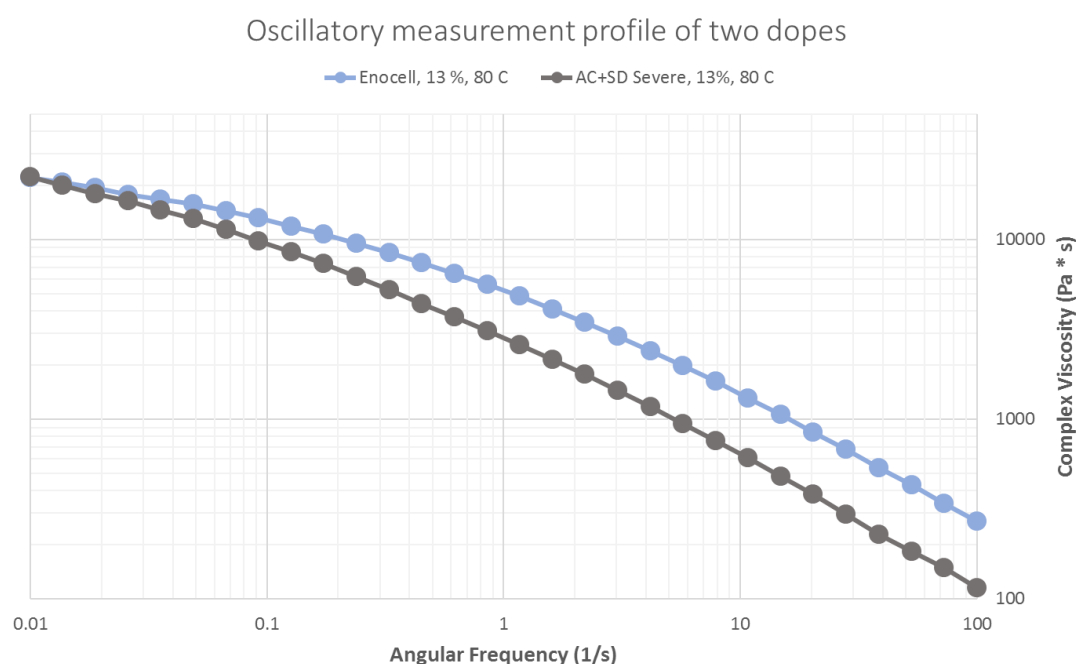


Figure 21, oscillatory complex viscosity comparison of Enocell 13 %, 80 °C and AC+SC Severe 13%, 80 °C on a double logarithmic plot.

7.4 Spinning trials

In this thesis 2 dry-jet wet spinning trials were conducted. The operational parameters of the spinning trials can be found in table 15. The initial spin-trial was conducted with the [DBNH]OAc-dope containing 10 mass-% of autohydrolyzed algal biomass. This initial spin trial ended unsuccessfully with no fibers produced. Instead of textile fibers, a bulbous mass of agglomerates

was produced. This mass can be seen in Images 7 and 8. After a short extrusion of the agglomerates the spinneret head became clogged and the extrusion of algal dope was halted completely.

Table 15. Operational parameters of the spinning trials

Raw material (pulp)	Pulp concentration (weight-%)	Number of holes in the spinneret	Diameter of holes in the spinneret (mm)	Spinning temperature (°C)	Dope reference temperature (°C)	η_0 (Pa*s)	ω at crossover point (1/s)	G at crossover point (Pa)
AC+SD-SEVERE_Full_DP-adjusted	13%	36	0.2	78	80	27780	0.147	824
AH	10%	36	0.2	80 – 105	90	688260	0.00529	688
Enocell	13%	4	0.2	76	80	23160	1.18	4020

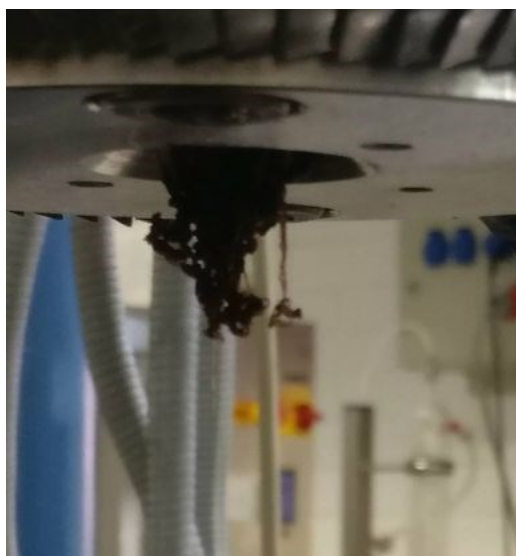


Image 7, a bulbous mass of agglomerates present at the spinneret head.

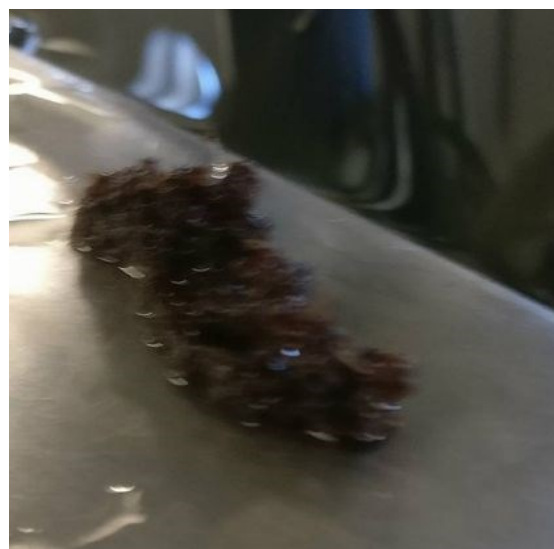


Image 8, a bulbous mass of agglomerates, collected from the bottom of the spin-bath

The second spin-trial was conducted with the AC+SD-severe 13% dope. In this trial textile fibers with DR 1 and DR 2 could be produced. After fibers with these draw ratios were collected, agglomerates appeared at the spinneret, the spinning unit piston started leaking around the temperature sensor, and spinning had to be subsequently halted. The production of fibers with higher draw ratio might have been possible, if the experimental setup had been better controlled and the leakage prevented. Images 9-11 depict the production process and the final produced fibers.

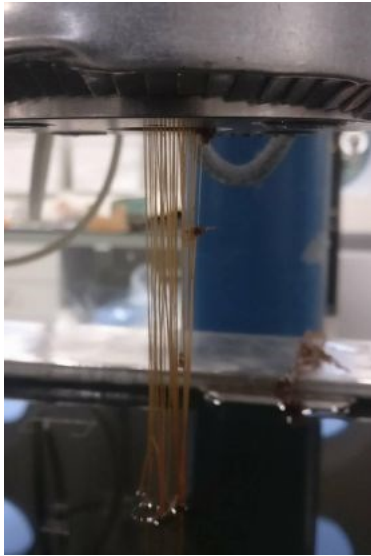


Image 9, Extrusion of algal textile filaments under no draw.



Image 10, Collection of algal textile fibers under DR 1.



Image 11, The produced algal textile fibers: on left fibers with DR 1, on right with DR 2

The fiber properties of these fibers are reported in table 17 below, and compared with conventional Ioncell-F fibers with similar draw ratios, prepared from Enocell 13% dope. The tensile properties and the dtex of fibers was measured by taking the average values of ten fiber measurements. Fiber properties were measured using the Vibroskop/Vibrodyn 400 – system (Lenzing instruments)

Table 16. Fiber properties of algal and enocell fibers.

Fiber	Test type	Titer (dtex)	Force on break (cN)	Elongation on break (%)	Tenacity cN/tex
Enocell DR1	dry	8.63	17.07	7.68	20.00
AC+SD-severe DR1	dry	16.72	26.34	23.22	16.50
Enocell DR1	wet	8.19	20.28	16.54	24.75
AC+SD-severe DR1	wet	16.21	17.18	23.50	10.83
Enocell DR2	dry	4.86	13.63	7.98	28.77
AC+SD-severe DR2	dry	3.55	7.68	9.40	21.88
Enocell DR2	wet	4.32	12.19	11.28	28.37
AC+SD-severe DR2	wet	3.19	4.95	9.29	15.5

Dry test were measured in air in standard condition room (23° C, 50 % Relative humidity), wet tests were measured with the fiber submerged in water. DR is abbreviation for draw ratio.

From Table 16 it can be observed that with low draw ratios the Enocell-fibers have superior fiber properties when compared to the fibers made out of algal cellulose. It can also be clearly seen that the titer of the algal cellulose fibers and the Enocell-fibers shows very different responses under a similar draw ratio. This is most likely the result of differences in visco-elastic behaviours of the dopes as they are being stretched in the air-gap. Before making any conclusions about the fiber properties of algae, it is worth considering that the algal dopes used in this study did not meet the ideal operation window of the spinning system, which has been optimized for Enocell production.

8. Conclusions

8.1 Conclusions on the literature section

Detailed usage of biological classification of algae, focusing especially on the evolutionary tree of algal biomass, may help in bringing sense to the world of algal biorefining. Grouping of species that are evolutionally more close to each other, and subsequently are more likely to include more similarities inside the group, than to more distant evolutionary groups seems to be prudent practice. Even though closely related species might show large variance in shape, growth environment and breeding behaviour, some aspects of the biochemical evolution, such as structure of certain cell wall polysaccharides, is unlikely to undergo massive changes in species that are evolutionally very close to each other. If one accepts this proposition, it would imply that the relevant categorizations for biorefining algae could be found from the already existing sub-categorizations of the biological scientific categorization.

Some investigations in this direction have already been done, with for example Bruce C. Parker (1970), attempting to uncover the evolutionary connections between algae by investigating the similarity of their cell wall chemistry. While Parkers main purpose was made obsolete by the advent of DNA-analysis, his study still has relevance. Parker focused on reporting the structural variety of cellulose present in various green algae and how this categorization matches the biological categorizations used in the field. For researching biological scientific categorisation of algae, this knowledge has become outdated, but for developing biorefining, such categorizations might still be extremely valuable, as this would allow to estimate the behaviour of yet unstudied algae in biorefining processes. However, the selection of the level of classification that would allow for condensation of relevant information in respect to biorefining, without becoming as overgeneralized is a scientific challenge that is yet to be overcome. Especially so, because large parts of the evolutionary history of algae has been rewritten in the last decades (Kim et al., 2014). The most significant reason for this rewriting is that new analytical methods, such as DNA-analysis, have provided novel insights on the structure of the evolutionary trees of algae. This has already led to reclassification of many algal species and even families of species, but the work on organising the algae into a well-defined group of biological organisms is far from complete. (Kim *et al.*, 2014)

8.2 Conclusions on the experimental section

This thesis demonstrated that it is possible to produce textile fibers from cellulose-rich, fractionated green algal biomass using the Ioncell-F method. A small amount of textile fibers was produced and

their properties were compared with Ioncell-F fibers made from more conventional feedstocks. The produced algal fibers were observed to have slightly worse technical properties than the more conventional prehydrolysis kraft pulp-based fibers.

From the sustainability perspective, usage of purely algal cellulose as a textile raw material might be a bit difficult. A potential problem is the need for a severe DP-reduction reaction for algal cellulose to achieve spinnable dope. Furthermore, it might even be impossible, if the LODP of the algal cellulose is too high, to ever produce dopes that would be in the optimal spinning range. Thus, what could be considered in the future is the usage of algal cellulose as a reinforcing material, for low DP cellulosic materials.

Michud (2016) reports the spinning behavior of the dopes is improved and the final fiber properties are improved as the amount of the high molecular weight fraction ($DP > 2000$) is increased in the cellulose substrate beyond 25%. This affects the relaxation or non-relaxation of the cellulose chains in the airspinning, and according to Michud allows formation of a more cohesive cellulose network during the fiber regeneration. The overall result is that higher DP-cellulose allows for production of thinner and higher tenacity fibers. This result implies that algal cellulose from *Cladophorales* might be used as a reinforcing agent in conventional Ioncell-F fiber production. However, to implement this idea in practice, further development on the recovery and purification of algal cellulose must be done, as even the purest algal cellulose prepared using the methodologies of this thesis had a low degree of purity (63 mass – % cellulose).

Interestingly enough, a company called Smartfiber AG, has recently started manufacturing a product called SeaCell, where a mixture of cellulose (85%) and seaweed powder (4%) has been spun into Tencel-type fibers using Lyocell technology. As these fibers have slightly worse technical properties (17-25 cN/tex wet tenacity) than conventional Tencel-fibers, it could be worth investigating, if by using *Cladophora glomerata* instead of *Ascophyllum nodosum*, this product could be replicated with better technical properties using the Ioncell-F technology.

The fractionation study conducted in this thesis did not succeed in isolating pure cellulose from algal biomass. However, the isolation procedures used in this thesis produced a material that was “good enough” for textile production with the Ioncell-F technology. Furthermore, the effects of a lighter chemical-load fractionation processes, than the ones found in literature on *Cladophora glomerata* biomass were observed and quantified. The fractionation processes investigated in this study present could be used in the future as lower-range estimate in future attempts to develop a

sustainable process for isolating algal cellulose. Finally, the LODP-behaviour of algal cellulose from *Cladophora glomerata* was examined and reported for the first time.

Finally, although it was left outside the scope of this thesis, the fractionation processes investigated in this thesis could merit further investigation as a potential fermentation broth for biorefinery purposes. As plain autohydrolysis succeeded in removing approximately 40% of the dry solids and almost all hemicellulosic sugars and most likely starch, the autohydrolysis liquid of *Cladophora glomerata* should be much more fermentable than the autohydrolysate liquids from terrestrial feedstocks, because algal biomass lacks lignin and the fermentation-inhibiting fractionation products cleaved from it during autohydrolysis.

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