Macroalgalae in the Biorefinery

A substance flow analysis and environmental assessment of an extraction process of the major components in *Saccharina latissima*

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A SUBSTANCE FLOW ANALYSIS AND ENVIRONMENTAL ASSESSMENT OF AN EXTRACTION PROCESS OF THE MAJOR COMPONENTS IN SACCHARINA LATISSIMA

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Abstract

A turn to more sustainable resources has lead the research during the last decades to algae. Algae is a resource that has been utilized for thousands of years offering a variety of possibilities. Nevertheless modern technology were able to uncover algae’s great potential and pave the way for alternative uses such as biofuel and biomaterial production. Towards that direction, ‘Seafarm’ aims in utilizing algae in the most efficient and sustainable way. For that purpose various steps have been established, including the biorefinery step which entail among other the extraction of carbohydrates from brown algae.

The current thesis is based on an extraction of carbohydrates from *Saccharina latissima*, a brown algae species, which was developed by Viktor Öberg during his master thesis at KTH. The aim of this work is to assist in the scaling up of that laboratory process by analyzing the basic steps and substances of the process, investigating its environmental performance and identifying improvement areas for theoretical optimization. The results of the aforementioned analysis include a substance flow analysis which reveals the basic steps of the process and constitute the basis for further analysis. The second step examines the environmental performance of the process based on the chemical selection. Hence the results are a risk assessment of chemicals with performance indicators for each chemical as well as the whole process. The final part provides a theoretical optimization of the process based on literature studies where the recommendations are divided in production optimization and environmental performance.

The above results constitute the basis of the analysis of the process and sets the foundations for scaling up the process at an industrial level. The current analysis in combination with an energy and economic assessment could be used for the designing of the process and its integration in the biorefinery.

**Key words:** algae, *Saccharina latissima*, extraction, carbohydrates, substance flow analysis, risk assessment, chemicals
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Table of Contents

Abstract ................................................................................................................................. ii
Acknowledgement ................................................................................................................ i
List of Chemicals ................................................................................................................... vi
List of Abbreviations ........................................................................................................... vii
1. Introduction ..................................................................................................................... 1
2. Aim and Objectives ......................................................................................................... 3
3. Methodology ................................................................................................................... 4
   3.1 Substance flow analysis ............................................................................................ 4
   3.2 Environmental Assessment .................................................................................... 6
   3.3 Theoretical Optimization ..................................................................................... 7
4. Background Studies ........................................................................................................ 8
   4.1 Eutrophication ........................................................................................................ 8
   4.2 Algae and potential utilization ............................................................................ 9
   4.3 Seafarm project ..................................................................................................... 10
   4.4 Potential use of brown algae ............................................................................. 11
      4.4.1 Biofuel production ....................................................................................... 11
      4.4.2 Biomaterials ................................................................................................. 12
      4.4.3 Food additives .............................................................................................. 12
      4.4.4 Medicine and Cosmetics ........................................................................... 12
   4.5 Chemical composition ........................................................................................... 13
   4.6 Extraction of Carbohydrates ............................................................................... 13
5. Results ........................................................................................................................... 15
   5.1 Substance flow analysis ....................................................................................... 15
   5.2 Environmental assessment .................................................................................. 20
      5.2.1 Assessment of chemicals ........................................................................... 20
      5.2.2 Waste Reduction Algorithm ........................................................................ 25
   5.3 Theoretical optimization ..................................................................................... 31
      5.3.1 Production optimization ............................................................................ 32
      5.3.2 Cleaner Production Applications ................................................................ 34
6. Discussion ....................................................................................................................... 35
7. Conclusions .................................................................................................................... 38
8. Recommendations for future studies .......................................................................... 39
References ......................................................................................................................... 40
Appendix I .......................................................................................................................... 44
List of Chemicals

Ca(OH)$_2$ - Calcium Hydroxide
CaCl$_2$ - Calcium Chloride
HCl - Hydrogen Chloride
MeOH - methanol
N - Nitrogen
Na$_2$CO$_3$ - Sodium Carbonate
NaOH - Sodium Hydroxide
P - Phosphorus
List of Abbreviations

AP - Acidification Potential
ATP - Aquatic Toxicity Potential
EPA - Environmental Protection Agency
FA - Focus Area
GWP - Global Warming Potential
HTPE - Human Toxicity Potential by Exposure
HTPI - Human Toxicity Potential by Ingestion
IUPAC - International Union of Pure and Applied Chemistry
MFA - Material Flow Analysis
NMR - Nuclear Magnetic Resonance
ODP - Ozone Depletion Potential
PCOP - Photochemical Oxidization Potential
PEI - Potential Environmental Impact
Ppm - Parts per Million
SFA - Substance Flow Analysis
TTP - Terrestrial Toxicity Potential
1. Introduction

Algae are photosynthetic marine organisms which grow in various aquatic environments in which constitute the base of the food chain. Algae is found as unicellular organisms which are categorized as microalgae or multicellular plant-like organisms categorized as macroalgae. Microalgae size varies from a few micrometers to a couple hundred micrometers while macroalgae, or so called seaweed, can reach many meters in length. Macroalgae can be further classified in three main groups according to their pigmentation: brown algae, red algae and green algae. (Demirbas 2010; Taelman et al. 2015)

Brown algae is usually large in size seaweed and its morphology consists of a long blade or lamina, the stipe, and the holdfast that fix the whole structure to the substrate. Among the above groups, brown algae demonstrates a high efficiency in utilizing light for photosynthesis resulting in high biomass productivity. That leads to an abundancy of brown algae in nearshore coastal areas with relatively low temperature water substrate. (Song et al. 2015)

Aquatic biomass is a material that have been studied for many decades now. Many of macroalgal substances have been used as nutritional additives or as animal feed. Other uses lie on cosmetic industry and even medicine production. The majority of aquacultures today stand in Far East countries such as China and Japan and their main purpose is the food industry (Spolaore et al. 2006). The last decade though, seaweed is studied for its potentials as an alternative biofuel feedstock and its potential use in material science. By combining current research with the existence knowledge, algae can be processed in a biorefinery issuing multiple products that used for various purposes. With the rise of third generation biofuels, algae cultivations can provide renewable, carbon neutral biofuels and the same time mitigate eutrophication in affected ecosystems. (Spolaore et al. 2006)

The current thesis is a study around the area of marine resources exploitation. More specifically, it is part of ‘Seafarm’ project, which deals with algae utilization for various purposes, such as, energy production, nutrients recovery and biomaterial production (SUBMARINER, 2015). ‘Seafarm’ is an interdisciplinary research project which aims in developing a methodology where brown macroalgae is utilized in the most efficient way in terms of substance extraction and utilization in a closed loop system that produces zero waste. The same time, sustainability needs to characterize all the different steps and processes which take part in the project. (Gröndahl Fredrik 2014)

The ‘Seafarm’ project is focused on brown algae which, as mentioned above, entail a group of kelp with similar pigmentation. Brown algae was chose due to its large quantities in the west coastal area of Sweden and its carbohydrate rich composition (Schiener et al. 2014). According to Fasahati et al., 2015 that group represents the first largest seaweed source which indicate the abundancy of that kelp group. Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta are some brown algae species. (Schiener et al. 2014)
The focus of the current thesis is on the biorefinery step. The concept of biorefinery includes a stream of biomass which yields a spectrum of valuable products using biological or thermochemical processing or a combination of both. The complexity of those processes lies on the biomass input on the one hand and the purity of the output products on the other hand. Potential products or a mixture of products may be either micronutrients like vitamins or macronutrients such as proteins, carbohydrates and fats. (National Non-Food Crops Centre 2007)

Regarding the carbohydrate extraction there has been work, into the framework of master thesis (Öberg, 2014), which utilize brown algae in order to extract carbohydrates in laboratory scale. That work provides a process which separates sufficiently the major components of *Saccharina latissima*. Hence, that process constitutes a basis to work on and develop.

![Extraction process flowchart](image-url)

Figure 3. Extraction process flowchart (Öberg 2014)
2 Aim and Objectives

The current thesis is entailed into the framework of research of ‘Seafarm’ project. The area of interest in that case is the biorefinery step and in particular, the carbohydrate extraction from *Saccharina latissima* samples which has been designed and tested in the laboratory.

The ultimate goal is to scale up that process from the laboratory scale to an industrial level. The current work constitutes the first step of the scaling up process which intends in providing information which are going to assist in the development of the extraction of carbohydrates at an industrial level. In order to achieve that, a substance flow analysis and an environmental evaluation of the reagents used in the process are going to be integrated and will provide the tools to perform a theoretical optimization of the process from a cleaner production perspective.

According to the above, the aim addressed by the study is the following: ‘Analyze the extraction process of the major components in *Saccharina latissima* in order to assist in scaling up the process at an industrial level’. That work is going to be conducted based on the following objectives:

- Identify the steps and flows of the process and analyze the substances that are present in the process.
- Examine the environmental performance of the process with focus on the chemicals used.
- Identify the improvement areas of the process and provide recommendations.
3. Methodology

The following analysis was performed based on the Öberg’s work as presented in his master thesis with title: ‘Macro Algae as a Renewable Resource: Extraction and characterization of the major components in Saccharina latissima’ in 2014. The raw data as well as the experimental results of the thesis were used in order to perform the substance flow analysis and the environmental assessment. The data included the flowchart of the extraction process as shown in figure 3, the type of chemicals that was used and the quantities mentioned on the flowchart and the description of the process.

A full literature review was required to establish a setting context for the research. Papers regarding biorefinery process, algae composition, SFA methodology and environmental tools were reviewed as a frame of reference during the study. Additional investigation methods include discussion with researchers related to the ‘Seafarm’ project as well as personal notes from meetings of the ‘Seafarm’ research team.

The spatial borders were defined by the laboratory sequence shown at figure 3. All the steps and streams from the raw material input to the output streams were included. Although no specific site was chosen for the process, the environmental assessment will be performed based on information and regulations from the Swedish environmental authorities (Naturvårdsverket). The information are in regard to the current environmental issues and the environmental objectives that have been established from the Swedish government. The temporal boundaries were required in order to calculate the flow of the streams. The available laboratory data were based on a 2 g dry algae input, hence an input flow of 2 g/s was established.

3.1 Substance flow analysis

In order to perform the substance flow analysis the comprehension of the various steps in the process is required. Additional literature around macroalgae content (Schiener et al. 2014) helped to acquire an insight of the process’ extracts and side streams. Subsequently the SFA methodology (Ayres & Ayres 2002, p91-101) is used to define the system and carry out the flow analysis. That type of analysis was selected since the analysis is at process level, hence the level of detail is high enough to follow the products and the reagents in each stream and by that to acquire the maximum amount of information.

The framework of SFA methodology is consisted of the following main steps: (i) the definition of the system, (ii) the quantification of the flows and (iii) the interpretation of the results. These steps entail a number of options that should be defined in the beginning of the analysis in order to meet the requirements of the study. (Ayres & Ayres 2002)
According to the above methodology the following steps were established in order to carry out the SFA:

1) Setting the system boundaries (spatial and temporal)
2) Identification of the substances
3) Identify the processes and the flows
4) Quantify the substances in each flow
5) Represent the flows and data graphically
6) Interpretation of the results

Defining the functional boundaries was the first task of the SFA. Although the concept of biorefinery is rather complex with multiple inputs and outputs, the specific case is a well-defined laboratory scale process with specific procedures. Hence, the process steps and streams are shown at figure 3. Since the process is not connected to a specific site or ecosystem, during the analysis it can be seen as an isolated system with completely defined input and output flows. Temporal boundaries, on the other hand, are not specified since most laboratory scale processes operate in batches. In order to overcome that problem and quantify substance’s flows, the inputs were defined as the mass or volume input during the experiments per time unit. The time unit that was selected was 1 second. Thus, the flows are measured in g/sec and volumes in cm$^3$/sec.

The following step is related to the identification of the substances. The key material is *Saccharina latissima* and the basic substance in order to scale up the process is the mixture of carbohydrates and in particular the extracts of mannitol, cellulose, laminarin and sodium alginate. Additionally to the carbohydrates, also the most important reagents as well as the water streams have been taken into account.

The next part of the analysis required the identification of all the different steps and flows of the process. For that purpose a detailed review of the experimental methodology and the characterization results of Öberg’s work was done. When it comes to the designing of the process a decision on the conditions of the first step need to be taken. The experimental process included batches of various temperatures and pH. In order to alter the pH values different reagents were used. The purpose of changing those parameters, as described above, is to examine the initial conditions that would lead to a better extraction and separation of the carbohydrates. According to the results of that Öberg’s work the yield of the process is maximized when the initial hydrolysis take place at a temperature of 40$^\circ$ C and a pH of 2. Thus, the analysis as well as the environmental assessments use those values.

The accounting type of modelling was used for the quantification of the flows since the SFA was based on the characterization results of the laboratory experiments. Thus, the numbers was used in order to calculate the separation coefficients of each step and the yield of hydrolysis. The calculations and visualization of the results were done in STAN2.5 software. This is a freeware software that was created specifically for substance flow analysis according to the Austrian standard ÖNorm S 2096.
The environmental assessment of the process is focused on the chemical used during the extraction. The risk assessment of the chemicals is held according to an algorithm developed by the Environmental Protection Agency (EPA) of United States. The software used for the assessment is using that algorithm for chemical process simulation for waste reduction and process design and is called WAR GUI. The input data in the software include all the input and output streams of the reagents according to the calculations from the SFA. The software has been used in many cases in order to determine the environmental performance of various processes. Some examples include the evaluation of a fermentation process for making penicillin (Hilaly & Sikdar 1995), the production of benzene from toluene (Smith et al. 2004) and ethanol generation from corn and sugar cane (Quintero et al. 2008).

In order to use WAR GUI software, in the current study, the first step required the input of the chemicals used in the extraction process. The chemicals were found in the database which is embodied in the software. During the second step the input and output streams were defined. It is important to mention that the same chemical may enter the process more than once in which case the quantities are added and a single input stream is considered. That is allowed since the point that a chemical enter the process has no effect to the Potential Environmental Impact (PEI). The same applies for substances that have more than one output, all the output of the chemical are added together. It should be also mentioned that not all the chemicals that enter the process have an output since chemical reactions occur. An example is the hydrogen chloride which enter the process but is neutralized in the most cases producing salts. Another parameter during the data input is that the program uses mass data which means that all the chemical streams were converted to mass flows. The input rate was grams per second (g/s) as used in the substance flow analysis.

The calculation of the PEI is performed based on the software and include the following categories: Human Toxicity Potential by Ingestion (HTPI), Human Toxicity Potential by Exposure (HTPE), Terrestrial Toxicity Potential (TTP), Aquatic Toxicity Potential (ATP), Global Warming Potential (GWP), Ozone Depletion Potential (ODP), Photochemical Oxidization Potential (PCOP) and Acidification Potential (AP).

The final part include the weighting of the impact categories. As mentioned before this is a feature that allows the evaluation of the categories according to the characteristics of a specific site. The weighting factor is used to normalize the results of each category which then will be added to create the total impact. Since PEI balance is a relative impact index, in our case the weighting of the impact categories would not have a substantial role. However, it was chosen to proceed including the weighting step in order to demonstrate the results according to the environmental objectives established from the Swedish environmental protection agency (Naturvårdsverket). That initiative defines 16 objectives regarding the environmental quality of Sweden and sets goals for future improvements in each objective (miljomal, 2015). Out of these objectives those which correspond to WAR algorithm categories were selected and studied in order evaluate each category and define an appropriate weighting factor. The selected objectives are the following:
• Reduced Climate Impact
• Clean Air
• Natural Acidification Only
• A Non-Toxic environment
• A Protective Ozone Layer

According to the latest report from the Swedish environmental protection agency only the last one is expected to achieve the desired goals and presents an environmental trend that leads towards the mitigation of this issue. Thus, the ozone depletion category is weighted with 1 while the rest categories have a weighting factor of 2. The only exception is the categories regarding human health risks (HTPI, HTPE) which are excluded since the current study examines only the environmental risks of the chemicals.

3.3 Theoretical Optimization

The third part of the analysis regarding the theoretical optimization is based on literature and include current solutions and methods that are used in industry in order to minimize costs and the environmental impact. That part may include the substitution of materials, stream recirculation or even changes in the process itself.
4. Background Studies

4.1 Eutrophication

As mentioned in the introduction, algae is capable of photosynthesis. It captures carbon dioxide and releases oxygen in the atmosphere. Apart from carbon dioxide algae also utilize nutrients that are found naturally in its environment. There is, however, an additional inflow of nutrients, such as nitrogen (N) and phosphorus (P), which ends up in the aquatic ecosystems. Those nutrients derive from chemicals that are used mainly for agriculture purposes since the increasing demand for food production and biofuel production from crops has led to the extensive use of fertilizers. As soon as these substances reach the coastal areas the ecosystem balance is disturbed and various interactions between the species in the food chain begin. That results to a phenomena known as eutrophication. (Cloern 2001)

Although aquatic ecosystems are dynamic systems and they generally absorb disturbances, the excessive and constant use of those substances during the past decades has stressed the ecosystems of the surround areas, sometimes in an extensive scale. Large aquatic ecosystems, such as the Baltic Sea, are congested by seaweed leading to bottom water hypoxia which have negative effects on the fauna (Karlson et al. 2002). As presented from Diaz, Selman and Chique at figure 1, eutrophication is a phenomena that has extended at global scale with a great proportion of the coastal areas suffering.

![World Hypoxic and Eutrophic Coastal Areas](image)

Figure 1. Hypoxic and eutrophic areas around the globe. (Diaz, Selman and Chique, 2011)
Mitigating an issue with such a global effect requires huge effort. Nutrient control is considered a necessity while various solutions have been proposed (Wang & Wang 2009). A rather alternative solution would be the removal of seaweed from the ecosystems using aquacultures. Cultivation and harvesting of algae would assist to the mitigation of the problem while the same time the collected material can be utilized unlocking algae’s great potential and offering a variety of possibilities as we are going to discuss in the following chapters.

4.2 Algae and potential utilization

The utilization of algae is not a new concept. Algae has been used for thousands of years mainly from Asiatic countries as food, in medicines or in their cosmetic products. Nowadays algae and its substances are still used in those industries. However technological advances have been able to uncover additional used of algae such as biofuel and biomaterial production. As a result, huge improvements have been made around the concept of algae cultivation. Aquacultures is one of the most rapid expanding food producing industries and the proportion of algae aquacultures grows bigger (Maisashvili et al. 2015). Brown algae as seaweed genre is divided in over 1800 species, however the main cultivated species in Europe nowadays are Saccharina latissima (sugar kelp) and Undaria Pinnatifida (Wakame) (Taelman et al. 2015).

Apart from a big variety of products and applications, algae also presents some important benefits when it comes to the cultivation itself. Algae does not compete with food production for arable land which means that those cultivations are free of social adversities related to food. Additionally, algae has a faster growing rate than land crops and grows in three dimensions instead of the traditional two dimension land cultivation. (Adams et al. 2009)

Another parameter in algae cultivation sites is their effect on marine biodiversity. Although the answer varies according to different cultivations and species, there have been expectations that the cultivation itself creates an artificial reef which will eventually increase biodiversity. (Ask & Azanza 2002; Wei et al. 2013)

Considering the above, algae cultivation seems as a promising solution for sustainable biofuel production, biomaterial production and numerous other applications. However, the cultivation and harvesting as well as the fermentation process itself have high cost nowadays. A lot of research regarding the optimization of different steps has been done (Richardson et al. 2014) and techno-economic analysis regarding the scalability of the production (Quinn & Davis 2015) still include high uncertainties.
4.3 Seafarm project

Since algae is abundant in the aquatic ecosystems and has been proven valuable in the modern society the next step include its efficient utilization. The ‘Seafarm’ project aims towards that direction taking into consideration the production of valuable products in an efficient and sustainable way. On figure 2 lies the general idea of ‘Seafarm’. The main steps include the cultivation of algae, the harvesting and preservation of the biomass, the biorefinery step, the biogas production and the use of the residue as fertilizers in land crops cultivation.

The whole project has divided in five focus areas which assess the various steps as well as the sustainability performance of the project. Focus area one (FA1), is related to the cultivation of algae in coastal areas. The exact locations and the structure of the farms are some of the parameters examined. The second focus area (FA2) intend to optimize the harvesting part as well as the transportation and preservation of the biomass. Next focus area (FA3) includes the biorefinery processes. Some of the processes are extraction of various substances, pre-treatment of the substances, if required, and conversion to valuable products. Focus area 4 (FA4) uses the waste stream of the biorefinery process in order to produce biogas through anaerobic digestion. The purpose of the last focus area (FA5) is to assess the sustainability performance of the project as a whole considering social, environmental and economic parameters. The current work is focused on the biorefinery step as shown in figure 2, hence the FA3. (Gröndahl Fredrik 2014)

![Diagram of Seafarm project](image)

Figure 2. The concept of ‘Seafarm’ Project
4.4 Potential use of brown algae

As mentioned above, macroalgae can be utilized for various purposes. The goal of ‘Seafarm’ project is to extract as much valuable products as it possible from brown algae. In order to achieve that, the biorefinery step plays a crucial role. By refining the biomass, carbohydrates can be separated from proteins and even combination of vitamins can be a potential product. There is a number of industries that may use the extracted substances as presented in the following chapters.

4.4.1 Biofuel production

Biofuel production is a great challenge of our times. Driven from the depletion rate and the unsustainable nature of fossil fuels, research on energy has took a turn to biofuels for some decades now. Although primary biofuels, such as wood, is a well-known energy source for men, their limited utilization in our modern life has dislocate them from the foreground. On the other hand, secondary biofuels derived from the processed biomass are much more adjustable fuels. (Dragone et al. 2010)

Biomass from vegetables and corn is the source for the so-called first generation biofuels. The production is mainly focused on biodiesel and bioethanol as well as biogas. What is used, basically, are oils and fats from vegetables that through a transesterification process produce biodiesel, and sugars from corn which are fermented from microorganisms to produce bioethanol. Although first generation biofuel production seems rather promising, it is however accompanied by important issues. The most significant one is that those processes share the same commodities which are also used for food production. Considering that, huge areas of arable land needs to be occupied for biofuel production leading to, in some cases, severe food shortages. With that being not acceptable and by changing the source for those processes we lead ourselves to the next generation of biofuels. (Naik et al. 2010; Dragone et al. 2010)

Second generation biofuels intent to use lignocellulosic biomass, the woody part of plants which do not compete with food production. Agricultural and forest harvesting residues or wood processing waste such as leaves, straw and wood chips are some of the raw materials that used during the second generation biofuel production (Naik et al. 2010; Dragone et al. 2010). Nevertheless, these biofuels have its own drawbacks as well. The complex polysaccharides that exist in those sources needs to be transformed in sugars in order to be ready for the fermentation process. That requires additional pre-treatment and processes that raise the cost. Although that generation fulfils the environmental and social criteria for sustainable biofuels, it is yet an uneconomic approach. (Dragone et al. 2010)

In order to overcome the above challenges a new organic source is required. A source that would not compete with agriculture and would provide an economically viable solution. With that in mind, a third generation of biofuels which utilize alternative feed stocks has emerged. New, specially engineered energy crops that promises to result in plants with properties more suitable for conversion into bio products is one possibility. The same time aquatic biomass is also considered. Research on renewable feedstock has increased
dramatically during the past decade, however only a 2.5% of nearly 30,000 papers is related to algae and oceanic biomass. The results so far indicate the existence of significant opportunities in terms of technology development for algal biomass utilization. (Kerton et al. 2013)

### 4.4.2 Biomaterials

The production of materials based on organic sources is another innovative solution that could decrease our environmental footprint. Polymeric materials can be natural polymers based on proteins and polysaccharides, semisynthetic polymers and synthetic polymers. Synthetic polymers are produced for many decades now and they are materials derived from petroleum industries. Natural polymers, on the other hand, are associated with renewable resources, hence they are characterized by biodegradability, availability and nontoxic nature. Bio-macromolecules such as polysaccharides are abundant in nature and present in all life forms and particularly in photosynthetic organisms like macroalgae. Biopolymers derived from organic sources can be used in various applications as adhesives, absorbents, lubricants, cosmetics, textile, good strength structural materials, etc. Brown algae could perform as an excellent source of carbohydrates for the production of biomaterials. (Zia et al. 2015)

### 4.4.3 Food additives

Another application that algae or its substances can be utilized is in food industry. Although seaweed is cultivated as food for centuries there have been researches for the potential use of algae substances as additives as well. Nutritional elements of seaweed such as proteins, lipids, carbohydrates, vitamins and minerals can be used for various products that consumed every day. Dietary fibers, for example undigested polysaccharides and alginate could be quite important due to their role in digestion process. (Holdt & Kraan 2011)

### 4.4.4 Medicine and Cosmetics

It is obvious that a number of industries are interested in algae extracts. Among them there are industries which traditionally use algae in their products. Medicine and cosmetic products include compounds that are found at seaweed such as polysaccharides and alginates. Algae in East countries is part of the culture and it is used for medicinal purposes for centuries. Nowadays, there has been a turn from artificial chemicals and people seek alternatives in many products. The same time advanced chemical analysis allows to
identify and isolate all the different compounds in algae making possible the commercial use of algae for multiple purposes. (Wang et al. 2014; Liu et al. 2012)

4.5 Chemical composition

Brown algae is an aquatic organism and as expected it is consisted from a large amount of water. The moisture content for most of the brown algae species is around 85%. The rest constitutes the dry mass which include carbohydrates, proteins, inorganic materials and traces of polyphenols. Carbohydrates are mainly polysaccharides with a small percentage of sugars. Polysaccharides such as alginate, laminarin, mannitol and cellulose compose a 63% of the dry mass in algae while proteins and amino acids are about 6% of dry mass. The remaining 31% are mostly inorganic substances such as phosphorus, nitrogen and metals. In addition, micronutrient analysis shows significant accumulation of Na, K, Ca, Mg, Sr and Fe in seaweed biomass as well as the non-metal iodine (I). (Schiener et al. 2014; Holdt & Kraan 2011)

Nevertheless, the above percentages may vary significantly according to the season in contrast to land crops which have a relatively steady composition. In the summer months it was observed a rise of the carbohydrate content with a simultaneous drop of the rest substances. Others factors that affect the biochemical composition are the environmental conditions and the maturity of the organisms. Keeping that in mind is of high importance since macroalgae may be utilized for different purposes according to its composition. (Schiener et al. 2014)

4.6 Extraction of Carbohydrates

There have been a lot of studies relating algae with biofuel production (Adams et al. 2009; Goh & Lee 2010; Kim et al. 2011; Fasahati, Woo & J. Jay Liu 2015) some of them focusing on the different pre-treatment methods and other on the fermentation optimization. Advances in chemical engineering has brought biorefinery processes into perspective combining it with algal biomass in order to produce a spectrum of valuable products.

Towards that direction, ‘Seafarm’ project integrates existing knowledge and ongoing research in a sequence of steps that most effectively utilize algal biomass. In order to achieve that, the different steps needs to be optimized and then integrated in the whole project. Research work on algae cultivation and harvesting, the biorefinery processes, the utilization of the side products as well as the remaining organics for biogas production is currently in progress. (Gröndahl Fredrik 2014)

Concerning the biorefinery step, research has led to different biorefinery industries regarding the different techniques used and the available feedstock (National Non-Food Crops Centre 2007; Schaidle et al. 2011). In relation to ‘Seafarm’ project there has been ongoing work on the extraction of carbohydrates from brown algae. Algal carbohydrates are of high importance because of their potential use in different products.
The sample that was used by Öberg were collected during late summer in 2013 on the west coast of Sweden. After harvesting, algae was mechanically crushed, mixed and kept frozen for transport and storage. The variables of the process was chosen to be the temperature and the pH of the initial extraction (step 1 as presented at figure 3). Three different temperatures and equal number of pH values were selected producing a total of 9 batches plus 3 control run tests. At figure 3 it is presented the flowchart of the process with the final products (laminarin, mannitol, sodium alginate and cellulose) as well as the reagents that was used. Streams A, B, C and D are side streams that include proteins, inorganic substances and reagents.

As mentioned above the main extracts of that process are alginate, mannitol, laminarin and cellulose.

Alginate is extracted as sodium alginate after the neutralization of the alginic acid with NaOH. Alginates are linear polysaccharides constituted by different proportions of two uronic acids (Andrade et al. 2004). Alginate salts form the cell wall of brown seaweed and therefore constitutes one of the main substances of algae. Alginate is used by various industries related to food processing, pharmaceuticals, feed and cosmetics. (Holdt & Kraan 2011)

Mannitol is a sugar alcohol which is found in many species of brown algae. Although it is present in small quantities and is heavily affected from seasonal variations, there is a big variety and diversity of applications that can utilize mannitol. Some of the industries that mannitol could be useful are pharmaceuticals, chewing gum, paint, paper, plastics and explosives. (Holdt & Kraan 2011)

Laminarin is another carbohydrate that exist in brown algae in significant quantities. It is a short polymer of about 20 - 25 glucose residues (Devillé et al. 2004). Laminarin, in contrast to the alginate and mannitol, has more limited industrial and commercial use, hence it is less valuable (Holdt & Kraan 2011). It is however easy to extract laminarin from brown algae by adding HCl since laminarin is not soluble to the most organic solvents (Devillé et al. 2004). The last carbohydrate that is extracted from brown algae is cellulose. Cellulose is a linear polysaccharide found in algae in very low percentages compared to wood (Song et al. 2015).
5. Results

5.1 Substance flow analysis

Substance Flow Analysis (SFA) is a specific brand of Material Flow Analysis (MFA) which deals with the analysis of the flows of specific chemicals in contrast to MFA which covers the analysis of bulk flows of materials such as wood, glass, plastics etc. (Brinzezu et al. 1997). Although the methodology is similar, the application of each tool may differ depending on the case. Typical example of SFA application is the studies on substance cycle in an ecosystem or a country. Nitrogen, phosphorous and various metals are some of the substances that have been studied extensively using a SFA (Finnveden & Moberg 2005). Nevertheless, regarding policy making SFA has been applied in both accounting and change-orientated studies (Finnveden & Moberg, 2005).

The designing procedure started with the main steps of the process (figure 3). Auxiliary steps such as centrifugation, settling and washing were also added in the flowchart. Next was filled the flows including the carbohydrate flows and reagents flows. The “I” indicates an input in the process and the “E” the output. The quantification part was done based on the characterization results of the experimental part of Öberg’s work. After filling all the flows with the different substances the software created the presentation of the quantified flows by the width of the arrows that connect the different steps as shown at figures 4 and 5. Figure 4 presents the process were flows are measured by the mass flow and figure 5 shows the flows by the volume flow. That separation was done through the restriction of the software to present mass and volume flows in the same flowchart.
Figure 4. Mass flow analysis of the extraction process (STAN2.5 software)
Figure 5. Volume flow analysis of the extraction process (STAN2.5 software)
In order to perform the substance flow analysis it is required to understand and analyze the process step and any physical or chemical steps that occur. The samples that were used for Öberg’s experimental process, as mentioned above, were collected from the west coast of Sweden in 2013. After the collection algae was mechanically crushed, mixed and kept frozen for transport and storage. Before the experiments the samples were thawed and dried for at least 48 hours at room temperature before each extraction. As seen on the figures the first step is the main extraction step, the hydrolysis of algae. At that step the algae sample is mixed with distillate water and HCl in order to create the acidic environment that required. Then the first centrifugation takes place and the aqueous supernatant is separated from the precipitate gel.

The gel contains a mixture of cellulose and alginate as long as other organic substances. In order to extract the desired carbohydrates water and dissolved Na₂CO₃ is added. Another centrifugation followed by a filtration step separate the alginate from cellulose. The solid phase with cellulose is washed with acetone in order to remove phenols and other organic residue and then washed again with water. The liquid phase after filtration is utilized to acquire sodium alginate. After the addition of HCl alginic acid is settled and another centrifugation separates the alginic acid from excess water. Then is added Methanol and solid NaOH in order to form sodium alginate. An additional centrifugation step generates a liquid waste stream and a heavier stream with sodium alginate. Finally a washing step with ethanol and methanol leads to a sodium alginate stream.

The supernatant phase from the hydrolysis contains the carbohydrates laminarin and mannitol. First the stream is neutralized adding solid Ca(OH)₂ and then the excess water is evaporated. More HCl is added in the mixture and after centrifugation the heavier part that includes proteins and amino acids is separated from the carbohydrates. In order to separate mannitol and laminarin ethanol is added. Mannitol is diluted in ethanol and centrifugation provide a stream which is neutralized with addition of Ca(OH)₂. Ethanol is removed and the stream is washed with acetone. The second stream is laminarin which is washed with methanol.

During the first step of the process it is used distilled water as mentioned before, however it is not specified if the rest of the water used in the process is also distilled. In any case, it is assumed that all the water that is used is distilled or at least it has low mineral content. The steps where HCl is added refer to a solution of HCl with molarity of 0,5 M. The calcium hydroxide as well as the sodium hydroxide are always added in solid phase. Acetone, ethanol and methanol are in liquid form.

It is important to mention that the product streams that involve the carbohydrates are not pure streams. An amount of the reagents accompany those streams and further purifications steps are required. In addition, traces of various carbohydrates is found in the product streams indicating the difficulty of complete separation of the substances (Öberg 2014). Due to luck of data, however, it is hard to determine the extent of those impurities and therefore the product streams were considered pure during the SFA calculations.

The four side-streams, named as F10, F42, F52 and F56 in figures 4 and 5, are also important for the scaling up of the extraction process. Although the process is focused on the extraction of carbohydrates, it results to the extraction of other substances of macroalgae as well. The stream of the protein and amino acid mix, for example, can be processed further and used for various applications as we described in the introduction. Another important element that will be analyzed at the theoretical optimization chapter
is the potential recirculation of those side-streams in order to enrich the product streams or to recover chemicals.
5.2 Environmental assessment

5.2.1 Assessment of chemicals

During an environmental analysis there is a big variety of tools that can be used according to the nature of the examined system and the object of the study. More specifically the object of the study can be categorized in one the following groups:

- Policies, plans, programs and projects
- Regions or nations
- Organizations, companies
- Products and services
- Substances

Once the object of the study has been specified, a distinction between attributional and change-oriented study needs to done. This is an aspect that defines the goal of the study and affects important decisions regarding the selection of the appropriate environmental tool and the boundaries of the analysis. An attributional study, basically, examines a system or product at a current state and attributes potential impact and consequences that derive directly from the system or product. On the other hand, a change-oriented study does not focus on a specific system but investigate the consequences of a decision. In the change-oriented case the boundaries normally extend beyond the system itself and take into account future repercussions as well. (Finnveden & Moberg 2005; Curran 2002)

The current study involve a laboratory scale procedure of a chemical extraction process. The goal is to examine the potential environmental impact of the process, hence it is an attributional study of a chemical process. The resources are excluded from the study, thus only the environmental impacts are going to be assessed.

Considering the above, the assessment is targeted on the chemicals used and their potential impact in the environment. In risk assessment of chemicals the dispersion of chemicals is often planned and forms part of its use. Methods and protocols for risk assessment of chemicals have been developed in several international fora, e.g. EU and OECD. An exposure assessment including a description of nature and size of exposed targets, as well as magnitude and duration of exposure, is combined with an effect assessment. The aim can be to define target values or acceptable risks. (Finnveden & Moberg 2005)

The chemicals that are involved in the analysis are the following:

- HCl – Hydrogen Chloride
- NaOH – Sodium Hydroxide
- CH₃OH – Methanol (MeOH)
- (CH₃)₂CO – Acetone
- C₂H₅OH – Ethanol
- Ca(OH)₂ – Calcium Hydroxide
During the laboratory experiments reagents were released into the environment since the quantities were negligible. In an industrial scale extraction though that would not be the case. In the current study, however, all the chemicals will be examined as potential stressors to the environment considering also the risk of release. In the next chapters follows and introduction of the above chemicals including their physical and chemical properties, commercial and industrial applications as well as effects in human health and the potential environmental impact.

5.2.1.1 Hydrogen Chloride – HCl

Hydrogen chloride solutions are used in numerous processes in various industries as well as during maintenance procedures. Some of its uses are in chemical industry for manufacturing of phosphoric acid, chlorine dioxide, ammonium chloride, vinyl chloride from acetylene, alkyl chlorides from olefins and fertilizers and in metallurgy industry during the production of tin and tantalum, as a lab reagent, and as a metal treating agent. Another application is as a neutralization agent in waste waters and to maintain pH balance in swimming pools, spas, etc. Hydrochloric acid is also used a cleaning agent to disinfect household areas and industrial buildings such as hospitals.

Hydrochloric acid is the solution of hydrogen chloride in the water and is quite corrosive, thus it needs to be handled diluted in water. Contact with metals produces hydrogen gas while in a case of fire the poisonous chlorine gas is emitted. Thus, the gas form of hydrogen chloride is non-flammable. Apart from water, it is soluble in benzene, alcohol, and ether but not in hydrocarbons. It reacts with almost all metals except mercury, silver, gold, platinum, tantalum, and certain alloys.

Hydrogen chloride can be found as colorless liquid or colorless to slightly yellow gas. Its solubility in the water is quite high forming a solution of hydrochloric acid. The molecular formula is HCl with a molar mass 36.46 g/mol and other common names are hydrochloride and spirits of salts. Hydrogen chloride has melting point is at -114.24°C, boiling point at -85.06°C and its density is 1.49 g/cm³.

Hydrochloric acid is a strong acid with both health and environmental risks once released in considerable quantities. Human exposure to concentrated solutions can cause circulatory collapse and asphyxia which may lead to death. Less concentrated solutions generate a number of effects such as inflammation, ulceration of the respiratory tract, skin burns etc. The environmental effects can be acute as well. Hydrochloric acid is toxic for all forms of life since it has a great effect to the pH value of the ecosystems. It is also a component of acid rain, thus it accelerates the decay of limestone buildings and other structures. Additionally hydrogen chloride contributes to the processes that cause photochemical smog. (Department of the Environment, 2015)
5.2.1.2 Sodium Hydroxide – NaOH

Sodium hydroxide is an inorganic substance also known as caustic soda and soda lye. It appears a white solid that can absorb moisture from air and generate heat. The solution of NaOH is used widely in industries and has a pH value higher than 7. The molecular formula is NaOH and has a molar mass of 39.997 g/mol. The density is 2.13 g/cm³ and the melting and boiling point is at 318 °C and 1,388 °C respectively.

Sodium hydroxide is highly corrosive and a powerful irritant once it make contact with human skin or inhaled. It can cause severe burning in the skin, eyes and lungs at high concentrations.

Sodium hydroxide is designated as hazardous substance for surface and ground water since it affects the normal pH value of the ecosystems leading to the death of marine life. Soil contamination might be also dangerous in case that sodium hydroxide is not neutralized after reaction with organic materials in the soil. (Salocks & Kaley 2003)

5.2.1.3 Methanol (MeOH) – CH₃OH

Methanol is volatile and highly flammable organic compound which mixes with water and many organic liquids. It is used broadly as an industrial solvent and as reagent for various chemicals. Other applications are as antifreeze or in paint strippers, aerosol and non-aerosol spray paints.

Methanol is found as a clear, colorless liquid with characteristic odor. Its molecular formula is CH₄O with a molar mass 32.04 g/mol and a density of 0.79 g/cm³. The melting point is at -97.6°C while the boiling point at 64.7°C. Other common names for methanol are methyl alcohol, wood alcohol and wood spirits.

Methanol may enter the human body by inhaling fumes or by absorption through the skin causing irritation of the eyes, nose, mouth and throat as well as vomiting, nausea, liver damage and muscle pain. Exposure to high concentrations might also lead to death. Environmental exposure to methanol may have various effects in animals, fishes and plants, though it does not accumulated in organisms. It is not associated with any major environmental issues. (Department of the Environment, 2015)

5.2.1.4 Acetone – (CH₃)₂CO

Acetone is mainly used as solvent in various industries for fats, oils, waxes, resins, rubber, plastics, lacquers, varnishes and rubber cements. Other applications include the manufacturing of chemical compounds, photographic films, plastics, fibers and drugs.

Acetone is another solvent with similar properties with methanol. The molecular formula is C₃H₆O and the molar mass 58.08 g/mol. The melting point is at -94°C, the boiling point
is at 56.5°C and its density 0.79 g/cm³. It is volatile liquid, highly flammable and mixes with mixes readily with water, alcohol, dimethylformamide, chloroform, ether and most oils. Other common names are dimethyl ketone, methyl ketone, 2-propanone, B-ketopropanone, dimethylformaldehyde and pyroacetic ether.

Acetone is a fire and explosion hazard once mixed with air and corrosive to iron and steel in the presence of moisture. It is also capable of dissolving plastic glasses frames, jewelries, pens and pencils, rayon stockings and other rayon garments.

Acetone can enter the human body by inhaling fumes or by contact with eyes and skin causing nausea, vomiting, drowsiness, unconsciousness and possibly coma. Kidney, liver and nerve damage occur in animals after long-term exposure.

Acetone has a slight toxic effect when exposed to aquatic life. It can cause membrane damage, a decrease in size and decrease in germination of various agricultural and ornamental plants. However it is not expected to accumulate in plants, animals or humans. Acetone can be transferred through the atmosphere and is also capable of moving into the groundwater from spills or landfills.

However, acetone is common substance in many domestic products. It is found in aerosol paints, architectural coatings, automotive and machinery paints and primers, furniture polish and cleaners, household hard surface cleaners, laundry pre-soaks, pet flea and tick removers, cockroach treatments, laundry starches, lubricating greases and oils, nail enamel and polish and polish remover, particleboard, paints (including interior clear finishes, undercoats and primers), varnish, paint and varnish removers and thinners, liniments for veterinary preparations, pharmaceutical preparations, shoe polish, sun tan lotions and oils, and in wood office furniture. (Department of the Environment, 2015)

5.2.1.5 Ethanol (EtOH) – C₂H₄OH

Ethanol is considered volatile and mixes with water and many organic substances. It is present in alcoholic drinks and it is used for pharmaceutical purposes, such as disinfections. Other applications are in cosmetics and in perfumes. It is an industrial solvent for fats, oils, waxes, resins, and hydrocarbons and it is used in the production of various chemical compounds.

Ethanol is one more organic substance which found as clear, colorless liquid. Like methanol and acetone, it is a good solvent and high flammable. Its molecular formula is C₂H₆O and it is also known as ethyl alcohol. Ethanol has molar mass 46.06844 g/mol and a density 0.79 g/cm³. The melting and boiling point is at -114°C and 78.5°C respectively.

Ethanol is relatively harmful in case of ingestion inhalation or by skin absorption, however at high concentrations it might cause dizziness and irritation to the eyes, skin and nose. Concentrations below 1000 parts per million (ppm) usually produce no signs of intoxication.

Ethanol can be transferred through water or air and can have an effect on a wide range of biota. In addition, ethanol act as contributor to the formation of photochemical smog. It is, however, oxidized quickly with carbon dioxide and water to other substances. Ethanol
is a good nutrient and energy source for microbes. In the absence of oxygen, this can lead to the formation of methane. (Department of the Environment, 2015)

5.2.1.6 Calcium Hydroxide – \( \text{Ca(OH)}_2 \)

Calcium hydroxide is used broadly in many industries such as in steel manufacturing and in pulp and paper industry. Additionally there are many environmental applications where calcium hydroxide is used for air pollution control and for treatment of sewage and industrial sludge. Another use is as a neutralization agent for phosphorous and nitrogen in wastewater.

Calcium hydroxide inorganic compound and is found as dry powder and is formed after the addition of water to calcium oxide. The molecular formula is \( \text{Ca(OH)}_2 \) and has molar mass 74.093 g/mol and density 2.343 g/cm³. The melting point is at 580° C, however a boiling point could not be determined since it disassociates when heated after the melting point.

Calcium hydroxide is transferred through water, hence the environmental assessment is focused on aquatic ecosystems. It is, however, slightly mixed with the water and reacts with \( \text{CO}_2 \) forming calcium carbonate. Calcium carbonate is incompatible with strong oxidizing agents, for example fluorine, boron trifluoride, and oxygen difluoride, and may lead to fire or explosion. Both calcium and hydroxides are abundant in nature and present low toxicity for the ecosystems. Hence, it is considered to be little risk associated with the presence of calcium hydroxide in the water environments. (Ecology 2005)

5.2.1.7 Sodium Carbonate – \( \text{Na}_2\text{CO}_3 \)

Sodium carbonate main use is at glass industry while other applications are in detergent powders and tablets for water softening in the washing process, in laundry additives, machine dishwashing products, surface cleaners, toilet cleaners and other household cleaning products.

Sodium carbonate is an inorganic compound which is found as white, crystalline hygroscopic powder. Its molecular formula is \( \text{Na}_2\text{CO}_3 \) and has a molar mass of 105.9888 g/mol. The melting point is 851°C but no boiling point can be determined since it decomposes when heated. The density at 20 °C is 2.532 kg/m³.

In water, sodium carbonate dissociates into sodium and carbonate increasing the alkalinity of the system and the pH value. Consequently the carbonate ions react with the water, resulting in the formation of bicarbonate and hydroxide. The ions are not transferred to the air, hence the environmental impact is focused only in aquatic ecosystems. Both the ions, though present low toxicity and have no adverse effect on the ecosystem. (No 2007)
5.2.1.8 Calcium Chloride – CaCl₂

Calcium chloride is an inorganic compound and is found as white powder. Its molecular formula is CaCl₂, and has a molar mass of 110.98 g/mol. The melting point of calcium chloride is 772°C while the boiling point is higher than 1600°C. The density is 2.16 g/cm³ at 25°C. Calcium chloride is used broadly in many industries as deicing agents (deicers), for road stabilization and dust control, for industrial processing, as accelerator in concrete and for oil and gas well fluids.

Calcium chloride is soluble in water and dissolves into ions, hence the environmental assessment is focused on the aquatic ecosystems. In case of soil contamination calcium binds to soil particulate or may form stable inorganic salts with sulphate and carbonate ions. Although there have been reports about soil toxicity there are not any available data to indicate any toxic results. Calcium chloride is not considered to accumulate in living organisms. (Sids et al. n.d.)

5.2.2 Waste Reduction Algorithm

In order to assess the above chemicals from the extraction process a software tool called WAR GUI was used. The software implements an algorithm called WAR, which stands for Waste Reduction, and was developed by environmental protection agency (EPA) scientists in order to evaluate environmental impacts of processes that use chemicals. The goal of the WAR program is to assist to the reduction of environmental and related human health impacts at the design stage. As shown in figure 6, WAR algorithm focuses only to the manufacturing process without considering other steps of the production process and the life cycle of the product. (U.S. Environmental Protection Agency, 2011)
The indicator used by the software is the Potential Environmental Impact (PEI) which is the effect of a chemical if it were emitted into the environment. The PEI, basically, quantifies the impact of the pollutants of a process providing a quantitative indicator of the environmental friendliness or unfriendliness of a manufacturing process. Thus it is a relative quantity. (Cabezas et al. 1999)

The software can evaluate the PEI of the streams entering the process, the PEI generated from the process as well as the PEI of the streams leaving the system. In figure 7 there are some of the indicators determined from WAR algorithm regarding a manufacturing process. A distinction of the indicators can be made between the energy production system and the chemical process. (Young et al. 2000)

However, the energy generation step was skipped since the study does not include an energy assessment of the process. In that case the flowchart in figure 7 changes in order to include only the chemical process and the indicators are delimited to the total PEI entering the system, the total PEI leaving the system and the total PEI generated within the system.
The chemical process include the PEI that enters the process and the PEI that leaves the system. The same applies for the energy production process. There are also the rates of waste energy emissions from both systems as presented in figure 7. The following list presents those rates and their symbols. (Young et al. 2000)

\[ l_{in}^{(cp)} \]: the PEI entering the chemical process (PEI/t)
\[ l_{out}^{(cp)} \]: the PEI leaving the chemical process (PEI/t)
\[ l_{in}^{(ep)} \]: the PEI entering the energy generation facility (PEI/t)
\[ l_{out}^{(ep)} \]: the PEI leaving the energy generation facility (PEI/t)
\[ l_{we}^{(cp)} \]: the PEI emitted from a chemical process associated with the waste energy (PEI/t)
\[ l_{we}^{(ep)} \]: the PEI emitted from an energy generation facility associated with the waste energy (PEI/t)

Another rate that is considered in a chemical process is the generation rate of PEI. Since there are chemical reactions, new compounds derive from the system while other are transformed, hence there is generation rate defined as following:

\[ l_{gen}^{(l)} \]: the total PEI generated within the system (PEI/t)

By looking the system as whole the following indicators can be used.

\[ l_{in}^{(l)} \]: the total PEI entering the system (PEI/t)
\[ l_{out}^{(l)} \]: the total PEI leaving the system (PEI/t)
The total PEI that leaves the system can be calculated from the equation 1 according to the potential environmental impact theory. (Young et al. 2000)

\[ I_{\text{out}}^{(t)} = I_{\text{in}}^{(t)} + I_{\text{gen}}^{(t)} \quad (1) \]

The calculation of the total PEI is based on eight categories as mentioned in chapter 3, two of them involving human health risk and the rest referring to environmental impacts. The impact categories are the following:

- Human Toxicity Potential by Ingestion (HTPI)
- Human Toxicity Potential by Exposure (HTPE)
- Terrestrial Toxicity Potential (TTP)
- Aquatic Toxicity Potential (ATP)
- Global Warming Potential (GWP)
- Ozone Depletion Potential (ODP)
- Photochemical Oxidation Potential (PCOP)
- Acidification Potential (AP)

In case that the chemicals and the rate of those chemicals entering and leaving the process are known, the WAR algorithm calculates the impact of each chemical in each impact category. The results of that calculation are shown in a graph which relates the PEI for each category as well as the whole process. With the weighting option of the categories the results can be focused on specific impact or impacts. That can be applied for example in case studies where the locations is important and the potential impact in a particular category or group of categories would be more significant.

After the completion of all the steps the graph in figure 8 was generated by the software.
The index presented in the graph is the output rate of the potential environmental impact which have the chemicals used in the extraction process. It is presented the PEI in each one of the impact categories as well as the total PEI after the normalization. Table 1 shows the exact PEI rate values as calculated from the software for each impact category. The values are before normalization, thus the sum is not equal to the total PEI. The complete report extracted from WAR GUI can be found at the appendix II.

Table 1. Output PEI rate of each category

<table>
<thead>
<tr>
<th>Impact Category</th>
<th>PEI/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTPI</td>
<td>10.30</td>
</tr>
<tr>
<td>HTPE</td>
<td>0.070</td>
</tr>
<tr>
<td>TTP</td>
<td>10.30</td>
</tr>
<tr>
<td>ATP</td>
<td>0.006</td>
</tr>
<tr>
<td>GWP</td>
<td>0</td>
</tr>
<tr>
<td>ODP</td>
<td>0</td>
</tr>
<tr>
<td>PCOP</td>
<td>41.8</td>
</tr>
<tr>
<td>AP</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>104.3</td>
</tr>
</tbody>
</table>
From both the table and the graph we can extract some conclusions regarding the environmental impact of the chemicals. First, the PEI rate in many of the impact categories are either zero or practically negligible. The only categories that present a significant potential are the terrestrial toxicity potential and the photochemical oxidation potential. According to the complete chemical report the photochemical oxidation potential is attributed exclusively to the organic solvents methanol, ethanol and acetone since they are volatile liquids and contributors to photochemical smog. The terrestrial toxicity on the other hand is affected by all the chemicals considered with the calcium chloride holding significantly the biggest share. It is clear that with the neutralization of the acids the PEI output is acidification free while the salts produced have no significant impact, especially considering the low quantities they produced. Thus the majority of the PEI output lies on the solvents methanol, ethanol and acetone which have a considerable impact to the environment and used in high quantities.
5.3 Theoretical optimization

As mentioned in chapter 2 this work aims to provide information regarding the scaling up of the extraction of carbohydrates at an industrial level. One the most challenging steps during a transition from a laboratory scale process to an industrial one is the designing of the process. That step may include economic, environmental and technical aspects.

The current chapter has a twofold aim. The first is to provide recommendations regarding the optimization of the process focused on the production. Since further experimentation is no option the optimization would be performed based on the results of Öberg’s work. The second aim is to suggest solutions regarding the handling of the chemicals in the process. Those suggestions would be based on a cleaner production thinking as we are going to discuss later.
5.3.1 Production optimization

Optimization of a chemical process is a rather complex task which on the one hand requires extensive knowledge of the particular process and on the other hand a relative experience of industrial processes. This chapter does not attempt to provide a full production optimization rather than to analyze some observations that was made during the review of Öberg’s work and the substance flow analysis of the previous chapter.

The first suggestion regards the preparation of the samples. In the experimental procedure it is mentioned that algae is dried out for 48 hours in room temperature before the extraction process. With that pre-treatment is achieved a reduction of the moisture content from an average 85% to a 7%. The water content reduction has been proposed at ‘Seafarm’ project as a technique for a more efficient transportation of algae after the harvesting. However, that treatment could aim to a 50 – 60 % reduction in order to increase the quantity of algae during transportation and decrease the cost. In the experimental samples though the motive of that pre-treatment is not stated. Additionally in an industrial scale a drying process in room temperate might be time demanding or even space demanding. Alternatively an increase of the temperature in a controlled environment in order to accelerate the process may require a lot of energy. Another interesting point is that the first step of the process includes the addition of distillate water. In case that the drying process is delimited, the water content of algae is increased and the addition of water is reduced. In laboratory scale the water input is not of high importance but at industrial scale the water quantity that needed for a processes is highly evaluated.

The second recommendation is focused on the side streams of the process. During the process there are 4 different streams (A, B, C and D in figures 4 and 5) which characterized as waste streams and streams with unwanted substances. Some of those streams, however, include the carbohydrates such as laminarin, mannitol and sodium alginate. Consequently that results to a decrease to the extraction yield. A countermeasure that can be applied in that case is the recirculation of those streams in different steps in the process in order to enrich the product streams and increase the yield (figure 9).

The side stream A contains proteins and an amino acid mix which is dissolved after the HCl addition. However, it was verified after NMR analysis that there is a quantity of mannitol. This quantity could be decreased with recirculation of that stream back to the dissolving step where it was produced. That way the product stream is enriched in mannitol. The side stream B include carbohydrates as well. In all the batches that were tested it was found quantities of laminarin and mannitol. Those carbohydrates could enrich the product stream F13, as shown in figure 9, and increase the yield of both mannitol and laminarin. However that stream includes also methanol which could not be recirculated back to the product stream. Thus, the side stream B needs first to be stripped out of the solvent which will be recovered and reused as we are going to discuss in the next chapter. On side stream C it was also found traces of mannitol but alginate as well. Although stream C could be recirculated back to stream F53 at the washing step the quantities of carbohydrates are not high enough and further data required in order to check if that recirculation would lead to a significant increase of the yield. The side stream D contain inorganic residue from algae and is free of carbohydrates according to the NMR analysis. Thus no action need to be done with that stream.
Figure 9. Recirculation of side streams
5.3.2 Cleaner Production Applications

The current chapter considers the concept of cleaner production in order to give recommendations of how the water and the chemical could be handled throughout the process. Cleaner production in general aims to present technical possibilities to minimize emission problems and deal with waste problems. (Persson, 2011)

One of the objectives towards that direction is the water and its efficient use in the industrial processes. Industries today use great amounts of water every year making water an expensive material. Thus it is strongly recommended the recycling of water for reuse in the same process.

In that case water can easily be recycled since the output of the water are quite clear from effluents in the most cases. The reason for that is that most water outputs derive from evaporation process and cannot carry any salts that are diluted. However some water outlets may have a pH higher or lower than 7 which is a result of ions diluted in the stream. Due to lack of data it is not sure which streams are those and how the pH has altered. If that is the case though it is quite easy to neutralize those streams and remove the salts in order to reuse the water. By that the water input in the system can be eliminated reaching practically zero.

The next recommendation that can be applied in the process is related to the chemicals. The effluents of the process are not many but they should be treated properly. Among them those with the highest environmental impact are the organic solvents methanol, ethanol and acetone. The rest include salts and ions that can be easily treated. The organic solvents, however, are volatile and can escape into the atmosphere contributing to the photochemical oxidization as shown in the chapter 4.2. The recommendation in that case is the recovery of the solvents from the streams and reuse in the process. The solvents are used mainly during washing steps and they leave the process as waste streams. Due to the lack of data though it is difficult to define what substances those streams include and how easily can the solvents stripped from the waste streams.

Another topic of discussion is around the selection of chemicals and more specifically the acids. There have been research on heterogeneous catalytic organic reactions where the use of solid acids seems to have great advantages. Mesoporous high surface area materials which act as acids play a significant role in green manufacturing. Those materials can be designed to provide different types of acidity as well as high degrees of reaction selectivity and they are widely used in large scale petrochemical processes as well as pharmaceutical chemicals manufacturing. Using solid acids the product isolation is simplified and the acid can be fully recovered. Additionally there is no need for neutralization factors which lead to salts or other hazardous waste. (Clark 2002)
6. Discussion

Exploitation of seaweed for various purposes has been applied even from ancient times. Today that industry can be evolved and promote both economic and environmental goals by providing a wide range of materials used in many products. The current work is entailed in ‘Seafarm’ project which aims towards that direction. The extraction of the raw materials for a large variety of industries as well as the mitigation of eutrophic and hypoxic ecosystems are only some of the objectives of the project. The focus of the master thesis lies on the extraction of substances, more specifically carbohydrates, and analyze a proposed process through a SFA and an environmental assessment of chemicals. In addition, it is attempted to propose measures and solutions for an increase of the yield and recommendations for a cleaner production. The final aim of the work is to put the basis and perform the initial steps for scaling up that process at an industrial level. During this chapter, the methodology and the results of the thesis will be discussed and comments related to uncertainties will be given.

The fact that the borders and most of the substances were well defined made it possible to analyze the process at a detailed level considering not only the product streams but the various reagents and the water content as well. That assists to both the analysis of the substances in the process as well as the environmental analysis. The borders, however, may not be so well distinct in a biorefinery step with many processes being integrated and interconnected. In that case new problems may arise, such as the need for products free of chemical impurities which might be harmful for other processes. On the other hand, existing issues could be resolved and unwanted side streams could be useful in another process in the biorefinery. From an engineering perspective, however, the flow analysis of a process is a necessary step at the early stages of the designing.

The input data was a problematic area of the study due to lack of the information for some chemical quantities and the qualitative characterization of the product streams. A typical example is the neutralization steps of the acids (figure 4) where the quantity of the neutralization factors is found after solving the equation of the neutralization reactions. In general, the primal analysis included the understanding of the reactions taking place in each case considering also chemicals from previous steps that remain in the stream. That was important also during the evaluation of the side streams which included a variety of substance.

The selection of algae’s flow rate is the system is an assumption that needs to be mentioned. The samples of algae in the developed process were 2 g, thus the flow rate selected to be 2 g/s. That inflow gives a total of 173 kg of algae processed every 24 hours. Since processes of a higher scale provides a lower yield in comparison to the same process in laboratory scale, an additional adjustment of the yield results from the SFA is required. Although that number does not correspond to an industrial scale process, it is a good indicator of the production potential avoiding big deviations from the laboratory process. This is important in the case of an economic or energy assessment where the energy return on investment is measured.
Although that in the SFA results it is not shown that the product streams include reagents or water, those streams are far from pure. Those uncertainties concerning the purity of the product streams derive after the characterization of the product and side streams. According to the results of Öberg's work the impurities include salts, volatile solvents, water and ever carbohydrates from other product streams. However the characterization was qualitative and the quantities were not determined, thus the SFA could not include those impurities. It is logical to assume that at an industrial level the impurities percentage would be much higher, however a product evaluation as well as an economic assessment can reveal if this is affordable.

Carbohydrates may exist in the reagents streams causing losses of product and decreasing the total yield of the process. More losses include reagents which cannot be fully recovered. Chemical recovery should be focused especially on volatile organic solvents which, as shown, hold the biggest part of environmental stress.

An assumption that needs to be mentioned involves the centrifugation of the stream F13 (figure 5). It is considered that stream F22 include only laminarin and a quantity of water while stream F14 consists of the ethanol and HCl and mannitol. In reality the water content in F22 is also acidic due to H⁺ ions that are diluted in it. However, there are not any information about the quantity of HCl that is found in the water, hence it is assumed that all the HCl is on F14 where it is neutralized with Ca(OH)₂. The equations of the chemical reactions as well as the calculations for the chemical requirements can be found in the appendix I.

Regarding the environmental performance of the process, the environmental tool was established after considering the size of the system and the impacts. Since the impacts of the natural resources and economic aspects were excluded, a risk assessment of the chemical substances was selected. The WAR algorithm that was used by the software assigns a performance indicator for each chemical based on its potential environmental impact. Therefore the results of the environmental assessment are qualitative and present the performance of a chemical in relation to another as well as their share in the total impact of the process. On the other hand, however, the tool gives the possibility for a comparative analysis where a variety of chemicals may be tested in order to examine the reduction of the total impact.

The indicator that is used for the chemicals is the potential environmental impact or PEI (figure 7). In order to calculate the PEI of a chemical substance it is simply considered that the total quantity of the chemical is emitted in the environment. An interesting point is that for the calculation of the total impact only the output streams of the process are considered. That is an important assumption since substances that are transformed into the process are not included in the results. An example is the input of acids and bases in the process which are neutralized creating salts. In that case the process is acidification free since there is no output stream that includes any acid or base. Consequently the results could not be used to assess impact of an accident. In addition to that, the environmental assessment would present different results in relation to the impact categories. For that reason, an additional literature review of the risks of all the chemicals in the process was performed.

Another parameter of the environmental performance results that should be discussed is the impact categories and their weighting as mentioned in sub-chapter 5.2.2. The software gives the possibility of assessing the chemicals in various categories which are mentioned in chapter 3.2. However the two categories which refer to health risk
assessment where excluding from the graph since the assessment of the human health risk does not belong in the objectives of the thesis. However there are results for those categories in the full report that was extracted from the software which can be found in the appendix II. The rest impact categories received a weighting factor of two except the ozone depletion category which received a factor of one. The weighting process took into consideration the environmental goals that have been established by the environmental protection agency of Sweden. In case of an alternative location with specific environmental demands, different weighting factors may be applied providing with different results. Thus it is important to justify the weighting of the categories according to the site of the process.

The results of the risk assessment indicate that the terrestrial toxicity (TTP) and the photochemical oxidization (PCOP) constitute almost exclusively the total impact of the process. According to the full report, all the chemicals in the process have an impact to the terrestrial toxicity with calcium chloride to hold the biggest share. The photochemical oxidization category, on the other hand, is affected exclusively from the volatile organic solvents. Considering that the PCOP category is responsible for around 80% of the total impact, it becomes clear that the organic solvents is the weak spot of the process and attracts the attention regarding the environmental performance of the process.

Considering the nature of the process and the selection of the chemicals one can see that there has been a careful study of the reagents used. According to the analysis, the neutralization process is a quiet important step and limits the environmental risks at a great extent. The weak point of the process which is the organic solvents is probably inevitable and the focus should be on the reuse of the solvents. However further analysis after the integration of the process in the biorefinery could reveal more weak spots.

The last part of the study regarding the theoretical optimization of the process include some interesting points as well. It is mentioned that there could be a recirculation of various side streams in order to increase the yield. An important parameter that should be considered is the impurities that are included in the recirculated streams. In the recirculation proposed on figure 9 the side streams include mainly salts which generally do not affect the products or the chemicals. However, other impurities may also be in those streams such as organic solvents. In that case it needs to be verified first that the product streams will not be affected. This is an important step in order to examine the quality of the final product.

Another point concerning the increase of the yield is the economic value of the products. Since there is no need for very pure products, a combination of products may be not only acceptable but even desirable. Ingrid Undeland, Professor at the Chalmers University of Technology, talks about multi-valued food ingredients aimed at different uses while Ulrica Edlund, Professor at the Department of Fiber and Polymer Technology at KTH suggest that we should settle for just a few valuable substances rather than extracting masses of them. Thus an economic assessment considering the industry demand and market values is required before the establishment of recirculation streams for the increase of the yield.

Finally it should be clarified that the results of the study assist in the scaling up process without those steps being the actual scaling up process. That means that neither the input data of the analysis nor the results are referring to an industrial process. On the contrary, the results are based on the laboratory process. Their importance is however high since they indicate the basic steps of the process as well as its weak spots and the improvement areas.
7. Conclusions

The current work include the initial steps in order to scale up the process providing results and information regarding the next steps of the analysis which should follow in order to fulfill the extraction of carbohydrates at an industrial scale. In order to do that a substance flow analysis and an environmental assessment of the chemicals in the process were performed. Additionally, an identification process of the major improvements areas lead to recommendations regarding the theoretical optimization of the process.

Through the SFA the main product streams and the basic steps of the process were pointed out. This is an important step that could assist future studies by presenting the process as well as product and auxiliary streams. The environmental assessment focused on the chemical selection and presented the environmental performance of the chemicals in relation to the total performance of the process. It became clear that the organic solvents constitute the biggest problem in the process’ performance and provided results that could be used in a comparative analysis in regard to chemical substitution. In the last part of the study recommendations for both the production process and the environmental performance of the process were developed. Thus it is considered that the work completed its aim through the objectives that were established at chapter 2.

The fore mentioned results does not correspond to the scaled up industrial process hence they are not representative for the industry. More steps are required including the integration of the process in the actual biorefinery step. Nevertheless they constitute a basis of work where further research needs to done.
8. Recommendations for future studies

This chapter focuses on further research that can be done in order to assist in scaling up the extraction of the carbohydrates from *Saccharina latissima*. These recommendations consist of the continuous work of that master thesis and include more analysis towards the scalability of the process at an industrial level.

A part of the following analysis include the energy assessment of the process. This is important in order to determine the potential costs in connection to the size of the process. There are various tools that can carry out an energy assessment which shown below (Pechsiri, 2015):

- Thermodynamic – Too complex, too much details
- Process modelling
  - Mechanistic models of flow, flow dynamics, mass balance and alkalinity equations
  - Statistical process modelling
- LCA
- Input – output analysis (offers flexibility)
  - Net Energy Ration (NET)
  - Energy Return On Investment (EROI)
  - Energy Renewability Efficiency (ERenEF%)

Those methods vary aim, level of detail and information required. Other tools are high in complexity such as thermodynamic energy analysis while other require real numbers for calculation such as the statistical process modelling. The input – output analysis however offers flexibility and is proposed in a big variety of chemical and physical processes.

Another important step is the economic evaluation of the process considering the energy required and the reagents that is used during the extraction. This step include various options as well and is up to the practitioner to choose the tool that best suits in that case. However the economic evaluation needs to be seen from the whole project perspective rather than the process in isolation.
References


Dragone, G. et al., 2010. Third generation biofuels from microalgae. *Current Research, Technology and Education Topics in Applied Microbiology and*


Öberg, V., 2014. 'Macro Algae as a Renewable Resource Extraction and characterization of the major components in Saccharina latissima', Master Thesis, School of Chemical Science & Engineering, KTH


Appendix I
Substance flow analysis

Assumptions regarding the chemicals:

- HCl is liquid solution with molarity 0.5M.
- NaOH is solid and has a 99% purity.
- Methanol is solution with concentration 98.5% v/v.
- Acetone is solution with concentration 99.5% v/v.
- Ethanol is solution with concentration 96.3% v/v.
- Ca(OH)$_2$ is solid and has an >99% purity.
- Na$_2$CO$_3$ is solid and has an >99.5% purity.
- CaCl$_2$ is solid and has an >99% purity.

Chemical reactions that take place during the process:

Neutralization of HCl:

2 HCl + Ca(OH)$_2$ → CaCl$_2$ + 2 H$_2$O  (1)

Reaction of alginate with HCl in order to produce alginic acid:

Alginate + HCl → Alginic acid + Cl$^-$  (2)

Reaction of alginic acid with NaOH in order to produce sodium alginate:

Alginic acid + NaOH → Sodium alginate + H$_2$O  (3)

Additional information in order to calculate the moles of the substances in the equations of the chemical reactions:

Ca(OH)$_2$ molar mass = 74 g/mol
CaCl$_2$ molar mass = 111 g/mol
NaOH molar mass = 40 g/mol

Equations used in order to transform the given quantities in moles:

\[ n = \frac{m}{M_r} \]  (4)  Where \( n \) are moles, \( m \) is the mass and \( M_r \) is the molar mass.

\[ molarity = \frac{n}{V} \]  (5)  Where \( n \) are the moles and \( V \) is the volume.
In order to find the moles of the neutralization factor in equation (1) the moles of HCl were calculated according to equation (5). After that the mass of Ca(OH)$_2$ was calculated using equation (4).
Appendix II
Chemical Assessment

List of chemicals:

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>IUPAC name</th>
<th>Molar Mass (g/mol)</th>
<th>Density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>Hydrogen Chloride</td>
<td>36.46</td>
<td>1.49</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
<td>39.997</td>
<td>2.13</td>
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<tr>
<td>CH₄O</td>
<td>Methanol (MeOH)</td>
<td>32.04</td>
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<tr>
<td>C₃H₆O</td>
<td>Acetone</td>
<td>58.08</td>
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<td>C₂H₆O</td>
<td>Ethanol</td>
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</tr>
<tr>
<td>Ca(OH)₂</td>
<td>Calcium Hydroxide</td>
<td>74.093</td>
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<td>Na₂CO₃</td>
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Environmental report (WAR GUI software)

Results file for extraction.war
File created on 28-Jul-15 at 19:25:10

----------------------------------------------------------------------------------------------
NOTES
Product stream(s) were not included in the calculations
Energy usage was not included in the calculations

----------------------------------------------------------------------------------------------
STREAM REPORT

Carbohydrate extraction from Saccharina latissima

<table>
<thead>
<tr>
<th>Name</th>
<th>Stream 1</th>
<th>Stream 2</th>
<th>Stream 3</th>
<th>Stream 4</th>
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Carbohydrate extraction from Saccharina latissima continued...
Flow rate  2.04E-01  9.00E-01  3.05E-01  4.10E+01  7.51E+01
HYDROGEN CHLORIDE  0.0000  0.0000  0.0000  0.0000  0.0000
SODIUM HYDROXIDE  0.0000  0.0000  0.0000  0.0000  0.0000
METHANOL  0.0000  0.0000  0.0000  0.0000  0.0000
ACETONE  0.0000  0.0000  0.0000  0.0000  1.0000
ETHANOL  0.0000  0.0000  0.0000  0.0000  1.0000
CALCIUM HYDROXIDE  1.0000  0.0000  0.0000  0.0000  0.0000
SODIUM CARBONATE  0.0000  1.0000  0.0000  0.0000  0.0000
CALCIUM CHLORIDE  0.0000  0.0000  1.0000  0.0000  0.0000

Carbohydrate extraction from Saccharina latissima continued...

Name Stream 11 Stream 12
Type Outlet Outlet
Flow rate 6.61E+01 9.00E-01

ENERGY USAGE REPORT

Case Energy Usage (MJ/sec) Fuel Type
Carbohydrate  0.00E+00 Coal

WEIGHTING PROFILE

Category HTPI HTPE TTP ATP GWP ODP PCOP AP TOTAL
Weight 0 0 2 2 2 1 2 2

ENVIRONMENTAL IMPACT REPORT

Total PEI Indexes
Case lout PEI/sec lout PEI/g Igen PEI/sec Igen PEI/g Ienergy PEI/sec Ienergy PEI/g
Carbohydrate  1.043E+2 N/A -8.508E+0 N/A 0.000E+0 N/A

Individual impact categories

Total output rate of PEI (PEI/sec)
Case Carbohydrate  1.03E+01 7.31E-02 1.03E+01 6.04E-03 0.00E+00 0.00E+00 4.18E+01 0.00E+00 1.04E+02

Total PEI leaving the system per mass of products (PEI/g product)
Case Carbohydrate  N/A N/A N/A N/A N/A N/A N/A N/A N/A

Total generation rate of PEI (PEI/sec)
Case Carbohydrate  -7.51E+01 -2.06E-01 -7.51E+01 -5.40E-03 0.00E+00 0.00E+00 -3.50E+01 0.00E+00 -8.51E+00

Total PEI generated within a system per mass of products (PEI/g product)
Case Carbohydrate  N/A N/A N/A N/A N/A N/A N/A N/A N/A

CHEMICAL REPORT

Carbohydrate extraction from Saccharina latissima

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<th>ODP</th>
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