# Potential of filamentous macroalgae and sessile invertebrates for bioremediation and valorization in the northern Baltic Sea

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Doctoral dissertation, to be presented for public discussion with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, in Lecture Hall P674, Porthania, on the 27th of August, 2020, at 12 o'clock.

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ISBN 978-951-51-6163-5 (paperback)

ISBN 978-951-51-6164-2 (PDF)

Unigrafia Oy

Helsinki 2020

# ABSTRACT

High nutrient concentrations caused by human activities is a global problem in fresh and coastal waters. Large areas of the Baltic Sea are also severely eutrophicated due to the past and present nitrogen (N) and phosphorus (P) emissions; consequently, ephemeral macroalgae exist in abundance. Nutrients incorporated in the marine biomasses have potential as part of a solution for amelioration of eutrophication in a world aiming towards a circular economy. Nutrients could be circulated back to the land and used for fertilization. Collected marine biomass also has potential for several other purposes.

In this thesis, I studied organisms adhering to the substratum in respect of their use for bioremediation and, furthermore, for valorization in the northern Baltic Sea's blue economy. Article I investigates the potential of filamentous macroalgae and sessile invertebrates for bioremediation. It studied the weight of the biomass attaching to the substratum, contents of particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP) in the invertebrate's fraction and macroalgae as well as concentrations of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) in the biomass. Article II focuses on the fatty acid concentrations and profiles of two green algae (*Ulva intestinalis* and *Cladophora glomerata*) and one red alga (*Ceramium tenuicorne*) in relation to ambient N and P availability. Article III reviews studies around the world on the potential of macroalgae in biofuel production.

Biomass on the artificial substrata is almost exclusively composed of the green algae *Ulva* spp. and *C. glomerata*, the red algae *Polysiphonia fibrillosa* and *C. tenuicorne*, the brown algae *Pylaiella littoralis* and *Ectocarpus siliculosus*, the blue mussels (*Mytilus trossulus*), the bay barnacles (*Amphibalanus improvisus*), and the hydroids *Cordylophora caspia* and *Gonothyraea loveni*. Over 95% of biomass is composed of sessile invertebrates; due to this and the small size of the northern Baltic Sea macroalgae, the removal of both invertebrates and macroalgae seems the most feasible practice in the current situation. Amelioration of small-scale nutrient loads is possible. The other most feasible utilizations for the total biomass would be as fertilizer as such or after composting and as additional feed material in biogas production.

The Cd content may limit the utilizable amount of biomass for the use as fertilizer as such. The Cd content varied according to the cultivation site and the incubation time. Limits set in the EU for the metals As, Cd and Pb may be exceeded if animal feed were composed exclusively of a certain fraction or fractions of biomass. Among the studied macroalgae, the lowest concentrations of all heavy metals were in *Ulva* spp., and it seemed to be a safe choice for solely algal-based feed applications regarding the measured metals.

Fatty acid composition is important in nutrition and in the refining of high value products. This study indicates that linoleic (C18:2n-6, LA) and arachidonic (C20:4n-6, AA) acid levels in *C. glomerata* could be regulated by nutrient manipulation. Elevated concentrations of these fatty acids were associated with higher N availability.

In practice, low biomass yield and lack of established infrastructure for cultivation, harvesting and utilization pose a challenge for a large-scale utilization of the northern Baltic Sea sessile organisms.

# TABLE OF CONTENTS

This thesis is based on the following articles, which are referred to by their Roman numerals:

I Suutari M, Leskinen E, Spilling K, Kostamo K, Seppälä J 2017: Nutrient removal by biomass accumulation on artificial substrata in the northern Baltic Sea. Journal of Applied Phycology 29:1707–1720. DOI 10.1007/s10811-016-1023-0.
The final publication is available at Springer via http://dx.doi.org/10.1007/s10811-016-1023-0.

II Suutari M, Ketola J, Tossavainen M, Leskinen E, Seppälä J: Concentrations of fatty acids in Baltic Sea macroalgae in relation to nutrient availability. Manuscript.

III Suutari M, Leskinen E, Fagerstedt K, Kuparinen J, Kuuppo P, Blomster J 2015: Macroalgae in biofuel production. Phycological Research 63:1–18. DOI 10.1111/pre.12078. Publisher John Wiley and Sons.

The authors' contributions to the articles

| | I | II | III |
|---|---|---|---|
| Original idea | MS, JS, KK, EL, KS | MS, EL, JS | PK, MS, EL |
| Designing the study | MS, JS, EL, KS, KK | MS, MT, EL | MS, JB, EL, KF, PK |
| Literature search | MS, EL | MS, EL, MT | MS, EL |
| Field work | MS, JS, KS, EL | MS, JaK, EL | – |
| Laboratory work | MS, EL, KK | JaK, MS, MT | – |
| Statistical analysis | MS, JS | MS, JS | – |
| Responsible for manuscript | MS | MS | MS |
| Contributions to manuscript (text, comments) | MS, EL, JS, KK, KS | MS, EL, JS, MT, JaK | MS, EL, JB, KF, JK, PK |

EL = Elina Leskinen, JaK = Janni Ketola, JB = Jaanika Blomster, JK = Jorma Kuparinen, JS = Jukka Seppälä, KF = Kurt Fagerstedt, KK = Kirsi Kostamo, KS = Kristian Spilling, **MS = Milla Suutari**, MT = Marika Tossavainen, PK = Pirjo Kuuppo

# ABBREVIATIONS

| | |
|---|---|
| AA | arachidonic acid (C20:4n-6) |
| ALA | α-linolenic acid (C18:3n-3) |
| As | arsenic |
| C | carbon |
| Cd | cadmium |
| $CH_4$ | methane |
| $CO_2$ | carbon dioxide |
| Cu | copper |
| DHA | docosahexaenoic acid (C22:6n-3) |
| DW | dry weight |
| EPA | eicosapentaenoic acid (C20:5n-3) |
| FA | fatty acid |
| FAME | fatty acid methyl ester |
| FAMEs | fatty acid methyl esters |
| FAs | fatty acids |
| $H_2$ | hydrogen |
| Hg | mercury |
| $KH_2PO_4$ | potassium dihydrogen phosphate |
| LA | linoleic acid (C18:2n-6) |
| N | nitrogen |
| $NaNO_3$ | sodium nitrate |
| $NH_3$ | ammonia |
| $NH_4$ | ammonium |
| $NO_2$ | nitrite |
| $NO_3$ | nitrate |
| P | phosphorus |
| Pb | lead |
| $PO_4$ | phosphate |
| POC | particulate organic carbon |
| PON | particulate organic nitrogen |
| POP | particulate organic phosphorus |
| PUFA | polyunsaturated fatty acid |
| PUFAs | polyunsaturated fatty acids |
| SFA | saturated fatty acid |
| SFAs | saturated fatty acids |
| TFA | total fatty acid |
| tot-N | total nitrogen |
| tot-P | total phosphorus |
| Zn | zinc |

# 1. INTRODUCTION

Persistent, high nutrient concentrations, i.e., eutrophication, induced by human activities is a global problem in fresh and coastal waters (McCrackin et al. 2017; Hamilton et al. 2018). Excess nutrients enhance primary production, which eventually leads to several changes in the aquatic environment, including oxygen depletion (Carstensen et al. 2014; McCrackin et al. 2017), increased turbidity (Bonsdorff et al. 1997) and changes in species abundances (Carstensen et al. 2014). In addition to the deterioration of the prevalent ecosystem, eutrophication often leads to negative economic impacts, for example, on fisheries and on recreational value via phenomena like anoxia that prevents fish reproduction and survival (McCrackin et al. 2017), and excess macroalgal wash ups on the beaches (Bucholc et al. 2014).

The primary cure for eutrophication would be to stop such activities that result in nutrient leakage into the sea. However, this is not easily done, because certain practices, like farming, are to be continued, anyway. Climate change also increases nutrient load via increased precipitation (Carstensen et al. 2014). In the Baltic Sea and in many other coastal areas around the world, sediment is so rich in nutrients of anthropogenic origin that the eutrophicated state of the marine environment is maintained via internal loading, regardless of reductions in the external load, such as municipal waste waters (Pitkänen et al. 2001; Andersson et al. 2014; Petersen et al. 2014; Puttonen et al. 2016).

Several practices can be realized on land to diminish nutrient leakage into the sea (Ahtiainen et al. 2014). Already existing consequences of eutrophication could be possibly ameliorated by removal of aquatic biomass (Hänninen 1996; Lindahl and Kollberg 2008; Huo et al. 2011). Cultivation of red macroalgae has improved the eutrophication status of both enclosed and open coastal sea areas in China (He et al. 2008; Huo et al. 2011). The term bioremediation in this thesis refers to the removal of aquatic biomass in order to remove harmful substances, such as excessive nutrients and heavy metals, from the water body.

Strong motivation for the prevention of eutrophication and alleviation of its consequences comes from legislation. The European Water Framework Directive gives guidelines for European water policy and requires all Member States to achieve good ecological and chemical status of surface waters (Directive 2000/60/EC). Its internal part, the Nitrates Directive, aims to protect waters from agricultural nitrates run off (Council Directive 91/676/EEC). The Marine Strategy Framework Directive requires Member States to achieve a good environmental status of their marine waters (Directive 2008/56/EC). These two Directives, along with the Helsinki Commissions Baltic Sea Action Plan (BSAP 2018), require countries to undertake active initiatives to reach a good environmental status of their marine areas. However, current measures have proven insufficient to achieve the set goals; therefore, new measures are needed. Cultivation, harvesting and utilization of marine biomasses could provide part of a solution for the requirements of The Renewable Energy Directive (Directive (EU) 2018/2001) and The European Commission's Circular Economy Action Plan (COM/2015/0614). However, the potential of the northern Baltic Sea macroalgal and invertebrate biomasses to provide solutions and novel measures to achieve the goals set forth by European and Baltic Sea policies is not yet fully investigated.

## 1.1 Potential of sessile biomass

Ambient conditions, like seawater temperature and the quantity and quality of solar radiation, affect the organism's chemical composition (Stengel et al. 2011; Fernández et al. 2015). The chemical composition of macroalgae and invertebrates enables their utilization in several ways, as well as the refining of a wide variety of compounds. Macroalgae and mussels can be used as fertilizer as such, or, after processing them (Lindahl and Kollberg 2008; Lill et al. 2012; Ammenberg and Feiz 2017; Alobwede et al. 2019), they are potential material for production of feed and food (Prou and Goulletquer 2002; Jönsson and Holm 2010; Rebours et al. 2014; Bikker et al. 2016) and a source for health-promoting products (Grienke et al. 2014; Parjikolaei et al. 2016). Macroalgae have potential as biosorbents in metal removal (Carrilho and Gilbert 2000; Lill et al. 2012) or as raw material for biofuels, as well as for chemicals and cosmetics (Fitton et al. 2015; Bikker et al. 2016). Instead of real tests or applications, evaluations on the use of sessile organisms for other purposes than food are mostly based on their chemical composition (Petersen et al. 2014; McCauley et al. 2016; Biancarosa et al. 2018), although some pilot-scale tests have been run, especially on biofuel research (Barbot et al. 2016) and on their use as fertilizer (Lindahl and Kollberg 2008).

Alleviation of eutrophication can be an opportunity for blue economy solutions that use marine resources in a way that provides economic value. Economic feasibility can often even be increased when the resource is used for multiple purposes (Baghel et al. 2016; Tedesco and Stokes 2017), for example, first for extraction of valuable compounds like pigments and lipids and then the remainder for biofuel and fertilizer (Baghel et al. 2016; Pechsiri et al. 2016; Tedesco and Stokes 2017). Resources in a circular economy are aimed to circle without losing them, often benefitting the environment as well (Geissdoerfer et al. 2017). Aquatic biomass has potential for both the blue and circular economies (Baghel et al. 2016).

## 1.2 Biomass of the Baltic Sea

At least 97% of the Baltic Sea suffers from severe eutrophication caused by past and present nitrogen (N) and phosphorus (P) emissions (HELCOM 2018). Eutrophication favors fast-growing, ephemeral macroalgal species that tend to have high nutrient uptake rates (Pedersen and Borum 1997; Karez et al. 2004). This makes them potentially efficient in bioremediation.

Heavy metal concentrations in the Baltic Sea sediment have been high during the past decades because of industrialization (Rainbow et al. 2000; Vallius 2014). Despite a trend towards general decrease, concentrations of arsenic (As), cadmium (Cd) and mercury (Hg) remain elevated in the sediments of the northern Baltic Sea (Vallius 2014). When mass-occurring, filamentous algae detach, loose algal masses are formed on the sea bottom creating hypoxic conditions (Vahteri et al. 2000; Rönnberg and Bonsdorff 2004), which may promote metal and P release from sediment (Voigt 2007; Andersen et al. 2017). Several macroalgal species, both living and non-living, are efficient heavy metal accumulators (Davis et al. 2003; Żbikowski et al. 2007; Lill et al. 2012; Gubelit et al. 2016; Zeraatkar et al. 2016). Depending on the further utilization of the cultivated biomass, heavy metals may restrict biomass use or heavy metals need to be removed prior to use (Nkemka and Murto 2010).

Fishery in the Baltic Sea is presently a considerable factor in nutrient removal from the sea area (Hjerne and Hansson 2002; Korpinen et al. 2018). There is no large-scale utilization of marine sessile organisms, especially not in the northern Baltic Sea (Gren et al. 2009; Malta and Agraso Martínez

2017). Additional options to remove nutrients are needed since the sea is largely eutrophic and the area affected by overload of nutrients has become larger compared to previous evaluation (Andersen et al. 2011; Fleming-Lehtinen et al. 2015). Bioremediation by biomass removal and utilization of the northern Baltic Sea macroalgae and invertebrates has been little studied, except for some assessments on blue mussels (Gren et al. 2009) and macroalgae (Lill et al. 2012; Gubelit et al. 2015). This thesis discusses these themes to fill in some of the gaps of knowledge.

# 2. AIMS OF THE THESIS

The aims of my thesis were to:

1. Study if filamentous macroalgae and sessile invertebrates could be used for bioremediation in the northern Baltic Sea
2. Study macroalgal fatty acid concentrations in relation to nutrient availability
3. Assess potential utilization capacity of the filamentous macroalgae and sessile invertebrates

In study I, we studied nutrient removal capacity of sessile organisms cultivated on non-seeded artificial substrata in brackish water in the northern Baltic Sea. Further utilization of harvested biomass as fertilizer and in biogas production is shortly discussed.

In study II, we studied the fatty acid (FA) content and composition of two green algae (*Ulva intestinalis* and *Cladophora glomerata*) and one red alga (*Ceramium tenuicorne*) in relation to N and P availability. The FA composition in nutrition is discussed.

Study III reviews the potential of macroalgae in biofuel production.

In this thesis, I assess bioremediation and utilization potential of common northern Baltic Sea macroalgae and invertebrates by reviewing the main findings from studies I and II and by inhering information of macroalgae in biofuel production from study III.

Part of the research reported here (I, II) was performed in the Baltic Sea Region Programme 2007-13 project Sustainable Uses of Baltic Marine Resources, SUBMARINER, which aimed to find new sustainable uses for Baltic marine resources.

# 3. MATERIALS AND METHODS

The Baltic Sea is shallow with a medium depth of 54 m, with a large catchment area (1 650 000 km$^2$) in relation to the surface area (390 000 km$^2$), and with limited exchange of water through the Danish straits (Andersen and Pawlak 2006; Myrberg et al. 2006). Almost the entire Baltic Sea is eutrophic (HELCOM 2018). The field experiments were performed in, and the species for the laboratory experiments were collected from, the northern Baltic Sea. The area has ice cover during the winter (Myrberg et al. 2006), no significant tidal water, and the water is low in salinity [(0-7 psu) (Andersen and Pawlak 2006)]. There is strong seasonal variation in species abundance (Takolander 2018), temperature and irradiance (Myrberg et al. 2006). Environmental conditions also vary between the years (Kiirikki and Lehvo 1997).

## 3.1 Description of the study area (I, II)

The experiment on biomass accumulation on artificial substrata (I) was performed in two localities on the coast of southern Finland (Fig. 1). In Rymättylä, Naantali, the cultivation substrata were placed in the strait of Hämmärö in close proximity to fish cages cared for by the Natural Resources Institute Finland (N60.18, E21.57). A ferry route and two commercial fish farms were in the same strait within a distance of 300 to 450 m from the experiment's set-up. The other set-up was located in Tvärminne, Hanko, in a nature preserve area in a strait formed by a few islands (N59.50, E23.15). The water depth at the study sites in Rymättylä was 10-13 m and 8-10 m in Tvärminne. Salinity averages during the study period were 5.2 in Tvärminne and 6.2 in Rymättylä. Temperature ranged from −0.3 to 24.2 °C in Tvärminne and from −0.2 to 23.7 °C in Rymättylä. The total phosphorus (tot-P) concentration in the water was higher in Tvärminne than in Rymättylä during the 2011 and 2012 growing seasons. The total nitrogen (tot-N) concentration did not differ between locations; both concentrations were higher in the 2011 growing season than in 2012.

The algae to study concentration of fatty acids (FAs) in relation to available nutrients (II) were collected from rocks in the depth of 0–0.2 m by the Tvärminne Zoological Station (N59.84, E23.25), Tvärminne (Fig. 1) and the nearby islands Joskär (N59.85, E23.26) and Halsholmen (N59.84, E23.26) in August 12, 2013 (*Ulva intestinalis* and *Cladophora glomerata*) and September 30 – October 1, 2013 (*U. intestinalis* and *Ceramium tenuicorne*).

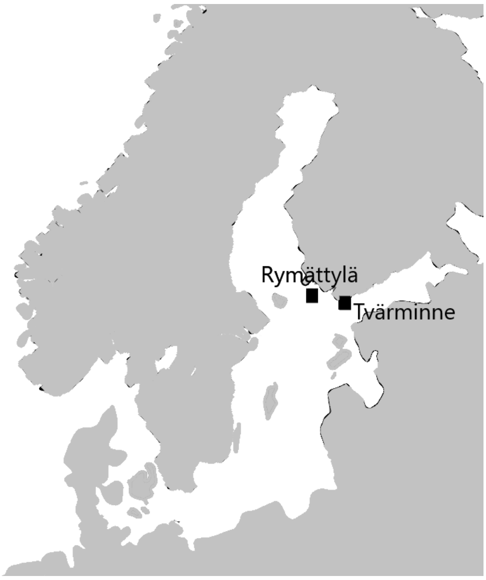


**Fig. 1.** Study sites Rymättylä and Tvärminne on the coast of Finland. In Rymättylä, the cultivation lines were situated in the strait of Hämmärö. In Tvärminne, the substrata were located in a strait formed by a few islands. Algae to study fatty acids were collected by the Tvärminne Zoological Station, Tvärminne, and the nearby islands Joskär and Halsholmen.

## 3.2 Biomass accumulation and concentration of nutrients and heavy metals in the biomass (I)

In both localities, Rymättylä and Tvärminne, new and clean net and rope substrata of the size 60.7 cm × 29.5 cm and 50.6 cm × 25.1 cm, respectively, were incubated in the sea from May 2011 until October 2011 and July/August 2012 (Fig. 2). A special set for following biomass gain on monthly bases was set in Tvärminne in May 2011 and sampled once a month from August to September 2012, excluding July 2012. This set is referred to as the biomass line later in the text. All installations were lowered a few meters below the surface for the winter period (Table 1).

**Fig. 2.** The substrata two weeks after installation. Photo by Milla Suutari.

**Table 1.** Schedule showing the installation, samplings, and lowering and raising the substrata in Rymättylä and Tvärminne during the 2011 and 2012 study period. The biomass line in Tvärminne was used to monitor biomass gain on a monthly basis on the substrata. Nutrient, heavy metal, species and biomass samples were taken in Rymättylä and Tvärminne in 5 to 14 October 2011 and 23 July to 1 August 2012. Table reshaped from study I.

| | 2011 | | | 2012 | |
|---|---|---|---|---|---|
| | Into the sea | Sampling | Lowering for the winter | Raising back to the surface | Sampling |
| Rymättylä | 19 May | 5-11 Oct | 16 Dec | 6 May | 30 July-1 Aug |
| Tvärminne | 16 May | 12-14 Oct | 29 Nov | 16 and 18 Apr | 23-27 July |
| Biomass line | 16 May | 9 Aug, 6-7 Sept, 4 Oct | 29 Nov | 18 Apr | 26 Apr, 23 May, 20 June, 28 Aug, 18 Sept |

The substrata collected from the sea were rinsed in ion exchanged water to remove loose material. The sampling area from which the attached organisms were scraped was 0.14 $m^2$ for the nets and 0.12 $m^2$ for the ropes. The nets were scraped on both sides and the ropes all around. The macroalgae were sorted by species into five groups according to the dominant taxa and put into separate plastic bags. The rest of the biomass (mainly fauna: blue mussels, bay barnacles, and polyps), consisting of particles of size > 80 μm, were put in a separate plastic bag. Size separation was performed by filtering the remaining biomass through a plankton net. Samples from the biomass line were also put in plastic bags but without sorting out macroalgae and invertebrates.

The sample bags were frozen as soon as possible either in dry ice or by placing them in a −85 °C freezer to avoid changes in organisms' chemical composition. When the initial freezing was performed, all the samples were stored at −20 °C until the laboratory measurements. All the handling and storage was performed using plastic items to avoid contamination with metals.

The samples were dried and weighed to measure the total and species-specific biomass gain. Nutrient analyses were made of samples collected from the net and rope substrata. Particulate organic carbon (POC) and particulate organic nitrogen (PON) contents of the attached biomass were analyzed using Leco TruSpec®Micro-analyzer. Particulate organic phosphorus (POP) of the attached biomass was analyzed using Thermo Scientific Aquakem 250 photometric analyzer according to Solorzano and Sharp (1980). Tot-P and tot-N were additionally measured from water samples collected next to each installation all through the growing seasons with 2- to 4-week intervals, and they were analyzed according to Grasshoff et al. (1983) and Solorzano and Sharp (1980). Salinity and temperature at the study sites were logged continuously (DST CT logger, Star-Oddi Iceland). The measurements of metal concentrations were performed for the samples collected from net substrata. Concentrations of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) were analyzed according to the methods described in Nóbrega et al. (2012) and standards USEPA (2007) and ISO 17294-2:2003 (ISO 17294-2) (I).

## 3.3 Macroalgal fatty acids (II)

The macroalgae were collected from natural populations in August 12, 2013 (*U. intestinalis* and *C. glomerata*) and September 30 – October 1, 2013 (*U. intestinalis* and *C. tenuicorne*). The algae were rinsed with seawater and all visible epiphytes were removed. A sample of each species, referred to as ''initial'' later in the text, was frozen at –80 °C for further analysis.

Macroalgal samples of 5 g wet weight per each aquarium were placed in 1 L plastic boxes covered with a thin mesh. The boxes were incubated in 30 L aquariums. The experiment in both August and October consisted of eight aquariums per species, comprehending four nutrient treatments using the macronutrients N and P. The nutrient treatments were Control, N treatment, P treatment, and N + P treatment. The aquarium water was mixed of ion exchanged water using Tropic Marine sea salt adjusting salinity at 5.8. The daily nitrogen additions (in N treatment and N + P treatment) were 80 μg $l^{-1}$ $NaNO_3$-N and the daily phosphorus additions (in P treatment and N + P treatment) were 17.7 μg $l^{-1}$ of $KH_2PO_4$-P. Besides that, small amounts of nitrogen (1.5 μg $NaNO_3$-N $l^{-1}$) and phosphorous (0.4 μg $KH_2PO_4$-P $l^{-1}$) were added daily to all aquariums.

During the experiments, ion-exchanged water was added to the aquariums to compensate for evaporation. The water was circulated using standard aquarium filters without filtering material

(Eheim 2006020). Illumination was set to follow the natural light rhythm (light:dark 18:6 in August and 16:8 in October (Philips TLD58W/965 and Philips TL-D 90 De Luxe Pro 58W/965). Light intensity both in August and October was 55 µmol $m^{-2}$ $s^{-1}$ (LI-COR LI-190R quantum sensor). The aquariums were placed in a temperature-controlled room with temperature during the experiments of 18.4±0.3 °C in August and 18.2±0.5 °C in October.

The experiments lasted for 10 days, August 13–22, 2013 and October 1–10, 2013. The nutrient concentrations, $NO_3$, $NO_2$, $NH_4$ and $PO_4$, in the aquariums were measured every second day before and after the nutrient additions. The analyses were made according to Grasshoff et al. (1983) and Solorzano and Sharp (1980). The macroalgae were rinsed with ion-exchanged water, weighed and frozen at –80 °C at the end of the experiments. Prior to lipid extraction, the samples were freeze dried, pulverized, and stored at –70 °C in gaseous N to avoid chemical transformations.

The extraction of lipids was performed as described in Natunen et al. (2017) with modifications from Parrish (1999) using chloroform-methanol extraction. FA methylation was performed in methanol-sulphuric acid solution (Christie and Han 2010; Natunen et al. 2017; Tossavainen et al. 2019). Olive oil (Supelco), methylated and analyzed along with the samples, was used as a control to the methylation process. The samples were analyzed using a gas chromatograph–mass spectrometer (GC-MS) (GCMS-QP2010 Series, Shimadzu). The retention times in a standard fatty acid methyl ester (FAME) mix (Larodan Fine Chemicals FAME 37 mix, 10 mg/mL, 99%) and mass spectra (http://lipidlibrary.aocs.org/) were used to identify the FAs. Since FA C18:0 was present in our samples, the deuterated FA C18:0-D3 (99%, Larodan Fine Chemicals, Sweden) was used as the internal standard for the quantification of fatty acid methyl esters (FAMEs). The total fatty acid (TFA) content was calculated as the sum of identified and quantified FAs. The proportions of saturated, monounsaturated and polyunsaturated FAs, as well as the ratios of n-6 to n-3 polyunsaturated FAs (PUFAs) were calculated. FAs were compared by the species and nutrient treatments.

## 3.4 Macroalgae in biofuel production (III)

The review covers information found using Google and Google Scholar web search engines, the Web of Science website and an article search in the Helka database (a shared collection and circulation service of the Helka libraries). The searches were centered on macroalgal chemical composition and factors affecting it, with focus on production of bio-oil, syngas, biodiesel, biobutanol, bioethanol, biogas and hydrogen ($H_2$). Literature searches were performed during the time period from February 2009 until July 2014. In total 174 journal publications, web pages and other references from the years 1950 – 2014 were reviewed.

## 3.5 Bioremediation potential and other utilizations (I, II, III)

Potential in bioremediation was estimated by measuring biomass and nutrient content by taxon on a certain area of substratum. This was compared to the input of nutrients into the Archipelago Sea and from fish farms and sewage treatment plants. Biomass on the biomass line is not included for the comparisons of total biomass between the locations and for estimations of bioremediation potential, because sampling times are different for biomass line in Tvärminne and the other substrata in Tvärminne (Table 1).

Assessment of suitability for different utilizations was performed by reviewing the newest information in literature and combining it with the analyses of metals, FAs and nutrients from studies I and II. Assessing the use in biofuel production was based on paper III and literature review.

### 3.6 Statistical analyses

Statistical analyses were performed using SPSS 21.0 statistical software (I, II, this summary). Comparisons between the type of substrata and locations were made for this summary using a different test type from the article I. In this summary I used independent samples two tailed t-test, which confirmed article I's results. Levene's test was used to test the equality of variances. Equal variances were assumed when p-value of the test was $\geq 0.05$. Biomass on biomass line is not included in biomass comparisons between the two locations, not in the original article (I) nor in this summary. It was also kept separate and tested separately from the comparisons on biomass on different substrata in Tvärminne (I, this summary). Test results from tests performed only for this summary are available in the text. Only significances are shown in this summary for the tests used in the original articles; otherwise test results can be found in the original articles. Confidence intervals of 95% were used for statistically significant differences.

## 4. RESULTS

### 4.1 Biomass accumulation and concentration of nutrients and heavy metals in the biomass (I)

Considering practical work with substrata, the ropes were more robust. The type of nets used in the experiment (I) broke easily and were unsuitable for a long-term cultivation. Furthermore, since the total biomass per area of substratum was similar or higher on ropes compared to nets (I), I will focus in this summary on growth and biomass on the rope substrata.

#### *4.1.1 Species composition*

The most abundant macroalgal species on the artificial substrata were the green algae *Ulva* spp. and *Cladophora glomerata*, the red algae *Polysiphonia fibrillosa* and *Ceramium tenuicorne*, and the brown algae *Pylaiella littoralis* and *Ectocarpus siliculosus*. There were also some individuals of the green alga *Cladophora rupestris* and red alga *Polysiphonia fucoides*. The most abundant invertebrates on the substrata were blue mussels (*Mytilus trossulus*), bay barnacles (*Amphibalanus improvisus*) and the hydroids *Cordylophora caspia* and *Gonothyraea loveni* (Table 2) (I).

#### *4.1.2 Total biomass*

The average net monthly gain was higher during the first growing season until October 2011, compared to the period from October 2011 to September 2012 (I). In October 2011, considering the ropes in Rymättylä and Tvärminne, there was no difference in total biomass between these locations, but the next summer's biomass on rope substrata in Rymättylä was higher [t(12)=3.302, p=0.006, 2-tailed]. In July/ August 2012, the total biomass on ropes was 3.3 kg m$^{-2}$ dry weight (DW) in Rymättylä, and 2.3 kg m$^{-2}$ DW in Tvärminne (I) (Table 2).

### *4.1.3 Macroalgal biomass*

Macroalgal biomass in the second growing season was clearly higher than in the first season, although still low compared to the total biomass. Algal biomass per m$^{-2}$ of rope substrata was 4.2 g DW (Rymättylä) and 4.9 g DW (Tvärminne), i.e., 0.3% of the total biomass on ropes both in Rymättylä and in Tvärminne in October 2011. *Ulva* spp. was the dominant species in both locations. Algal biomass on ropes had increased to 77.9 g m$^{-2}$ DW in Rymättylä, and 108.1 g m$^{-2}$ DW in Tvärminne by the end of July/beginning of August 2012. These were 2.4% and 4.8% of total biomass, respectively. At this point, *C. glomerata* formed the highest biomass in both places (I) (Table 2).

**Table 2.** Species composition and biomass on the rope substrata, g DW m$^{-2}$ and kg DW ha$^{-1}$ substratum, in Rymättylä and Tvärminne in October 2011 and July/ August 2012. Incubation of the substrata started in May 2011. Biomass of the invertebrates was not measured by species.

| | | October 2011 | | July/ August 2012 | July 2012 |
|---|---|---|---|---|---|
| | | Rymättylä | Tvärminne | Rymättylä | Tvärminne |
| Brown algae - | *Pylaiella littoralis* (Linnaeus) Kjellman | 0.1 | 0 | 3.4 | 5.8 |
| Phaeophyceae | *Ectocarpus siliculosus* (Dillwyn) Lyngbye | | | | |
| Red algae - | *Polysiphonia fibrillosa* (Dillwyn) Sprengel | 0.3 | 0 | 1.4 | 0.001 |
| Rhodophyta | *Polysiphonia fucoides* (Hudson) Greville | | | | |
| | *Ceramium tenuicorne* (Kützing) Waern | 0.5 | 0 | 4.1 | 0.1 |
| Green algae - | *Cladophora glomerata* (Linnaeus) Kützing | 0.8 | 0.6 | 68.5 | 101.8 |
| Chlorophyta | *Cladophora rupestris* (Linnaeus) Kützing | | | | |
| | *Ulva* spp. Linnaeus | 2.4 | 4.4 | 0.4 | 0.3 |
| | **Macroalgae in total, g DW m$^{-2}$** | **4** | **5** | **78** | **108** |
| Bivalves - Bivalvia | *Mytilus trossulus* (Gould, 1851) | | | | |
| Maxillopoda | *Amphibalanus improvisus* (Darwin, 1854) | | | | |
| Hydrozoans - | *Cordylophora caspia* (Pallas, 1771) | | | | |
| Hydrozoa | *Gonothyraea loveni* (Allman, 1859) | | | | |
| | **Invertebrates in total, g DW m$^{-2}$** | **1427** | **1430** | **3208** | **2162** |
| | **Total biomass (algae + invertebrates), g DW m$^{-2}$** | **1431** | **1435** | **3286** | **2270** |
| | Total biomass (algae + invertebrates), kg DW ha$^{-1}$ | 14310 | 14352 | 32859 | 22702 |

### *4.1.4 Nutrients*

During the study period from May 2011 to September 2012 (no water samples were taken during the winter period between November 2011 and March 2012), both the tot-N and tot-P concentrations in water were higher in year 2011 (N: $p < 0.001$, P: $p = 0.001$). Tot-P concentration in water was higher in Tvärminne than in Rymättylä ($p < 0.001$); similarly, POP was higher in total biomass as well as in *C. glomerata* and filamentous brown algae in Tvärminne ($p = 0.037$, $p = 0.003$, $p < 0.001$, respectively) (I). The average P content, covering the samples from both years and locations, was similar in all macroalgal species and the invertebrate fraction with a variation of 2.2–3.0 g kg$^{-1}$ DW.

*Ulva* spp. and *P. fibrillosa* had the highest POC and PON contents. POC content in *Ulva* spp. was 344 g kg$^{-1}$ DW and in *P. fibrillosa* 322 g kg$^{-1}$ DW. Temporal variation in algal nutrient concentrations was observed, unlike in the invertebrates. The highest PON contents were measured in *Ulva* spp. with more than 44 g kg$^{-1}$ DW in 2011. The following year, it was clearly lower, 12.2–14.4 g kg$^{-1}$ DW, and the average PON content, with both years included, was higher in *P. fibrillosa* (41.2 g kg$^{-1}$ DW) compared to 29 g kg$^{-1}$ DW in *Ulva* spp. Covering both years, invertebrates had low average carbon (C) (172 g kg$^{-1}$ DW) and N (18 g kg$^{-1}$ DW) contents compared to macroalgae.

In macroalgal and invertebrate fractions, the mass C/N ratio varied from 7 in *C. tenuicorne* and filamentous brown algae in 2011 to 22 and 27 in *Ulva* spp. in 2012. In Rymättylä, on ropes the total biomass mass C/N ratio decreased a little from 11 in October 2011 to 8 in July/August 2012. In Tvärminne, the mass C/N ratio in total biomass on ropes rose from 9 in October 2011 to 11 in July 2012 (Table 3).

The atomic N/P ratios were highest in *P. fibrillosa* (Table 3).

**Table 3.** The mass C/N ratios and atomic N/P ratios in the invertebrate and macroalgal fractions as well as in the total biomass on the substrata.

| | | Invertebrates | | *Ulva* spp. | | *Cladophora glomerata* | | *Polysiphonia fibrillosa* | | *Ceramium tenuicorne* | | Filamentous brown algae | | Total biomass | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| C/N mass ratio | Rymättylä | 11 | 8 | 8 | 27 | 8 | 19 | 8 | 9 | 7 | 10 | 7 | 14 | 11 | 8 |
| | Tvärminne | 9 | 11 | 8 | 22 | 8 | 15 | * | 8 | * | 11 | * | 12 | 9 | 11 |
| N/P atomic ratio | Rymättylä | 15 | 21 | 35 | 13 | 19 | 20 | 40 | 39 | 25 | 31 | 31 | 20 | 15 | 21 |
| | Tvärminne | 14 | 21 | 34 | 23 | 23 | 14 | * | ** | * | 22 | * | 13 | 14 | 20 |

*species were not present on the rope substrata

**calculation was not possible, due to the absence of the measurement of POP content

The total attached biomass contained 200 kg N and 29 kg P ha$^{-1}$ substratum in Rymättylä, and 273 kg N and 43 kg P ha$^{-1}$ substratum in Tvärminne after the first growing season, a period of 5 months. The next summer, 3.8 and 1.4 times as much N, kg ha$^{-1}$ substratum was bound in the total biomass in Rymättylä and Tvärminne, respectively. The P content in the total biomass was 2.8 times as much in Rymättylä, whereas in Tvärminne the P content was slightly lower compared to the first year (Table 4a). P content in percentages did not change between the years, neither in the total biomass nor in the macroalgal biomass. In total biomass, the highest share of N was in Rymättylä in 2012. In macroalgal biomass, the highest percentages of N were in 2011 (Table 4b).

**Table 4. a**) Total and macroalgal biomass, kg DW ha$^{-1}$ rope substratum, as well as N and P content, kg ha$^{-1}$ in the total and macroalgal biomass of rope substrata after five and 14.5-month incubation periods in Rymättylä and Tvärminne. Calculations refer to the area of substratum. **b**) N and P content, % in the total and macroalgal DW biomass of rope substrata after five and 14.5-month incubation periods in Rymättylä and Tvärminne.

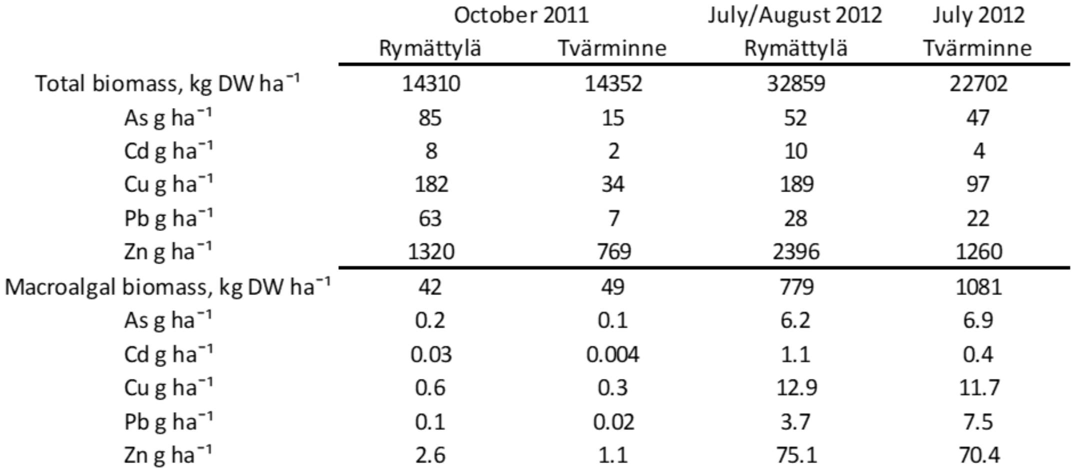
| **a**) | October 2011 | | July/August 2012 | July 2012 |
|---|---|---|---|---|
| | Rymättylä | Tvärminne | Rymättylä | Tvärminne |
| Total biomass, kg DW ha$^{-1}$ | 14310 | 14352 | 32859 | 22702 |
| N kg ha$^{-1}$ | 200 | 273 | 753 | 369 |
| P kg ha$^{-1}$ | 29 | 43 | 81 | 40 |
| Macroalgal biomass, kg DW ha$^{-1}$ | 42 | 49 | 779 | 1081 |
| N kg ha$^{-1}$ | 1.6 | 2.1 | 13.6 | 20 |
| P kg ha$^{-1}$ | 0.1 | 0.1 | 1.4 | 3.2 |

| **b**) | October 2011 | | July/August 2012 | July 2012 |
|---|---|---|---|---|
| | Rymättylä | Tvärminne | Rymättylä | Tvärminne |
| Total biomass, DW | | | | |
| N, % | 1.4 | 1.9 | 2.3 | 1.6 |
| P, % | 0.2 | 0.3 | 0.2 | 0.2 |
| Macroalgal biomass, DW | | | | |
| N, % | 3.9 | 4.3 | 1.8 | 1.9 |
| P, % | 0.3 | 0.3 | 0.2 | 0.3 |

### *4.1.5 Heavy metals*

Concentrations of As, Cd, Cu, Pb and Zn in algae were taxa dependent and were, on average, far lower in the invertebrate fraction than in macroalgae (I). Among the studied macroalgae, the lowest concentrations of all heavy metals were in *Ulva* spp., and it seemed to be a safe choice for solely algal-based feed applications regarding measured metals. Cd, Cu and Zn concentrations were higher in Rymättylä than in Tvärminne (Cd: $p = 0.042$, Cu: $p = p < 0.001$, Zn: $p = 0.005$) (I). Table 5 presents metals calculated as g $ha^{-1}$ of the area of substratum.

**Table 5.** Total and macroalgal biomass, kg DW $ha^{-1}$ substratum, as well as the As, Cd, Cu, Pb and Zn contents, g $ha^{-1}$ substratum, in the total and macroalgal biomass of rope substrata after five and 14.5-month incubation periods in Rymättylä and Tvärminne. Calculations refer to the area of substratum.



| | October 2011 | | July/August 2012 | July 2012 |
|---|---|---|---|---|
| | Rymättylä | Tvärminne | Rymättylä | Tvärminne |
| Total biomass, kg DW $ha^{-1}$ | 14310 | 14352 | 32859 | 22702 |
| As g $ha^{-1}$ | 85 | 15 | 52 | 47 |
| Cd g $ha^{-1}$ | 8 | 2 | 10 | 4 |
| Cu g $ha^{-1}$ | 182 | 34 | 189 | 97 |
| Pb g $ha^{-1}$ | 63 | 7 | 28 | 22 |
| Zn g $ha^{-1}$ | 1320 | 769 | 2396 | 1260 |
| Macroalgal biomass, kg DW $ha^{-1}$ | 42 | 49 | 779 | 1081 |
| As g $ha^{-1}$ | 0.2 | 0.1 | 6.2 | 6.9 |
| Cd g $ha^{-1}$ | 0.03 | 0.004 | 1.1 | 0.4 |
| Cu g $ha^{-1}$ | 0.6 | 0.3 | 12.9 | 11.7 |
| Pb g $ha^{-1}$ | 0.1 | 0.02 | 3.7 | 7.5 |
| Zn g $ha^{-1}$ | 2.6 | 1.1 | 75.1 | 70.4 |

## 4.2 Macroalgal fatty acids (II)

The differences in macroalgal FA content found in this study were more related to taxonomy than nutrient availability. It seemed that n-6 series FAs linoleic (C18:2n-6, LA) and arachidonic (C20:4n-6, AA) acids could be regulated in *C. glomerata* by nutrient manipulations. Elevated concentrations of these FAs were associated with higher N availability.

*Ulva intestinalis* contained the highest percentage of PUFAs, and its polyunsaturated FA/saturated FA (PUFA/SFA) ratio was higher than this ratio in *C. glomerata* or *C. tenuicorne*. *U. intestinalis* also contained the highest proportion of α-linolenic acid (C18:3n-3, ALA) (% of TFA), a precursor for the synthesis of n-3 FAs eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic (C22:6n-3, DHA) acids (Pereira et al. 2012). The essential FAs ALA and LA were the most abundant among the PUFAs in *U. intestinalis*.

EPA was present in all the three species but at the highest proportion (% of TFA) in *C. tenuicorne* and in the highest concentration in *C. glomerata* (II).

Palmitic acid, C16:0, was the most abundant FA in all species (II). The highest TFA content, 7 – 8.7 mg $g^{-1}$ DW, was in *C. glomerata*. All three species had n-6/n-3 ratios of 0.4–0.5 (II). Seasonal variation was seen when the TFA content in *U. intestinalis* was significantly higher in August than in October ($p < 0.01$) (II). The FA profiles of *U. intestinalis* were very similar in both seasons (II).

### 4.3 Macroalgae and other biomass in biofuel production (I, III)

Considering the macroalgal chemical composition and generally high carbohydrate concentration but negligible lignin content, the most suitable conversions might be fermentation into biobutanol or bioethanol and anaerobic digestion to produce biogas or hydrothermal gasification to produce syngas (III, this thesis). However, in the northern Baltic Sea, the filamentous macroalgal biomass per area is so low (I) that solely macroalgal-based biofuel production seems unrealistic. Production of biofuels from sessile biomass also needs an optimization of the practically the whole production chain, including transportation, storage and extraction technologies. This is especially crucial in the northern Baltic Sea, since there is no large-scale utilization of macroalgae or sessile invertebrates at all. Multiple utilizations or combined production of several products is important in order to reach economic feasibility (III). Low mass C/N ratio of macroalgal and total biomass is not optimal for biogas production via anaerobic digestion (I).

## 5. DISCUSSION

### 5.1 Biomass on the substrata

All the species present on the substrata are common in the southern coast of Finland (Hänninen 1996; Vahteri et al. 2000; Lehvo and Bäck 2001). The higher biomass on the rope substrata in Rymättylä compared to Tvärminne in 2012 may be due to both salinity and temperature. The mean salinity was higher in Rymättylä (6.2) compared to Tvärminne (5.2) (I). A large part of the biomass on the substrata is composed of blue mussels that live close to their lower salinity limit in the northern Baltic Sea (Vuorinen et al. 2015). Blue mussels grow larger in higher salinity (Westerbom et al. 2002; Gren et al. 2009), and indeed, the mussel biomass has been reported to decline along with the decreasing salinity from the Archipelago Sea, where Rymättylä lies, to Tvärminne and continues to decline towards the eastern Gulf of Finland (Westerbom et al. 2002). Faster growth rate of blue mussels, driven mainly by higher salinity, also existed in the Archipelago Sea compared to Tvärminne (Westerbom et al. 2002). Other environmental factors, like predation, may also have had impact on differences between the locations in the amount of biomass on the substrata. Low temperature reduces the growth of the mussels (Westerbom et al. 2002). The water temperature in the Archipelago Sea is higher in comparison to the Gulf of Finland (Westerbom et al. 2002). During the years of this investigation, the mean water temperature in Rymättylä was 18.8 °C between the June 14, 2011 and October 12, 2011, and 17.6 °C in Tvärminne. The next year, during the period from May 25 to July 30, mean water temperatures were 15.1 °C and 13.4 °C in Rymättylä and Tvärminne, respectively (I).

The difference in the dominant macroalgal species between the years (Table 2) may be the result of macroalgal species' seasonality in the northern Baltic Sea (Kiirikki and Lehvo 1997; Lotze et al.

1999). This means that, since samples were taken in different seasons, *Ulva* spp. was dominant in October and *Pylaiella littoralis* and *Cladophora glomerata* in July/August, even though the incubation time also differed. Varying environmental conditions between the years and outplacement of the substrata, in addition to seasonality, also impacted on community structure and species abundance during the given time period (Kiirikki and Lehvo 1997; Kraufvelin et al. 2007).

It is likely that the dominance of green algae persists from summer to summer despite the annual and local scale variation in algal abundance. This is supported by previous studies in the northern Baltic Sea (Kiirikki and Lehvo 1997; Golubkov et al. 2018), as well as by the assessed influences of climate change (Takolander 2018). *C. glomerata* has previously been reported as the dominant species in Tvärminne (Kiirikki and Lehvo 1997), and it is characterized as a common species in the northern Baltic Sea (Ólafsson et al. 2013). *C. glomerata* and *Ulva* spp. are the dominant algal species in the uppermost part of the littoral zone in the easternmost and heavily eutrophic part of the Gulf of Finland (Golubkov et al. 2018).

## 5.2 Filamentous macroalgae and invertebrates as nutrient offsets

The effectiveness of cultivation as a bioremediation measure depends on several factors, such as the growth rate of the organisms targeted in the measure and the scale of cultivations in relation to the amount of nutrient removal, as well as the frequency of biomass removal (Carmichael et al. 2012; Verhofstad et al. 2017; Wei et al. 2017). Mussels' ability to filtrate and bind nutrients in particulate organic matter from the water column depends largely on external variables, such as temperature and salinity (Carmichael et al. 2012). Tissue N and P content in macroalgae are determined more by macrolagal species thallus morphology than by nutrient levels in their environment (Bucholc et al. 2014).

The atomic N/P ratio of biomass (Table 3) shows that more N than P is removed by the biomass growing on artificial substrata. A decrease in this ratio could be observable even in the local sea water with large-scale cultivations (Bucholc et al. 2014). In China, cultivation of macroalgae on a large scale has improved the state of entire sea areas (He et al. 2008; Huo et al. 2011). Improvements were obtained by cultivations of red macroalga *Porphyra yezoensis* with 800 kg DW ha$^{-1}$ a$^{-1}$ from a farming area of 300 ha and of *Gracilaria verrucosa* from a farming area of 7.5 ha (He et al. 2008; Huo et al. 2011). Results from the southern Baltic Sea indicate that local improvements in the eutrophication status are possible in sites where beach-cast plant biomass is collected (Bucholc et al. 2014). The C, N and P contents in macroalgal and invertebrate fractions in study I were at the same range as those in Bucholc et al. (2014), with the exception of *Polysiphonia fibrillosa* with a higher content of N (I).

The cultivation of mussels (*Mytilus* sp.) in the Baltic Sea seems to be an efficient tool for controlling eutrophication (Gren et al. 2009), but at least cost efficiency may not be true in the northern areas of the sea where growth is slow (Hedberg et al. 2018). Additionally, the impact of deposits from the mussel culture on biogeochemical processes at the sea bottom needs more investigation (Gren et al. 2009; Stadmark and Conley 2011). Several unwanted environmental consequences are possible when a large-scale mussel cultivation is practiced. Intensive cultivations may lead to changes in phytoplankton communities, collapse of the zooplankton community, and even to plankton blooms or starvation and death of natural mussel populations. Especially in the northern Baltic Sea, where there is no previous knowledge of intense cultures and their impact on the unique environment, cultivation impacts must be thoroughly investigated and monitored (Hedberg et al. 2018).

The biogeochemical processes affected by biodeposits seem either to promote bioremediation or to release nutrients from sediments, depending on local conditions, such as the sediment type (Carmichael et al. 2012). Assimilation into tissues has been estimated as the dominant removal rate for N until harvest size in oysters (*Crassostrea virginica*) cultivated in the northeastern USA. After that, enhanced denitrification by the presence of oyster cultivations can become more important. Scaling up local measurements for bigger installations in different sea areas must be carefully considered, since different environmental conditions and cultivation density may impact on growth rate or physiological state and, furthermore on bioremediation capacity. For example, the oxygen concentration in water may decline at high density cultivations, leading to a reduced nutrient assimilation by bivalves (Carmichael et al. 2012).

Both in Rymättylä and Tvärminne, the substratum area of one ha would produce a harvest of approximately 14 000 kg DW in total biomass after an incubation period of five months from May until October. The next year the total biomass was 33 000 kg DW $ha^{-1}$ substratum in Rymättylä, and 23 000 kg DW $ha^{-1}$ substratum in Tvärminne (Table 2). The annual emissions originating from fish farms in the Archipelago Sea were 2 286 kg N and 294 kg P per operating farm (Mäkinen 2008) if nutrient emissions were shared evenly among the farms operating in the area. Results from Rymättylä show that to ameliorate N and P loads from one farm would require the substrata surface area of almost 11.5 ha in the first growing season. If the cultivation lines consisted of one-meter long rope substrata with similar distances between the ropes as were now in one substratum, 1150 approximately 100- meter-long cultivation lines fulfilled this need. If lines were in 2 m distance from each other, 23 ha of sea surface would be covered. Only 3.6 ha of substrata surface area and consequently 7.2 ha of sea surface area would be required to remove the same amount of nutrients in Rymättylä in the middle of the second growing season (July/August). For similar reductions in Tvärminne, the required surface areas in comparison to Rymättylä were somewhat smaller in the first growing season, and twice as much in the second year. The installations' structure should be designed to promote as high a biomass as possible for a certain area of the sea surface to maximize effectiveness on bioremediation. Blue mussels do grow well in the Baltic Sea at least to the depth of 4 m (Minnhagen 2017). Since most of the biomass on the substrata is composed of blue mussels, we can make a conservative estimate that if the substrata consisted of three meter long ropes, then only 7.6 ha and 5.6 ha of sea surface would be required in Rymättylä and Tvärminne, respectively, at the end of the first growing season to ameliorate nutrient loads from one farm. The sea surface-area demand would be less than 2.5 ha in Rymättylä and less than 5 ha in Tvärminne during the next growing season (in July/August). These could already be considered as feasible surface-area demands. A sea surface area of 350 ha for mussel production has been seen as a realistic area demand on the west coast of Sweden (Lindahl and Kollberg 2008).

Depending on the location and incubation time, an N discharge from two sewage treatment plants of the Finnish Capital region (HSY 2019) could be ameliorated by harvesting the total biomass from a substratum area of 1 400-5 200 hectares. The required substratum area to ameliorate P load from these treatment plants (HSY 2019) only is much less, 284-793 ha. Given the preceding structure just described, with 3 m long cultivation lines, the sea surface-area demand for P removal would still be 190 ha at a minimum. Table 6 presents the required substratum areas to ameliorate fish farm and sewage treatment plant nutrient loads. Annual anthropogenic P load into the Archipelago Sea was, on average, 531 000 kg in years 2008-2012 (Ymparisto.fi). A substratum surface area of 6 600 ha would be needed to ameliorate this. Due to wide area demands, it is clear that the whole human-induced nutrient input to the Baltic Sea cannot be ameliorated by cultivating sessile organisms on the artificial substrata.

**Table 6.** Areas of substratum, ha, required to ameliorate nutrient loads from an average fish farm and the capital region sewage treatment plants in Finland.

| | Required substratum area to ameliorate nutrient load | | | |
|---|---|---|---|---|
| | October 2011 | | July/August 2012 | July 2012 |
| | Rymättylä | Tvärminne | Rymättylä | Tvärminne |
| Average annual discharge from a fish farm 2 286 kg N + 294 kg P* | 11.5 ha | 8.4 ha | 3.6 ha | 7.4 ha |
| Discharge from sewage treatment plants in Finnish Capital Region 1 048 000 kg N + 23 000 kg P** | 5 240 ha | 3 839 ha | 1 392 ha | 2 840 ha |

*in 2006 (calculated from Mäkinen 2008)

**in 2018 (HSY 2019)

Removal of all the attached biomass after the first growing season seems the most feasible practice (I), regardless of the higher content of incorporated nutrients on the second summer, to minimize cultivation-related labor. The labor cost is the major share of the total annual costs in mussel cultivation for bioremediation purposes (Petersen et al. 2014). Bioremediation costs of mussel cultivations are reported to increase the further north in the Baltic Sea the cultivation is practiced (Gren et al. 2009). Much work is needed in the autumn and spring, if cultivation installations were kept underwater over the winter in the northern Baltic Sea, to lower and lift cultivations to prevent the ice from destroying installations during wintertime. However, despite the increasing labor costs in overwinter cultivations, Petersen et al. (2014) estimated N mitigation being the most cost efficient when installations were incubated until May of the next year. This practice resulted in N removal approximately 1.5 times that of the previous autumn (Petersen et al. 2014). Our installations incorporated 3.8 and 1.4 times as much N in July/August compared to October, in Rymättylä and Tvärminne, respectively (Table 4a). Mussel cultivations have survived for several years in the Åland archipelago without submerging (Minnhagen 2017). Since sessile organisms grow several meters below the surface (Minnhagen 2017), an option might also be to install the substrata well below the surface, then to lift them close to the surface the next spring. The actual costs and work hours should be calculated to really understand cost-effectiveness in the northern Baltic Sea.

The mussels in long-line mussel cultivations in Denmark start to fall off the lines after a one-year incubation period (Petersen et al. 2014). Since algal species growing on the substrata are seasonal (Kraufvelin et al. 2007; Takolander 2018), they also detach in the end of their life cycle; consequently, the nutrients bound in them slowly diffuse back into the sea water as the algal biomass decay. Preventing this and avoiding the potential extra labor related to lowering the cultivations supports the idea of an autumn harvest.

Due to apparently high cultivation costs, other utilizations for the biomass should be discovered than just bioremediation alone. Abatement costs for nutrients via mussel farming in the Baltic Sea have been estimated to be lower in the southern part of the Baltic Sea, and even reaching zero, if mussels are sold for food or feed (Gren et al. 2009). The abundance of the blue mussels may decrease in the future due to the predicted decline in salinity in the northern Baltic Sea, including in Finnish waters

(Vuorinen et al. 2015; Westerbom et al. 2019). Blue mussels would no longer be potential factors in bioremediation solutions in these areas in that case. However, sessile invasive species, such as the zebra mussel *Dreissena polymorpha*, might extend their distribution and abundance in the future (Leppäkoski et al. 2002) and become more significant in nutrient offsetting. *D. polymorpha* already inhabits the Baltic Sea and lives in areas with salinity too low for the blue mussels (Oganjan and Lauringson 2014). Filamentous macroalgae in the northern Baltic Sea are so small that their significance in nutrient offsetting seems minimal even if their abundance increased in the coming years (Table 4).

## 5.3 Fertilizers

Biomass from the sea collected for bioremediation purpose can be part of a circular economy when, for example, nutrients of land origin are recycled back to the land as a fertilizer (Seghetta et al. 2016). Composted macroalgae and mussels (Chmielewská and Medved' 2001; Lindahl and Kollberg 2008), macroalgae and mussels as such (Ammenberg and Feiz 2017; Alobwede et al. 2019), macroalgal extract (Shahbazi et al. 2015), or the remaining biomass or process water after fuel conversion processes (Cherad et al. 2013; Pechsiri et al. 2016) could be used for fertilization. Carbon dioxide ($CO_2$) produced in anaerobic digestion and hydrothermal gasification (Cherad et al. 2014, III) can also be used to fertilize aquatic cultures (Cherad et al. 2013; Ferella et al. 2017). Study I shows that total biomass on the substrata could be used to fertilize several crop plant species so that the recommendations of fertilization for these species are fulfilled (I).

Basic requirements in Finnish agriculture allow the use of 325 kg P $ha^{-1}$ farmland during a period of five years; in the horticulture of certain plants, 560 kg P $ha^{-1}$ in five years is allowed (Finnish Food Authority 2019). An annual permitted total N on farmland is 170 kg $ha^{-1}$ in manure and organic fertilizers containing manure (Government Decree 1250/2014; Finnish Food Authority 2019). Biomass should be collected from a substratum area of 0.5-0.6 ha to reach the same amount of N in Tvärminne. This biomass would contain 18-27 kg P depending on the incubation time. A substratum area of 8.5 ha would have been required in Tvärminne 2012 to collect the same amounts of nutrients from macroalgal fraction. Rymättylä and the 2011 growing season in Tvärminne required wider areas. Reaching 170 kg N in Rymättylä from the total biomass would require a substratum area of 0.2 or 0.9 ha with 18 and 25 kg P in 2012 and 2011, respectively. However, since the annual average limit of Cd in farmland in Finland is 1.5 g Cd $ha^{-1}$ (7.5 g $ha^{-1}$ per 5 years) (Ministry of Agriculture and Forestry of Finland 2011), the Cd may restrict the usable amount of biomass and, consequently, the fertilization of farmland. 170 kg N is utilizable only from the total biomass and macroalgal fraction collected in Tvärminne at the end of the first growing season (Table 7), but 170 kg N in macroalgal fraction requires a substratum area of 80 ha in comparison to 0.6 ha for the total biomass. This shows that, depending on the cultivation site, an autumn harvest may be advisable to maximize the utilizable nutrients in relation to heavy metals.

These estimations are based on nutrient and heavy metal contents in biomass (I). Composting or some other processing of material was not tested. The high content of blue mussels in the total biomass may cause a strong odor problem with their direct use as a fertilizer (Olrog and Christensson 2008). Due to the high calcium content of mussel and bay barnacle (Ullmann et al. 2018) shells, the total biomass is likely to also have a liming effect on soil (Olrog and Christensson 2008; Spångberg et al. 2013). High concentrations of heavy metals, especially Cd, are possible in the digestate from anaerobic digestation in the Baltic Sea macroalgae, but removal of heavy metals is possible (Nkemka and Murto 2010; Nkemka 2012).

**Table 7.** The amount of total biomass, kg DW, that contains 1.5 g Cd, as well as the N and P contents, kg, in this amount after five and 14.5-month incubation periods in Rymättylä and Tvärminne.

| | October 2011 | | July/August 2012 | July 2012 |
|---|---|---|---|---|
| | Rymättylä | Tvärminne | Rymättylä | Tvärminne |
| Total biomass, kg DW | 2646 | 13245 | 5144 | 8253 |
| Cd, g | 1.5 | 1.5 | 1.5 | 1.5 |
| N, kg | 37 | 252 | 118 | 134 |
| P, kg | 5 | 40 | 13 | 15 |

Macroalgae improve the soil structure in addition to their fertilizing effect (Harlén and Zackrisson 2001). It would be advisable to also measure the salt content of biomass when used directly as a fertilizer and to rinse it, if necessary. Macroalgae have successfully been used in the cultivation of leek in the Åland Islands (Harlén and Zackrisson 2001).

## 5.4 Biofuels

Macroalgae can be used to produce several types of biofuels (III). Paper III review the potential of macroalgae as raw material in bio-oil, synthesis gas, biodiesel, biobutanol, bioethanol, biogas and $H_2$ production, and study I also reviews producing biogas from the biomass growing on artificial substrata in the northern Baltic Sea.

The chemical composition of macroalgae is often especially suitable for biogas, biobutanol and bioethanol productions because of the high carbohydrate content (III). The water content of fresh macroalgae is high, even over 90% (McDermid and Stuercke 2003); due to this, macroalgae are suitable for conversion processes designed for wet biomass: anaerobic digestion (Milledge et al. 2019) and hydrothermal processes (Schumacher et al. 2011; Cherad et al. 2014). Blue mussels (*Mytilus edulis*) also have a high moisture content (around 60% with the shells, 80% in meat) (Nkemka 2012; Colombo et al. 2016). Drying prior to conversion for biofuel is possible, but if not performed naturally under the sun, it demands extra energy or chemicals and is an extra step in the production process (Herrmann et al. 2015; Milledge et al. 2019). Macroalgal biomass was very low in this study compared to the total biomass on the substrata; thus, in the following chapters, I will evaluate those biofuel conversions that seem the most feasible for the total biomass on the substrata and can tolerate high water content. Evaluation of feasibility is based on information received from papers I, III and the literature searches for this thesis.

### *5.4.1 Biogas*

Biogas production via a microbiological process occurs when methanogenic bacteria digest biomass in anaerobic conditions (Vergara-Fernández et al. 2008). Gröndahl et al. (2009) have analyzed the Baltic Sea macroalgae and blue mussels in a combined use for bioremediation and biogas production.

Based on the net energy budget for the full process chain and calculated on a unit mass of N, blue mussels in the Baltic Sea seem effective in nutrient recovery but not in biogas production, whereas macroalgae were efficient in biogas production (Gröndahl et al. 2009). Biogas production from blue mussels requires almost as much or even more energy than what is achieved from the produced biogas (Gröndahl et al. 2009; Ammenberg and Feiz 2017). Results by Gröndahl et al. (2009) are largely determined by different harvesting methods for algae and mussels, with mussel harvesting being more energy intensive. Due to the current very poor energy balance of mussels in anaerobic digestion (Gröndahl et al. 2009; Ammenberg and Feiz 2017), one can also assume a poor balance for the total biomass. I suggest that conversion of the total biomass for biogas should be investigated anyway, since the northern Baltic Sea macroalgae are small and their proportion on the substrata was low (I).

The mass C/N ratio is crucial in biogas production via anaerobic digestion, in which enough C in relation to N ensures optimal biogas yield (Habig et al. 1984; Marquez et al. 2014). Too high N content may lead to formation of excess ammonia ($NH_3$), thus inhibiting biogas production. Lack of N reduces bacteria growth (Marquez et al. 2014). A favorable mass C/N-ratio in the digester is suggested to lie between 25 and 30 (Marquez et al. 2014). Total biomass had a mass C/N ratio of 10 in both years in our study. The only fraction that reached the optimal ratio or had it close to optimal was *Ulva* spp. in 2012 (Table 3). Due to the low C/N mass ratio, mixing with higher C-containing material is a preferred solution for biogas production (I). Optimization of the anaerobic digestion process has been shown to have a large impact on decreasing the greenhouse gas emissions and on improving the energy budget of the whole supply chain of cultivated kelp (*Saccharina latissima*) to biogas and fertilizer production in western Sweden (Pechsiri et al. 2016). Upscaling of the cultivation area also improved energy returns on investment and decreased the greenhouse gas emissions per unit of produced biogas (Pechsiri et al. 2016).

Previous studies from the Baltic Sea confirm the necessity of the addition of carbon-rich material when macrophytes and macroalgae are used for anaerobic biogas production (Dubrovskis et al. 2012; Bucholc et al. 2014). Another factor that needs to be considered is that a high concentration of bioavailable heavy metals in the raw material may reduce methane ($CH_4$) yield (Nkemka and Murto 2010).

### *5.4.2 Synthesis gas*

From the technical point of view, hydrothermal gasification might be one of the most suitable conversion methods for the total biomass attaching to the substrata.

The method is tolerant of the ash content and alkali salts (Cherad et al. 2014), both of which can be relatively high in macroalgal biomass (Schumacher et al. 2011, III). In fact, inorganic salts appear to corroborate the formation of high gas yields, since the salts act as catalysts promoting the gasification (Schumacher et al. 2011; Cherad et al. 2014). Conversely, they also cause fouling and slagging of ash (Ross et al. 2008; Cherad et al. 2013) if not handled with technical solutions (Cherad et al. 2014). Macroalgae, like meat of mussels (*Mytilus* sp.) (Fernández et al. 2015), are also often rich in carbohydrates (III), which is suitable for hydrothermal gasification (Cherad et al. 2014). Especially gasification in supercritical water results in near to complete gasification of feedstock (Cherad et al. 2014) and seems efficient with macroalgae (Cherad et al. 2014, III). Gas produced via this method consists primarily of $H_2$, $CO_2$ and $CH_4$ (Schumacher et al. 2011; Cherad et al. 2014) and can further be used to produce other products (Spivey & Egbebi 2007).

Constituents of biogas and synthesis gas can be used to produce several compounds or be separated and used for distinct purposes (Cherad et al. 2014; Ferella et al. 2017; III). Side products of these processes, such as the process water or digestate, can also further be used as fertilizers, for example (Cherad et al. 2014; Pechsiri et al. 2016).

## 5.5 High value products

Nutritional supplements, cosmetics and pharmaceuticals (Pereira et al. 2012; Pimentel et al. 2018) are potential algal and mussel high-value products (Pereira et al. 2012; Fernández et al. 2015). Concerning the FAs, the amounts and ratios of PUFA/SFA and n-6/n-3 series FAs are especially significant for these fields (Simopoulos 2006; Pereira et al. 2012).

Human nutrition should include more PUFAs than saturated fatty acids (SFAs) (Pereira et al. 2012; McCauley et al. 2016). *Ulva intestinalis* was the most favorable for its PUFA content and the ratio of PUFAs/SFAs, in this sense.

Vertebrates are not able to synthetize n-3 FAs EPA and DHA sufficiently for their needs; therefore, their intake from feed and food is important (Burdge and Wootton 2002; Burdge et al. 2002; Pereira et al. 2012). EPA is often the most abundant PUFA in macroalgae (Pimentel et al. 2018). EPA was the most abundant PUFA in *Ceramium tenuicorne* in study II and the second most abundant PUFA in *C. glomerata*. The highest concentration of EPA in our study was measured in *C. glomerata*.

*C. glomerata* is the choice with TFA contents of 7 – 8.7 mg $g^{-1}$ DW (II) when only maximum TFA content is looked for. Low n-6/n-3 ratio, which was detected in all three species – *C. glomerata, U. intestinalis* and *C. tenuicorne* (II) – is considered favorable for human health (Pereira et al. 2012). The FA composition and content may remarkably change seasonally in macroalgae (II, III), whereas seasonal variation in DHA content in blue mussels (*M. edulis*) has been documented (Fernández et al. 2015).

Other algal compounds, in addition to FAs, with commercial interest are pigments, lipids, proteins, polysaccharides and phenolic compounds (Stengel et al. 2011). Blue mussels (*M. edulis*) also have potential in production of nutritional supplements due to their protein, lipid and carbohydrate composition (Fernández et al. 2015). Since the algal biomass on the substrata was low, high-value products might be the most feasible utilization if algae alone were utilized. Algae differ widely in their chemical composition based on their taxonomic entity (Stengel et al. 2011). Due to this, and since the dominant species were the green algae, in both locations and years, applications solely based on algae might be the most effective when designed suitably for the green algae *C. glomerata* and *Ulva* spp. However, separation of these two species from the other algae may be difficult. Dominance of one species might be obtained by adjusting the incubation time, location and substratum material, as can be indicated from study I's results.

## 5.6 Restrictions of the use of biomass

### *5.6.1 Heavy metals*

Not only macroalgae (Chan et al. 2003; Akcali and Kucuksezgin 2011), including *C. glomerata* (Chmielewská and Medved' 2001), *P. littoralis* (Lill et al. 2012) and *U. intestinalis* (Gubelit et al.

2016), but also barnacles [*Balanus amphitrite*, current name *Amphibalanus amphitrite* (WoRMS 2018)] (Rainbow and Wang 2005) are reported to accumulate heavy metals.

Heavy metal concentrations in the surface sediments of the northern Baltic Sea have declined during the past decades (Vallius 2014). However, As, Cd and Hg remain at levels harmful to the environment in some areas (Vallius 2014). Eutrophication may enhance heavy metal bioavailability when decaying organic biomass causes anoxia in bottom sediments, releasing heavy metals bound in sediment (Gubelit et al. 2016). Cd, Cu and Zn also bind strongly in the algal tissue in around pH 7 (Carrilho and Gilbert 2000). Hypoxic conditions in the Baltic Sea may result in a pH as low as 7.2 (Ulfsbo et al. 2011), and climate change is predicted to generally decrease the pH of the Baltic Sea (Takolander 2018). Cd and Zn concentrations in all fractions (the red algae are excluded from the comparison, since we had no metal samples of them from Tvärminne) were higher in Rymättylä than in Tvärminne in our study. Higher overall heavy metal concentrations in Rymättylä than in Tvärminne remain unclear. Heavy metal concentrations vary from site to site in the northern Baltic Sea (Voigt 2007), and they should always be checked prior to utilization of the biomass.

Limits set for certain metals were exceeded if feed or fertilizer were composed exclusively of a certain fraction or fractions of biomass on the substrata. The content of As in filamentous brown algae exceeded the limit for a certain type of animal feed, as determined in Directive 2002/32/EC of the European Parliament and of the Council. The content of Cd exceeded the limits for several types of animal feed in all the other taxa, either of the year or the location, except in *Ulva* spp. Pb also exceeded thresholds for certain types of animal feed, except in invertebrates, *Ulva* spp. and *P. fibrillosa* (Directive 2002/32/EC). The limit values of Cd for a farmland fertilizer were exceeded in *P. fibrillosa* and filamentous brown algae (Ministry of Agriculture and Forestry of Finland 2011).

### *5.6.2 Availability of biomass and technology*

Besides heavy metals, another potential restriction on the utilization of macroalgal and invertebrate biomass is the current lack of established technology to handle marine biomasses other than fish in the northern Baltic Sea. Processes must be adjusted to handle new biomasses, most likely in annually varying quantities.

The naturally occurring seasonal variation in the abundance and chemical composition of organisms in the northern Baltic Sea pose a challenge for utilization. Physical conditions during winter (ice, storms) may also prevent harvesting of biomass and cut the continuous supply of biomass. Furthermore, if the availability of the required biomass or compound is not continuous, this may interfere with the production process and finally restrict commercialization of the product.

## 5.7 Establishment of the cultivations

### *5.7.1 Selecting a site for the installations*

Biomass cultivation infrastructure should be located so that it does not conflict with other activities on the site, such as boat traffic or fishing. Environmental conditions should be suitable for installing cultivation infrastructure and collecting biomass, i.e., the water depth, water current and openness to wind and waves should be such that they do not prevent cultivation operations. Table 8 presents issues to be considered when selecting a site for the cultivations, as well as practical features for the substratum. The effectiveness of the system increases in bioremediation if light and temperature are not limiting the growth (Lamprianidou et al. 2015). One should take installation in the low heavy metal location into consideration when the biomass collected is going to be further utilized and the target is not the removal of metals. This may mean lower nutrient concentrations in the water in some locations, since eutrophication and high heavy metal concentrations seem to be interlinked (Voigt 2007). However, biomass with low heavy metal concentration is potential raw material for food and feed applications, fertilizer (I), and for several biofuel production techniques (III). Study I indicated that macroalgae can grow on thin growth substratum, but the sessile fauna was not present on it. Installations should be incubated two meters below the surface if the aim is to grow mussels and avoid barnacles and algae (Hamilton et al. 2013).

A controlled way to promote biomass cultivations would be to include potential sites in the Maritime spatial plan that is under development and going to be completed in 2021. Sites that are clearly unsuitable could be avoided in this way. Monitoring of the environmental impacts of the cultivations should still be carried on, because there is very little information on their impact, especially in the northern Baltic Sea with its unique environmental characteristics (Hedberg et al. 2018). After all, finding space for large-scale cultivations will be especially difficult close to the shore where nutrient loads occur. The Archipelago Sea has narrow waterways and a lot of traffic. Traffic is high also in the Gulf of Finland. Nutrient-induced changes closer to the shore might not be alleviated if cultivations are located in the open sea. Several small-scale cultivations at a distance from each other make utilization of the harvested biomass difficult. Local utilization of harvest seems recommendable for small-scale cultivations.

**Table 8.** Issues to be considered when selecting the right site for the installation to collect biomass and practical features for the substratum. Importance of harmful substances depends on the application of how the biomass is about to be treated.

| Site of installation |
|---|
| - appropriate in view of the investigation<br>- enables desired quality of biomass (impact of nutrients/harmful substances)<br>- not in the way of other activities, e.g., traffic<br>- vulnerability of nearby habitats<br>- ease of installation, ease of growth collection<br>In view of further use:<br>- distance to the end user<br>- eventual harmful substances |
| Substratum |
| - robustness, resistant to the power of water movement<br>- shape and structure, supports good growth of attached organisms<br>- easy to handle<br>- reusable, sustainable |

*5.7.2 Planning the production chain*

Prior to a full-scale establishment of the cultivations for bioremediation and other purposes, basically the whole production chain must be carefully planned. Figure 3 presents the steps prior to establishment of commercial cultivation. Steps for production of high-value products, such as ingredients for cosmetics, may differ from biomass production for bioremediation, fertilizer and biofuels, uses that only require high quantities of biomass (Fig. 3).

When a decision to start cultivations is made, the first step for both purposes is to survey suitable cultivation sites while considering the ecological aspects and impacts to the other marine uses. Since the quality and quantity of available biomass depends on the site (I), the factors affecting it also need be evaluated. Second, the structure of the installations ought to be designed for easy maintenance and harvest and for maximal quantity of desired biomass. Besides the installations, the equipment and a boat needed for harvesting also ought to be efficient and easy to handle. Third, it should be studied whether the installations must be lowered, removed or if they can stay as they are over winter. Harvesting methods for the biomass composing of both invertebrates and algae could probably be adopted or modified from the harvesting methods used for mussels, since blue mussels are the main component of biomass. Fourth, since both the yield and the chemical composition of the biomass depend on the season and harvesting frequency, they both should be optimized. The fifth question to be solved is the logistics of storage and transport of the harvested material to the end users on land.

Utilization of biomass for high-value products starts by identifying valuable components in algae or in other biomass (Fig. 3). The next step is to identify the factors influencing the accumulation of the valuable components and how the accumulation could be maximized. Commercial-scale extraction may need improvements to the current technology, adjusted to the Baltic Sea species, although several macroalgal compounds are commercially utilized today (White and Wilson 2015; Malta and Agraso Martínez 2017). Establishment of the commercial-scale production chain may also involve the environmental impact assessment, environmental permits and authority inspections.

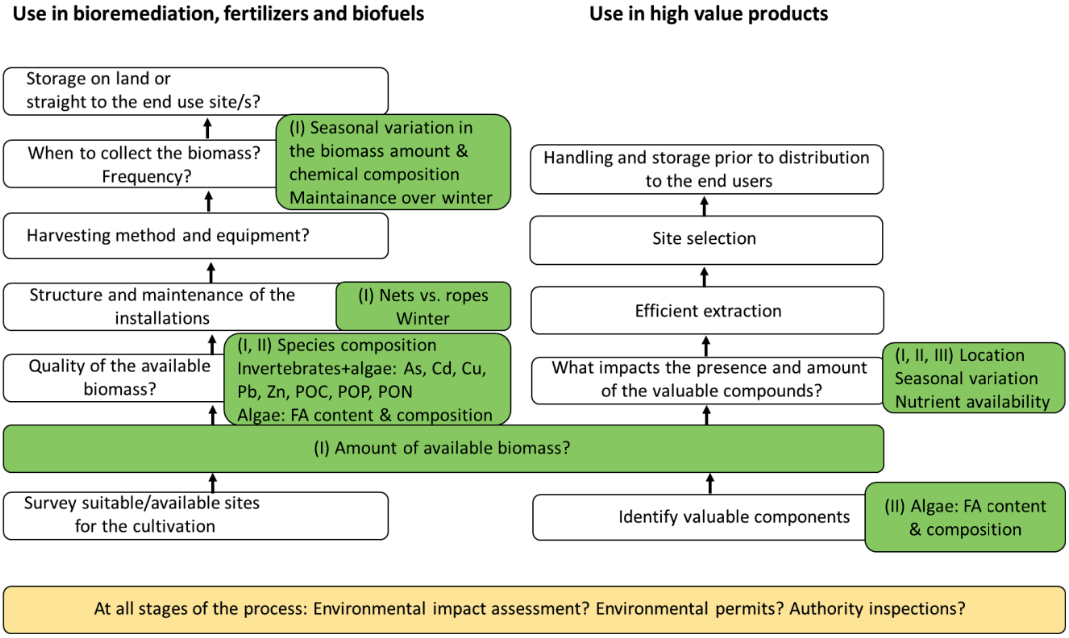


**Fig. 3.** The key steps to be solved out prior to a full-scale establishment of the cultivations for bioremediation and other purposes. Text in the green circles identifies which issues were investigated in studies I-III.

Value in bioremediation can be calculated by comparison to the other bioremediation measures. Costs related to bioremediation are likely to decline if a chain of multiple utilizations for the produced biomass can be realized (Baghel et al. 2016; Tedesco and Stokes 2017). Baghel et al. (2016) demonstrated refining six products of commercial value from one macroalgal sample without compromising each product's yield. Tedesco and Stokes (2017) also report $CH_4$ content in biogas after extraction of high-value compounds close to the yield of unextracted biomass. A profitability analysis by Baghel et al. (2016) has resulted four times higher profit for a stream of products compared to the agar production alone from the red macroalga *Gracilaria corticata*. The stream of multiple products from the northern Baltic Sea biomass should be investigated. Meanwhile, due to the general lack of infrastructure for mussel- (Ammenberg and Feiz 2017; Minnhagen 2017) and macroalgal- (Balina et al. 2017) based products or other utilization in the Baltic Sea, the most likely profitable utilization for the total biomass might be a direct use as a fertilizer, as has also been estimated for the blue mussels (Ammenberg and Feiz 2017). This does not require establishment of processing plants and not necessarily even a continuous substrate yield. Mussels' fertilization effect has been tested in Sweden with the results on the crop yielding ca. 85% compared to the chemical fertilizer (Olrog and Christensson 2008). Another option would be using the collected biomass in a local-scale biogas plant as an additional substrate. Use of blue mussels as animal feed has also been estimated to be more profitable than their use in biogas production (Ammenberg and Feiz 2017). The small size of the mussels and macroalgae in the northern part of the Baltic Sea makes reaching economic sustainability even more difficult compared to the southern part of the sea.

In summary, challenges to achieving the economical sustainability of biomass production in the northern Baltic Sea are the small size of the mussels and macroalgae, the lack of cultivation and utilization infrastructure, cold wintertime, seasonality in the amount and chemical composition of macroalgal biomass and seasonality in chemical composition of mussel biomass. Even a lack of knowledge of the abundance of utilizable algae (Balina et al. 2017) and a lack of knowledge of the utilizable chemical components hinder currently a wide-scale utilization of sessile organisms in the northern Baltic Sea. Figure 3 represents which steps were investigated in this thesis.

## 5.8 Assessment of the methodology used in the studies

Commonly used methods to study organisms' nutrient offset capacity are particle removal by deposit feeders, nutrient assimilation and biogeochemical processes brought about by invertebrate or algal assemblages (Carmichael et al. 2012). The total biomass on a certain area also impacts an organism's bioremediation capacity (Petersen et al. 2014). The effectiveness of biomass removal in bioremediation can be estimated by comparing its costs to the costs of other actions against eutrophication (Gren et al. 2009; Petersen et al. 2014). The ice cover during winter may create an extra workload and require special solutions and careful evaluation of the most feasible incubating period for the substrata.

The biomass yield and tissue (including the shell and gut content) nutrient content in this study were used to evaluate the bioremediation potential of macroalgae and sessile invertebrates. Tissue nutrient content is commonly considered a reliable method, since measuring assimilation by particle removal or filtration rate is difficult, and biogeochemical processes vary according to local conditions (Carmichael et al. 2012). Studies on sediment N and P fluxes are recommended when cultivating biomass for bioremediation purposes (Stadmark and Conley 2011).

$NO_2$, $NO_3$, $NH_4$ and $PO_4$ were measured in study II's aquarium tests, since they represent nutrients N and P in the forms readily available for macroalgae (Corey et al. 2013).

Heavy metal concentration in biomass can be used to estimate usability of biomass in solutions in which heavy metals may cause harm or when there are limits for it in the end product (Petersen et al. 2014; III).

Temperature and salinity affect growth and chemical composition of algae and mussels (Westerbom et al. 2002; Gren et al. 2009; Stengel et al. 2011), so these parameters were used as background information for differences in the biomass on the substrata.

It would be interesting to focus in the future on the bioavailability of nutrients and heavy metals from the collected marine biomass for crop plants in the northern climate. The impact of biomass on the soil structure should also be studied. The actual costs of the cultivation and harvesting, including labor cost, should be compared to the value attainable from the collected biomass.

# 6. CONCLUSIONS

On the DW basis, sessile organisms naturally attaching to the artificial substrata in the northern Baltic Sea consisted mainly of the invertebrates blue mussels (*Mytilus trossulus*) and bay barnacles (*Amphibalanus improvisus*) and the hydroids (*Cordylophora caspia* and *Gonothyraea loveni*). The size of the organisms is small and their biomass per area on the substratum of both invertebrates and macroalgae is low in the northern Baltic Sea in comparison to the oceanic and southern Baltic Sea sessile organisms. Due to the low biomass and the practically complete lack of infrastructure to collect and refine this biomass, the most suitable utilizations might currently be 1. removal of nutrients from the sea, 2. use as fertilizer as such or after composting, and 3. as additional feed material in biogas production in locations where the distance is short from the harvesting site to the plant. Utilization of the total biomass is advisable, since macroalgal biomass formed less than 5% of the total biomass.

Amelioration of small-scale nutrient loads can be accomplished by removing biomass from the substrata. A large-scale amelioration would require such a wide substratum and sea surface areas that the area demand seems impractical. Therefore, non-seeded artificial substrata with naturally attached organisms is not a solution for amelioration of the whole human-induced nutrient input to the Baltic Sea. Costs related to the operation were not calculated in this study, and the actual costs and overall workload should be calculated to really understand cost-effectiveness in the northern Baltic Sea. Solutions that include several utilizations are likely to be economically most feasible.

The utilizable amount of the total and macroalgal biomass depended strongly on the Cd content in biomass for their direct use as a fertilizer. The Cd content varied according to the site and to the incubation time of the substrata being higher in Rymättylä than in Tvärminne. As, Cd, Cu, Pb and Zn in algae were taxa dependent and, on average, far lower in the invertebrate fraction than in macroalgae.

Combining with other feed material is recommendable for biogas production by anaerobic digestion, because, as the only feed, the mass C/N ratio both in macroalgae and in total biomass was low, enabling formation of excess $NH_3$, which is toxic to methanogenic micro-organisms, thus inhibiting biogas production.

The limit values for Cd, set in the EU regulation, were exceeded for several types of animal feed, depending on the time and location of sampling, but the limit was never exceeded in *Ulva* spp. The content of As exceeded the limit for a certain type of animal feed in filamentous brown algae. Pb exceeded thresholds for certain types of animal feed, except in invertebrates, *Ulva* spp. and *Polysiphonia fibrillosa*.

The macroalgal FA concentrations were more related to taxonomy than nutrient availability. Only in *Cladophora glomerata* did it seem that n-6 series FAs, LA and AA could be regulated by nutrient manipulations. Elevated concentrations of these FAs were associated with higher N availability. The LA is an essential FA in vertebrates' nutrition. *Ulva* spp. seems promising to human and animal nutrition with respect to both its metal content and the FA profile and composition. *U. intestinalis*, *C. glomerata* and *C. tenuicorne* all had low n-6/n-3 ratio, which is considered favorable for human nutrition.

The naturally occurring seasonal variations, not only in the abundance and chemical composition of organisms in the northern Baltic Sea but also in their physical conditions (ice, storms), determine the feasible harvesting time for the biomass. The right time may vary according to what type of biomass is required.

In conclusion, sessile biomass collected from the northern Baltic Sea has potential for several utilizations. However, development of the processes from cultivation to utilization and a cost-efficiency analysis for different utilizations are badly needed. Low biomass is a challenge for utilization.

# 7. ACKNOWLEDGEMENTS

This thesis work was financed by The Foundation for Research of Natural Resources in Finland, Neste Oil Ltd., Maa- ja vesitekniikan tuki ry, and the European Regional Development Fund through the Baltic Sea Region Programme 2007-13 project Sustainable Uses of Baltic Marine Resources, SUBMARINER.

The field work was performed in the vicinity of Tvärminne Zoological Station, University of Helsinki, Hanko and the Finnish Game and Fisheries Research Institute, Rymättylä. I would like to thank the personnel of both stations. I also want to thank Markku Riittonen for the boat and assistance during the installation of the experiment in Rymättylä. Thank you to Ari Ruuskanen and Lari Järvinen for your help after winter when the submerged installations had to be located.

I would especially like to thank my supervisors, PhD Elina Leskinen, PhD Kirsi Kostamo and PhD Jukka Seppälä. Thank you for your support, guidance, patience and all the time you have given throughout these years. Thank you also for contributing to the papers included in this thesis. Elina, I especially thank you for your endless positivity, effort and feedback considering this work. Thank you, Kirsi, for the great tips regarding the direction of this work and seeing the big picture. Jukka, thank you for your support with the laboratory and field work and for providing me the excellent facilities of the Finnish Environment Institute, as well as a number of laboratory workers. Without your belief in my work, I would never have completed it.

Many thanks to Prof. Kimmo Kahilainen for acting as custos and to Prof. Emer. Ilppo Vuorinen for acting as my opponent. I am grateful for the pre-examiners of my thesis, Prof. Anna-Stiina Heiskanen and Prof. Veijo Jormalainen.

Sincere thanks to the members of my thesis advisory committee, Prof. Jorma Kuparinen, Prof. Hannu Lehtonen, and Prof. Jukka Horppila, for having faith that I am on a right path with my thesis work.

Warm thanks to my co-authors Marika Tossavainen, Kristian Spilling, Kurt Fagerstedt, Jorma Kuparinen, Pirjo Kuuppo, Jaanika Blomster and Janni Ketola. Thank you for investing your time and expertise towards my PhD work. Kristian Spilling, thank you also for giving a hand in the field. I am very grateful to Marika Tossavainen, who shared her expertise in fatty acids. I want also to thank Jukka Pellinen for the instructions regarding laboratory work in the AlmaLab, University of Helsinki.

Warm thanks also for you all not yet mentioned: people who participated in the laboratory and field work. You kept up a good spirit, and we had a lot of laughs and good conversations.

Thanks to my great colleagues at the university and the Finnish Environment Institute, it was always nice to come to work.

I wish to express special thanks to Jaanika Blomster, who originally guided me to the path of PhD studies. Also, warm thanks to Kaj Kronström for encouraging me to start with this topic.

Thank you also for my parents for the summers in the archipelago. They were the best summers ever and especially when accompanied by Aila, Jussi and Nelli the dog. I believe those summers ignited my love towards the Baltic Sea.

And finally, Matti, thank you for everything.

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