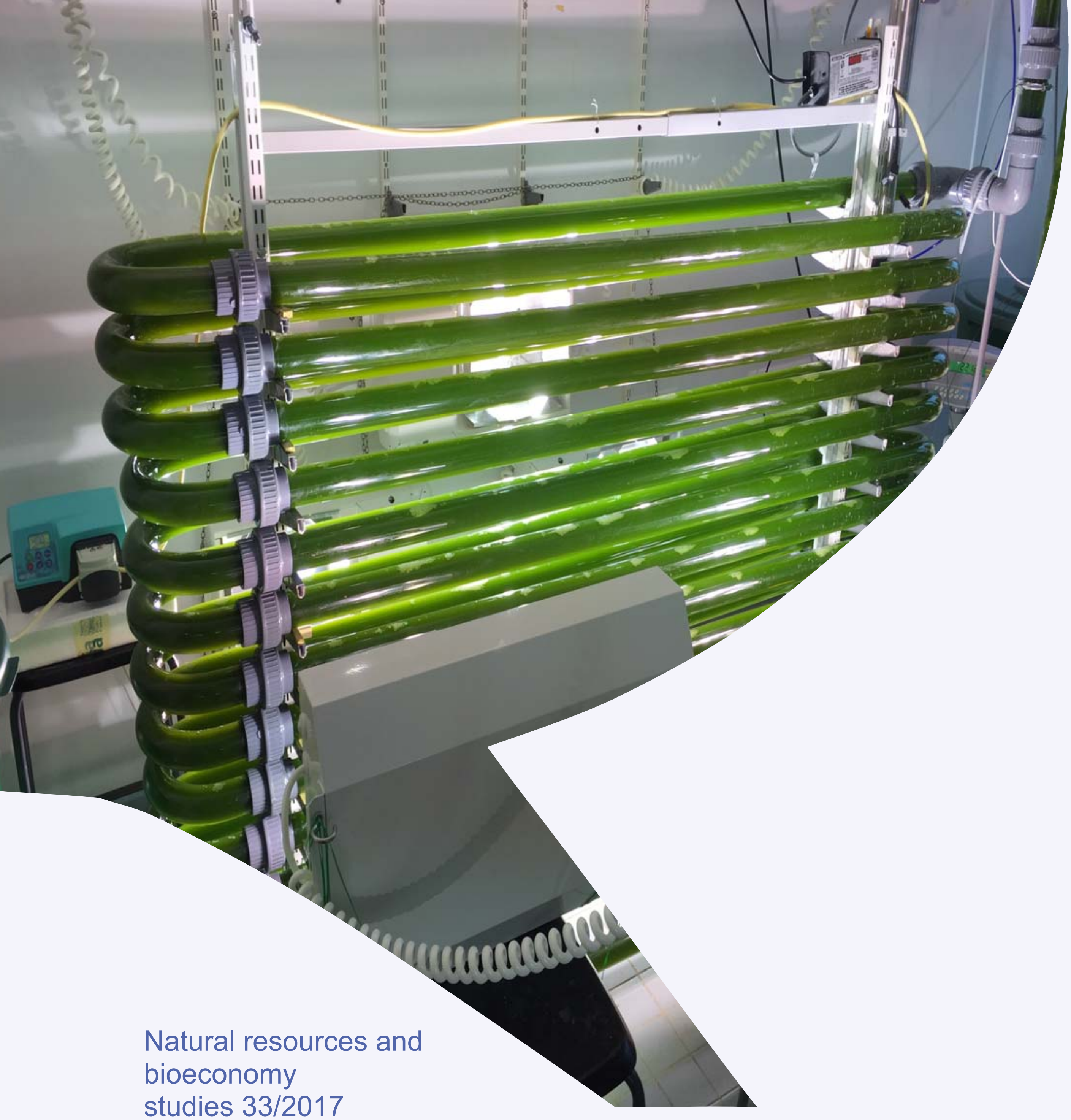

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Utilization of microalgae in industrial symbiosis, focus on Finland

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Abstract

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Large-scale cultivation of algae in Finnish conditions is challenging. Seasonal variation in weather conditions in terms of light and temperature invites to select closed photobioreactors for algal cultivation. Furthermore, the photobioreactors need to be placed indoors. Artificial illumination is needed because sunlight is not sufficient for year-round cultivation in Finland. Methods of cultivation, harvesting and dewatering will be discussed.

Algae can be cultivated in wastewaters for purification, for uptake and recycling of nutrients, and for production of algal biomass. Wastewaters from municipalities, industries and agriculture can potentially be utilized. The most important factor in wastewaters is their nutrient content.

One possible place for growing algae is in recirculating aquaculture, where algae can potentially be used to remove ammonium. Cultivation of algae in greenhouses in combination with plants might allow utilization of light energy that is currently wasted at the corridors. Algae might also be applied in underground tunnels (for example in landfills) that have a constant temperature and in which wastewater is available. However, artificial illumination is the only available light source.

Combination of algal cultivation with existing industries offers the possibility to combine wastewater purification with production of lipids, biogas and fertilizers from the algal biomass, following the biorefinery concept. Pulp and paper industry produce excess heat, carbon dioxide and wastewater, all of which can be utilized for growing algae. Unfortunately, industrial wastewaters may not have enough nutrients, and therefore it might be necessary to add some of the nutrients or to combine industrial and municipal wastewaters.

Biodiesel, bioethanol and biogas are the most commonly discussed energy products but several non-energy products can also be obtained from algal biomass. Microalgal lipids, pigments, proteins and carbohydrates are potential high value non-energy products. Fish feed and fertilizers may also be produced. Production of high-value compounds usually requires genetic modification of the algae.

In conclusion, industrial algal cultivation in Finland is technically possible when combined with the use of wastewaters and with the exploitation of existing infrastructure. Use of indoor spaces and artificial light is necessary for all options. The economic feasibility of algal cultivation remains to be elucidated.

Keywords: Microalgae, photobioreactor, greenhouse, valuable compounds, production environment, integrated solutions, exploitation

Summary in finnish

Levien teollinen ja laajamittainen viljely Suomessa on haastavaa. Vuodenaikojen ja sään vaihtelu suosii sisätiloihin asennettavien suljettujen fotobioreaktorien valintaa levänkasvatusmenetelmäksi. Koska auringon valo ei riitä ympärivuotiseen kasvatukseen Suomessa, tarvitaan myös keinovaloa. Raportissa tarkastellaan kasvatusmenetelmiä, biomassan keruuta ja vedenpoistoa.

Leviä voidaan kasvattaa jätevesissä, jolloin ravinteiden kierrätys ja biomassan tuotto yhdistyvät luontevasti jätevesien puhdistukseen. Kaupunkien, teollisuuden ja maatalouden jätevesiä voidaan hyödyntää. Tärkein tekijä jätevesissä on niiden ravinnepitoisuus.

Yksi mahdollinen paikka levän kasvatukseen on kiertovesikalankasvatuslaitos, jossa levää mahdollisesti voidaan käyttää ammoniumtypen poistamiseen. Levänkasvatus voidaan myös mahdollisesti yhdistää kasvihuoneviljelyyn, jolloin viljelyrivien väliin kohdistuva ja hukkaan menevä valoenergia saadaan hyötykäyttöön. Muita mahdollisia levänkasvatuksen sovelluskohteita voivat olla kaatopaikkakompleksien yhteydessä sijaitsevat maanalaiset tunnelit, joissa vallitsee tasainen lämpötila ja joissa on jätevettä saatavilla. Tosin tunneleissa keinovalo on ainoa mahdollinen valonlähde.

Levänkasvatuksen yhdistäminen olemassa olevaan teollisuuteen tarjoaa mahdollisuuden tuottaa jäteveden puhdistamisen yhteydessä leväbiomassasta biojalostamotyyppisesti useita tuotteita kuten lipidejä, biokaasua ja lannoitteita. Sellu- ja paperiteollisuus tuottaa jätevesiensä lisäksi hiilidioksidia ja lämpöä, joista molempia tarvitaan levien kasvattamiseen. Teollisuusjätevesissä ei tosin ole välttämättä riittävästi ravinteita, minkä vuoksi voi olla tarpeen lisätä osa ravinteista tai yhdistää jätevesiin yhdyskuntajätevesiä.

Biodiesel, bioetanoli ja biokaasu ovat yleisimmin esitetyt levistä saatavat energiatuotteet, mutta leväbiomassasta voidaan jalostaa energiatuotteiden lisäksi myös muita orgaanisia molekyylejä. Lipidit, pigmentit, proteiinit ja hiilihydraatit ovat mahdollisia korkean lisäarvon tuotteita. Myös kalanrehua ja kasviravinteita sisältäviä lannoitteita voidaan tuottaa. Korkean lisäarvon tuotteiden valmistaminen vaatii yleensä levien geneettistä modifioimista.

Yhteenvetona voidaan todeta, että levän teollinen kasvatus Suomessa on teknisesti mahdollista kun se yhdistetään jätevesien hyötykäyttöön ja kun käytetään olemassa olevaa infrastruktuuria. Sisätilat ja keinovalo ovat tarpeelliset kaikissa tarkastelluissa vaihtoehdoissa. Levän kasvatuksen taloudellinen kannattavuus jää ratkaistavaksi.

Contents

1. Cultivation and harvesting technologies of algae

1.1. Cultivation of algae

Microalgae can be successfully cultivated in open ponds or closed photobioreactors (deVree et al., 2016). Some types of cultivation systems are presented in Fig. 1.

Figure 1. Outdoor cultivation systems (from top to down: raceway bond, horizontal tubular and vertical tubular) in operation at AlgaePARC facility, in the Netherlands. (Photos by courtesy of Dr. Rouke Bosma at Wageningen UR, AlgaePARC).

A traditional open pond is the simplest method for cultivation of microalgae, and open ponds are still widely applied in industrial algaculture. Temperature and other environmental conditions fluctuate in an open pond both diurnally and seasonally.

An open pond may consist of circular ponds or looped channels, often in a form resembling a raceway ("raceway pond", Carvalho et al., 2006). The pond is typically ~30 cm deep, and a paddle wheel is used for mixing and circulation. Baffles are placed in the bends to guide the flow. Evaporation cools an open pond but the loss of water through evaporation can be significant. Furthermore, a large part of added carbon dioxide escapes to the air from an open pond (Chisti, 2007).

Disadvantages of open systems are the (1) contamination cannot be controlled, (2) it is difficult to maintain the environment constant and (3) harvesting is costly because the algal suspension must be kept thin. Very often, the contamination problem is solved by cultivating an organism that can grow and dominate the culture in highly selective conditions. Examples of such organisms are the cyanobacterium *Arthrospira* (spirulina) that grows in pH exceeding 10, and the green alga *Dunaliella salina* that grows in brine. Due to low cell density, a very large volume of the culture must be treated for harvesting. High harvesting costs substantially increase the final cost of the product (Carvalho et al., 2006).

Contaminants, infections, grazers and environment can be more easily controlled in a closed photobioreactor. Furthermore, several times higher cell mass productivity can be achieved than in an open system. However, the advantages of closed systems come together with higher costs of both investment and running, compared to costs of open systems (Carvalho et al., 2006).

Three basic types of closed system shapes have been applied: tubular (Fig. 1), flat panel and fermenter-type. Tubular and flat panel reactors can harvest sunlight efficiently whereas fermenters can only be used with artificial illumination. In tubular and flat panel reactors, the algae are enclosed in a transparent unit consisting of small diameter tubing or a flat panel. The transparent light harvesting structure maximizes penetration of light to the culture by maximizing the ratio of illuminated area to volume. In addition to the light harvesting unit the reactors have a gas exchange system in which CO_2 is added to the culture and excess O_2 is removed. The culture is pumped through the system (Carvalho et al., 2006), and the volume of the gas exchange unit should be relatively small. Biomass can be harvested either continuously or after batch cultivation.

The pumping of the culture through the photobioreactor is often achieved with an airlift that can simultaneously act as a gas exchange unit. The tubes can be plced at the same horizontal level or on top of each other; also helical tubing has been applied (Carvalho et al., 2006).

Tubular photobioreactor is the most commonly applied photobioreactor type in experimental outdoor mass culture systems. There are still several problems in large scale tubular photobioreactors like temperature control and removal of oxygen along the tubes (Ugwu, 2008). Tubes of a photobioreactor are generally 10 cm or less in diameter because light would not penetrate to a thick tube containing a high concentration of pigmented algae. High biomass concentration, in turn, is necessary for high biomass productivity of and for easy harvesting (Chisti, 2007).

Sunlight is free but its intensity fluctuates due to the day/night cycles, seasonal changes and changes in weather. These fluctuations in irradiance can be prevented or mitigated by applying artificial lighting, which usually causes an increase in productivity (Blanken et al., 2013). Artificial illumination in a photobioreactor is expensive but nonetheless, artificial illumination is being used even in industrial algaculture, particularly for high-value products (Chisti, 2007). Electricity is one of the main expenses in artificial illumination, so saving electricity by using led lights is important.

Intensity and wavelength of light are the most important parameters affecting microalgal growth in a photobioreactor. Excessive intensity causes photoinhibition, whereas low light levels limit growth rate (Carvalho et al., 2011).

Production costs of existing microalgal plants are published very seldom (Sun et al., 2011), and the lack of existing facilities makes approximations uncertain (Acién et al., 2012). Cyanobacteria have been produced for centuries in open ponds in warm climate but there is little experience on production of cyanobacteria or algae on a larger scale under outdoor conditions in moderate climate.

One of the biggest existing photobioreactors is situated in Klötze, Germany, where 130-150 t dry biomasses is produced yearly in a vertical tubular photobioreactor with glass tubes. The total length of the tubing is 500 km and the volume is 700 m^3, and the tubing is situated in a 1.2 ha glasshouse (Spolaore, 2006). A report on algae and bioeconomy by Lunkka-Hytönen et al. (2016) gives a comprehensive review of present algal research in Finland, Europe and outside of Europe.

1.2. Harvesting of algae

There are three major challenges in the harvesting of microalgae. The first is the dilute nature of the algal broth, typically less than 0,5 g/L in an industrial system, due to which large volumes of culture need to be handled to recover algal biomass. Secondly, algal cells are typically only 2-20 µm wide, which makes it difficult to separate them from the liquid. Thirdly, these small cells generally have an electronegative surface charge in a wide pH range. In addition, the variety in size, shape and motility among different algal species makes is difficult to develop a single technique suitable for the recovery of all species (Wang et al., 2015). There are three major challenges in the harvesting of microalgae. The first is the dilute nature of the algal broth, typically less than 0.5 g/L in an industrial system, due to which large volumes of culture need to be handled to recover algal biomass. Secondly, algal cells are typically only 2-20 µm wide, which makes it difficult to separate them from the liquid. Thirdly, these small cells generally have an electronegative surface charge in a wide pH range. In addition, the variety in size, shape and motility among different algal species makes is difficult to develop a single technique suitable for the recovery of all species (Wang et al., 2015).

Algal cells can be harvested by various methods, including centrifugation, sedimentation, flocculation, filtration, flotation, or by a combination of these methods. None of the harvesting methods is considered superior, and the best method depends on the algal species. Disadvantages of current harvesting methods include high cost, high energy consumption, or the requirement for a time-consuming process (Wang et al., 2015).

The harvesting cost usually contributes 20-30% of the total cost of an algal cultivation process (Wang et al., 2015). Microalgae are typically harvested in two steps. First, the algal suspension is thickened to 2-7% dry weight content and secondly, dewatered to 15-25% total suspended solids (Fig. 2). In addition to the species, the type and value of the end product affect the choice of the harvesting method. For example, food and feed production call for non-toxic harvesting methods (Barros et al., 2015).

Microalgae can be harvested with chemical, mechanical or even with electric methods. Two or more methods can be combined to lower the cost of harvesting (Barros et al., 2015). Figure 2 presents common harvesting and drying techniques.

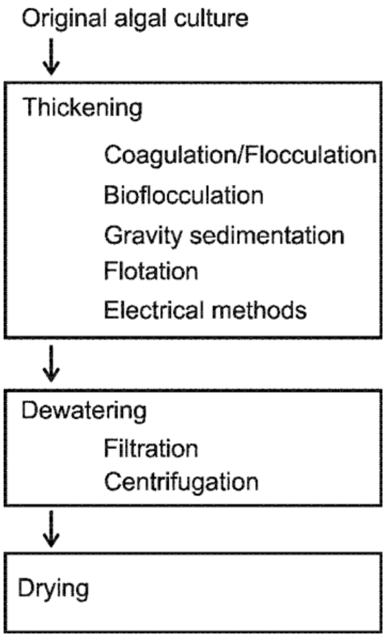

Figure 2. Harvesting techniques (Barros et al., 2015).

1.2.1. Thickening procedures

Microalgae are usually harvested in two phases, starting with a thickening procedure and then dewatering the thickened slurry. Alternative thickening methods include gravity sedimentation, coagulation-flocculation, flotation and electrical methods. Current research focuses largely on optimization of the thickening processes (Barros et al., 2015).

Coagulation-flocculation can be done either chemically or biologically but chemical coagulant/flocculant materials are usually applied in industrial processes. Coagulation-flocculation can easily handle large volumes, and the same method can be applied to several species. Gravity sedimentation is usually used to separate the thickened slurry from the growth medium (Barros et al., 2015).

Many algae have extracellular structures like glycoproteins or exopolysaccharides that allow the algal cells to agglutinate. Agglutination may also occur in response to changes in environmental conditions, and autoflocculation can often be induced by increasing pH. Filamentous cyanobacteria like *Arthrospira* and filamentous algae (e.g. some diatoms) may easily attach to each other and then form large flocks or sediment. Autoflocculation has been studied as a possible alternative for thickening. An obvious advantage is that autoflocculation occurs without added flocculants. Transformation of cyanobacteria or algae with machinery for cellulose biosynthesis is one option (Kawano et al., 2011). In this case, it may be possible to induce autoflocculation with a physical or chemical stimulus.

Chemical flocculants can be replaced by bacterial exopolysaccharides. In this bioflocculation method, heterotrophic, exopolysaccharide-producing bacteria are cultivated together with the algae (Barros et al., 2015).

Microalgae and cyanobacteria that do not contain gas vesicles can often be gravity sedimented, which is an energy efficient and cheap thickening method and therefore suitable if the value of the end product is low. Gravity sedimentation is, however, very inefficient if the specific gravity of the algae is low, and therefore a coagulation/flocculation step may be required before sedimentation.

Flotation means thickening by bubbling the slurry with gas. Flotation can be applied to algae that have a low specific gravity due to small size or presence of gas vesicles. Flotation is relatively rapid and it can be done in a relatively small vessels and the method is fast. Flotation is more costly than sedimentation, and flocculants are often required (Barros et al., 2015).

Electrolytic thickening takes advantage of the negative surface charge of microalgal cells, which makes the cells to move toward the positive pole in an electric field. Electrolytic thickening uses a sacrificial electrode that is electrolytically oxidized to generate coagulants which destabilize the original emulsion and triggers flock formation. Microalgae become aggregated upon their arrival to the anode (Pragya et al., 2013). The principles of flotation, coagulation and flocculation can be applied to the electrolytic process. Electrolytic thickening methods are not in widespread use (Barros et al., 2015).

Magnetic micro or nanoparticles can be used for thickening of algal slurry, or even for separation of harmful algae from natural waters (Wang et al., 2015). Naked Fe_3O_4 nanoparticles act as flocculants and attach to algae with electrical interactions but magnetic particles can also be coated with cationic substances to increase the strength of the interaction with the algal surface. Magnetic particles, together with attached algae, can be collected using a magnetic drum, and the particles can, at least in principle, be separated from the algae with acid treatment and filtration (Wang et al. 2015).

1.2.2. Dewatering procedures

The microalgal slurry is dewatered mechanically by filtration and/or centrifugation and often finally dried (Barros et al., 2015). Filtration is done with a porous membrane through which water is forced with pressure. The filter must be continuously washed to prevent clogging. The cell size defines the filtering method; vacuum filtering with filter aid powder that improves the filter performance is suitable for large algae while micro or ultrafiltration with a vacuum is required for small algae and unicellular cyanobacteria. Vacuum filtering is a relatively expensive method and the membrane needs to be replaced quite often due to fouling. Tangential flow filtration is cheaper and it has been shown to be able to recover 70-89 % of algae (Pragya et al., 2013). Vacuum filtering may damage the algal cells whereas tangential flow filtration keeps the cells intact.

Centrifugation is a rapid and expensive dewatering method that can only be used for dewatering for high-value end products. Centrifugation can be used for most microalgae, with the exception of those containing gas vesicles. Centrifugation is typically used after thickening but centrifuges that can handle the algal culture as such have also been developed (Barros et al., 2015).

2. Cultivation of algae in municipal, industrial and agricultural wastewaters and purification of wastewaters using algae

The treatment of wastewater requires chemicals, and the current technologies are energy demanding (Gouveia et al., 2016). These facts promote development of new methods for wastewater processing, such as use of microalgae. Algae can be grown in wastewaters for purification of wastewaters and for recycling of nutrients. Biofuels, fertilizers and other possible products like high-value products like proteins and lipids can additionally be produced. Algae can also be used to remove heavy metals or other toxic substances from wastewater. Production of biofuels alone is not economically viable, but the biomass should be used for several high-value products, as in biorefinery solutions. Microalgae can, in principle, be used to treat several types of wastewaters like municipal wastewater, livestock wastes and industrial wastes.

The use of algae may offer several potential advantages for wastewater treatment, such as (1) part of the oxygen needed for the heterotrophic bacteria can be provided through microalgal photosynthesis, which reduces the energy cost of aeration; (2) greenhouse gas emissions can be reduced due to consumption of CO_2 by the algae, and (3) useful algal biomass can be produced and the nutrients of the wastewater can be recycled (Gouveia et al., 2016). Furthermore, combined nitrogen that is currently converted to N_2 via nitrification and denitrification can be converted to reusable algal biomass. Despite these potential advantages, no industrial applications of the use of microalgae in wastewater purification exist.

High biomass productivity and high tolerance to wastewater are the most important requirements for algal strains considered for commercial production of microalgal biomass with wastewater. If the algal biomass will be used for biodiesel production, then the algae should show high lipid content and productivity (Chen et al., 2015). Pittman et al. (2011) suggests that algal biofuel production in connection to wastewater purification might be economically feasible.

Like plants, algae need mineral nutrients, of which nitrogen and phosphate are the most important and most expensive. The nutrient that is first depleted from the growth medium limits growth, and thus it is important that the stoichiometry of the nutrient content of the culture medium matches the stoichiometry of the nutrients in the algal biomass. The mean nitrogen to phosphorus ratio of marine planktonic biomass is 16:2 (Redfield, 1934; the ratio of C:N:P of 106:16:1 is called the Redfield ratio but the original paper shows the ratio of 15.7:1.88 for nitrogen and phosphorus). However, in individual species, the N:P ratio varies from 8 to 45 (Christenson and Sims, 2011). Addition of nitrogen or phosphate to a wastewater can be necessary to adjust the stoichiometry, which facilitates removal of the more abundant nutrient (Klausmeier et al., 2004).

Various green algal (Chlorophyta) species of *Chlorella*, *Scenedesmus*, *Chlamydomonas*, and *Botryococcus* have been found suitable for wastewater treatment (Abinandan and Shanthakumar, 2015). In addition, *Phormidium* has been reported suitable for treating municipal wastewater (Rawat et al., 2011). Algal communities can be more efficient than one species because the species differ in nutrient requirements and other characteristics. For example, a consortium of 15 strains removed over 96 % of nutrients from wastewater (Chinnasamy et al., 2010). Furthermore, filamentous cyanobacteria, including the edible cyanobacterium *Arthrospira* (spirulina), appear to be good candidates for wastewater purification because they grow rapidly and are easier to harvest than single-celled species (Markou and Georgakakis, 2011).

Wastewaters tend to contain both organic and inorganic pollutants that the wastewater-purifying algae must tolerate. An extensive review by Abdel-Raouf et al. (2012) listed *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium* as the algal genera that best tolerate organic pollutants. Inorganic pollutants are mostly heavy metals, and especially copper is highly toxic to both algae and cyanobacteria.

Algae that tolerate particular metals can also be used to remove these metals from waste waters (phytoremediation). Many algal biomasses bind metals efficiently and may therefore offer potential for cleaning metal pollutants (Pb, Cd, Hg, Sc, Tn) or other toxic elements (As, Br) from wastewaters. Binding of metal cations depends on the negative surface charge of the cell wall of many algae. Such binding is called adsorption or biosorption to distinguish it from actual uptake of metals to the algal cells. Biosorption depends on time, temperature, pH, charge density and concentrations of the metal in question and other ions and nutrients. The species and other characteristics of the biomass, and even culture age may affect (Kumar et al., 2016).

2.1. Municipal wastewaters

Wastewater purification with algae has most often been studied by using municipal wastewaters. Municipal wastewaters can be nutrient-wise suitable for algal growth, with nitrogen and phosphate concentrations up to 10-100 mg/L (Chen et al., 2015).

Traditional purification of municipal wastewaters consists of a primary and a secondary phase, optionally followed by advanced tertiary and quaternary steps (Cai et al., 2013). In the primary treatment, physical or chemical methods are used to remove substances that can be easily removed. The secondary treatment removes dissolved organic material and colloids by biological or chemical treatments. Dissolved ammonium is often converted to molecular nitrogen by nitrification and denitrification during the secondary step. Sometimes the secondary effluent derived from these stages is directly discharged although the secondary effluent still contains a lot of nitrogen and phosphorus and therefore may cause eutrophication. The effluent may also contain other pollutants like heavy metals (Cai et al., 2013). A tertiary treatment process aims at removing organic ions, ammonium, nitrate and phosphate. Tertiary treatment can be accomplished biologically or chemically, and it is approximately fourfold as expensive as primary treatment (De la Noüe et al., 1992). Removal of heavy metals, soluble minerals and remaining organic compounds, in turn, is eight to sixteen times as expensive as primary treatment (Abdel-Raouf et al., 2012). Advanced processes employ several different methods. Anaerobic digestion can recover some of the energy of the wastes, but various methods like post-aeration, pond sedimentation, filtration, membrane separation and activated carbon treatment can be used (Cai et al, 2013).

Microalgae can be applied in the tertiary and quaternary purification steps because the growth of microalgae is usually limited by nitrogen or phosphorus and algae therefore remove these nutrients efficiently from the medium. A second reason why microalgae are interesting for wastewater purification is that some algae to can remove toxic organic compounds and heavy metals (Abdel-Raouf et al., 2012). Toxicity of heavy metal ions against different algal species determines the potential remediation capacity of the alga, but heavy metal tolerance can be highly strain specific (Zeraatka et al., 2016). However, if algae remove toxic metals, there are restrictions for the use of the biomass. For example, use as a fertilizer is not possible.

The composition of sewage wastewater can remain relatively stable, which makes it possible to use it for cultivation of algae. Sewage stabilization ponds might be suitable for cultivation of microalgae, and these ponds are readily available, thus diminishing the investment costs of the cultivation (Cheah et al., 2016).

Algae may be more efficient than conventional tertiary treatment in removal of nutrients and metals (Chen et al., 2015). For example, *Chlorella* sp. removed 93.9%, 89.1% and 80.9% of ammonia, total nitrogen and total phosphorus from raw concentrate, respectively (Li et al., 2011).

Mixtures of several (waste)water sources are often suitable for the use of algae for wastewater purification. The testing of mixtures is further promoted by the fact that many industrially interesting algae are marine species whereas wastewaters are fresh water. The marine microalga *Nannochloropsis* was successfully cultivated in a 1:1 mixture of seawater and municipal wastewater, with flue gases with 15 % CO_2 as a source of carbon (Jiang et al., 2011).

2.2. Industrial wastewaters

Industrial wastewaters typically contain less nitrogen and phosphorus than municipal wastewaters but lots of heavy metals. High rate of metal removal can only be reached by using a microalgal species or strain that adsorbs metals efficiently (Cai et al., 2013). Toxic metal ions are present in effluents from e.g. textile, leather, tannery, electroplating, galvanizing, metallurgical and paint industries (Ahluwalia and Goyal, 2007). Effluents from the cellulose industry act as important aquatic pollutants in developing countries (Ali and Sreekrishnan, 2001), but these wastes are efficiently treated in Finland.

Most research on the use of algae with industrial wastewaters focuses on removal of metals and organic pollutants (Chen et al., 2015). The high metal concentrations, organic toxins and low nitrogen and phosphorus concentrations in industrial wastewaters tend to inhibit algal growth and thereby limit the possibilities to grow microalgae in industrial wastewaters (Chen et al., 2015). Anaerobic treatment may be used to obtain effluent with a higher ammonium concentration than original industrial wastewater. For example, a recent study showed that effluents obtained from anaerobic treatment of the biosludge of a wastewater treatment plant of a pulp/paper mill combinate can be used to grow *Nannochloropsis* (Polishchuk et al. 2015).

2.3. Agricultural wastewaters

Agricultural wastewater originates mainly from livestock production, and the waters have generally higher nitrogen and phosphorus concentrations than municipal wastewaters. In fact, the nutrient concentrations of agricultural wastewater are often too high for microalgae, which results in the need of dilution. The N to P ratio is typically 2-8 in wastewaters of cow or pig farms (Cai et al., 2013). The dominant form of nitrogen is usually ammonium, which is toxic in high concentrations and at high pH because the chemical balance between ammonium (NH_4^+) and ammonia (NH_3) favors the toxic ammonia at high pH. Furthermore, agricultural wastewaters are often turbid and colored, which hinders the penetration of light to the medium (Wang et al., 2010). Furthermore, the nitrogen to phosphorus ratio tends to be too low for microalgae. Due to the above characteristics, agricultural wastewater usually needs to be diluted before it can be used for growing algae (Chen et al., 2015).

3. Special features of Finnish climate, industry and agriculture

3.1. Climate

The Finnish climate sets special requirements for cultivation of microalgae, as weather varies a lot both seasonally and diurnally. Temperature and light conditions in Finland are very challenging. Outdoor cultivations can be run only for a couple of months a year, which restricts the use of open pond type cultivations.

3.2. Industry

Pulp and paper mills are a very important branch of industry in Finland. Secondary water streams from the cellulose industry can be combined for cultivation of microalgae. Wastewater from pulp and paper industry may contain a lot of carbon (cellulose) but little bioavailable nitrogen and phosphorus. To ensure the availability of nitrogen required for biological wastewater purification, it is often necessary to add fertilizers or to combine municipal wastewater streams with those from a pulp or a paper mill. Sufficient amounts of municipal wastewater are seldom available because pulp and paper factories are located in the countryside and because transportation of wastewater is too expensive. Production of microalgal biomass can be a new area of business for pulp and paper mills that suffer from the decreasing trend in the demand for paper (Gentili, 2014).

Flue gases from the mill can be used as free carbon dioxide for microalgae; such use of flue gases would also reduce CO_2 emissions. When the photobioreactor is placed in a glasshouse, excess heat from a pulp or a paper mill can be used to heat a greenhouse (Saeid and Chojnacka, 2015). In 2008, forest industry used 25.3 TWh/a as electricity and 63.3 TWh/a as heat, together 88.6 TWh/a (23 % of Finland’s energy consumption). In comparison to these numbers, paper industries sold out only very little excess heat, 0.32 TWh/a. Excess heat is most commonly bound to exhaust vapors, flue gases, wastewaters, cooling waters, gas outlets and condensation heat of mechanical cooling (Työ- ja elinkeinoministeriö, 2010).

3.3. Agriculture

Agro-industrial wastewaters include potato processing wastewater, swine wastewater, livestock wastewater, dairy wastewater, slaughterhouse wastewater and fish farm wastewater. High ammonium concentration is a common characteristic of agro-industrial wastewater. Some agro-industrial wastewaters have high COD levels, and thus it may not be easy to directly treat the original wastewaters by microalgae. Most studies on microalga-based treatment of such wastewaters are conducted with appropriate dilution of the original wastewater or by employing anaerobic digestion as a pretreatment. Under optimal conditions, microalgae can remove over 90 % of ammonium (Wang et al., 2016).

4. Case studies of algal cultivation in Finnish conditions

4.1. Case 1. Recirculating aquaculture

Recirculating Aquaculture Systems (RAS) are land-based, almost closed systems with high level of water re-use (Burr et al. 2012). RAS farming is a globally fast developing form of aquaculture. In a RAS, water from the fish tanks is mechanically filtered, treated in a nitrifying biofilter and a gas exchange system and returned to the tanks after addition of oxygen and pH control. The effluent volume of a RAS is small, which enables efficient nutrient removal and minimizes the eutrofying impact when compared to traditional net cage cultivation.

Up to 99 % of water is recycled in a RAS, and RASs are used especially when water availability is restricted (Badiola et al., 2012). A RAS must control a large number of factors, including the rearing temperature, concentrations of O_2 and CO_2, ammonium, nitrite, nitrate, pH, salinity, and solid dirt (Dalsgaard et al., 2013). In practice, the control of concentrations of ammonium and oxygen causes the biggest investment and running costs.

Fish produce ammonium as a by-product of their protein metabolism (Collos and Harrison 2014). The ammonium is liberated from the fish gills, feces, urine and unused feed. Removal or conversion of ammonium is an important step in the wastewater treatment of a RAS, since ammonium is toxic to fish. Ammonium is usually removed via oxidation to nitrate by a biofilter containing nitrifying bacteria (Schreier et al. 2010). Biofilters are problematic because the nitrifying bacteria produce off-flavors that affect fish quality. The most important off-flavor compounds are geosmin and 2-methylisoborneol that give earthy and musty off-flavors to the fish (Burr et al. 2012). Because of these off-flavors the fish need a depuration phase in fresh water before selling. Depuration generates expenses because large amounts of pure water are needed and because fish do not grow during depuration. Up to 30 % of the potential revenue may be lost annually due to off-flavor problems (Burr et al. 2012).

The off-flavor problem could be solved by replacing the biofilter by other means of ammonium removal. Cultivation of autotrophic photosynthetic organisms like cyanobacteria, microalgae or plants is a potential option, as most autotrophs can use ammonium in their metabolism. The microalgal option is discussed here.

Cultivation of algae in the recirculated water offers both pros and cons when compared to a bacterial biofilter. In addition to removing ammonium, autotrophic organisms remove other mineral nutrients and CO_2 and produce O_2. Furthermore, contrary to a biofilter, algae do not need aeration, as ammonium is incorporated in biomass instead of being oxidized to nitrate. On the other hand, algae need light for growth, which consumes energy and sets special requirements for the facilities. The resulting algal biomass may have economically interesting uses, whereas the biomass of a bacterial biofilter cannot be harvested or utilized.

With the use of extensive artificial illumination, algal biomass can be grown at high density, which would improve the economy of the system. On the other hand, harvesting of algae is expensive, especially if the recirculated water should be completely free of algal cells. Very high water flow even above 1 m^3 per second is one of the major challenges in using algae to remove ammonia and CO_2 from the RAS water. One solution is to keep algae separate from water flow using a semipermeable membrane that allows the algae to receive the nutrients without being washed away (Ojanen et al., 2015).

The combination of a RAS and autotrophic organisms is often termed aquaponics, and the idea has been tested in a few cases. Michels et al. (2014) grew *Tetraselmis suecica* in a fish farm wastewater and found that removal efficiencies of nitrogen and phosphorus could be increased to 95.7% and 99.7%, respectively, by adding phosphate to the water to adjust the stoichiometry. Kloas et al. (2015) presented a combination of tomato cultivation in aquaponic system and fish production in a glasshouse, and Rakocy et al. (2004) have results from several years from a pilot-scale experiment with fish tanks and several plant species.

4.2. Case 2. Greenhouse cultivation

One option to cultivate year around and continuously microalgae in Finnish conditions is the combination of a photobioreactor and a heated greenhouse. Even in that case artificial lighting is needed for successful cultivation. In addition, building of a greenhouse is likely to be too expensive for algae production alone and thus the unit will be economically uncompetitive compared to other production regions with favorable climatic conditions. The construction costs of large glasshouses in Finland without any equipment are estimated to be 80-90 € m^{-2} (Jukka Tuominen, Puutarhaliitto ry/Kauppapuutarhaliitto, personal communication). Moreover, technical equipment, lighting and heating for the greenhouse are needed.

A potential approach is to take algal cultivation into current greenhouse production with available top- and interlighting. The nutrient delivery can be integrated into crop cultivation based on split-root fertigation (Jokinen et al. 2011). The approach gives flexibility to adjust the nutrient concentration appropriately for algae as well.

An example of tomato production combined to algal production in a greenhouse is presented (Fig 3). In the greenhouse the space between the rows of tomato crop is needed for harvesting and maintaining the plants, and this area could be used for growing algae. The photobioreactor is designed as a rectangular-tubular combination of flat panel and tubular designs and placed on the floor between the tomato rows. If the available area of the greenhouse is 20 ha, then the manageable area for a photobioreactor could be the half of this area (10 ha). The height of the photobioreactor is estimated to be 5 cm. The rate of biomass generated is predicted to be 0.5 $gL^{-1}day^{-1}$. Using these assumptions the algal biomass production in this kind of system would be 250 kg dry biomass day^{-1}.

Figure 3. A photobioreactor combined with tomato cultivation in a greenhouse.

Figure 4 presents the monthly electricity consumption of lighting (top- and interlighting) calculated for the above described case (Timo Kaukoranta, personal communication). A production area of 20 ha would consume 13.8 GWh/year. Even if the tomato plants shade the floor, a photosynthetic photon flux density of 300 µmol/m^2 would be reached on the floor.

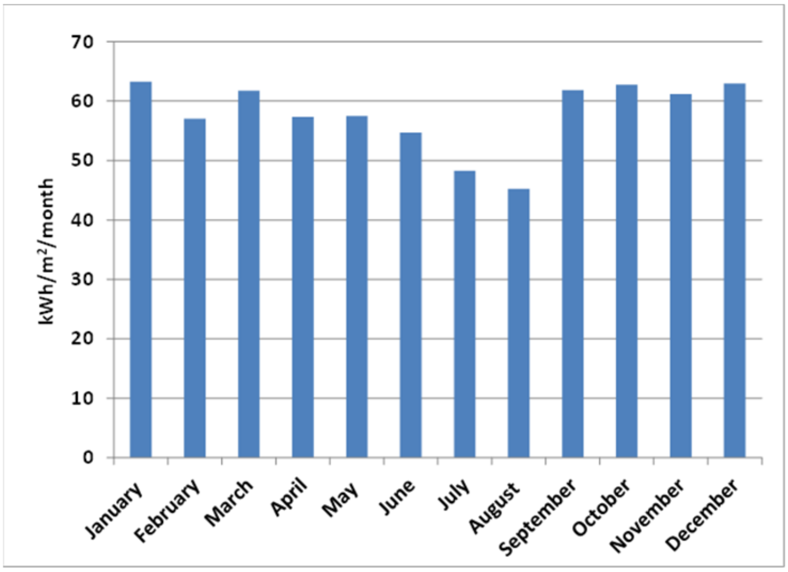

Figure 4. Monthly electricity consumption of lighting in combined cultivation of tomato and algae in a greenhouse.

The above presented co-cultivation of algae and a greenhouse crop might be a realistic option in Finnish conditions. Practical experiments are, however, needed to verify the productivity of the algae and the economic feasibility of the overall production model.

One possibility is also to place the photobioreactor on the upper part of the greenhouse space. However, the photobioreactor would then shade the crop plants significantly and thereby reduce the crop yield.

4.3. Case 3. Ämmässuo Waste Treatment Centre in Espoo, Finland

This case study evaluates algal cultivation in Ämmässuo Waste Treatment Center, assuming that existing infrastructure and waste streams are used. The Ämmässuo center produces wastewaters, carbon dioxide, excess heat and electricity, which could be exploited by algae.

The Ämmässuo Waste Treatment Center treats biowaste, ash and slug from a waste-to-energy plant, collects and utilizes landfill gases, treats and performs final disposal of landfill. A maintenance tunnel and a maintenance channel could be used for algal cultivation. Both are underground, so artificial lighting is needed. The tunnel is about 850 m long and 4.8 m wide but because of an explosion risk, only 170 m of it can be used for cultivation of algae. The temperature is about 24° C.

The temperature in the 3 m x 1 km maintenance channel is about 18° C, and part of the channel is outdoors. The channel is constructed as a three-floor tunnel where the floors are open from bottom to up. The channel is well ventilated and there is no risk of explosion.

All wastewaters from the site are conducted to a wastewater stadium. Due to the availability of several different wastewater streams and the stable temperature, Ämmässuo would be a suitable location for a pilot scale algal growth facility.

Three wastewater streams, named as TAL1, KAI1/2 and BioWasher, from the Ämmässuo site, have been investigated by University of Turku (Dimitar Valev and Esa Tyystjärvi) for algal growth. TAL1 represents a combination of waste streams accumulated at the waste treatment plant site. KAI1/2 is groundwater that is found to be contaminated with leachate seeping through the bottom structures of the old landfill, and BioWasher is water used in the cleaning of exhaust air from a composting plant. The results showed that KAI1/2 (alone or mixed with TAL1) seems to suit for growing the green alga *Neochloris oleoabundans.* BioWasher was found to contain too much ammonium and too little other nutrients for growing algae directly in it.

4.4. Case 4. Pulp and paper mill

Kouhia et al. (2015) presented calculations for a biorefinery producing high-value microalgal products in a system integrated to a traditional pulp/paper mill. Fertilizer and biogas would be produced from secondary process streams. The presented biorefinery process is validated with mass balances, using data from a Scandinavian pulp and paper mill. The results suggest that the process is technically viable.

The biorefinery is designed to produce several products in parallel (wasteless use of biomass) and to utilize secondary streams that otherwise would have to be disposed of (Fig. 5). Potential products are algal lipids, then biofertilizers and finally biogas or biomethane. In addition, oxygen and ash reject are produced. The consumption of oxygen in bleaching or combustion processes at the pulp and paper mill has been suggested (Kouhia et al., 2015), but the amount of oxygen produced by an algal reactor is probably too small for technical use. The ash can be used as an additive in concrete production (Kouhia et al., 2015).

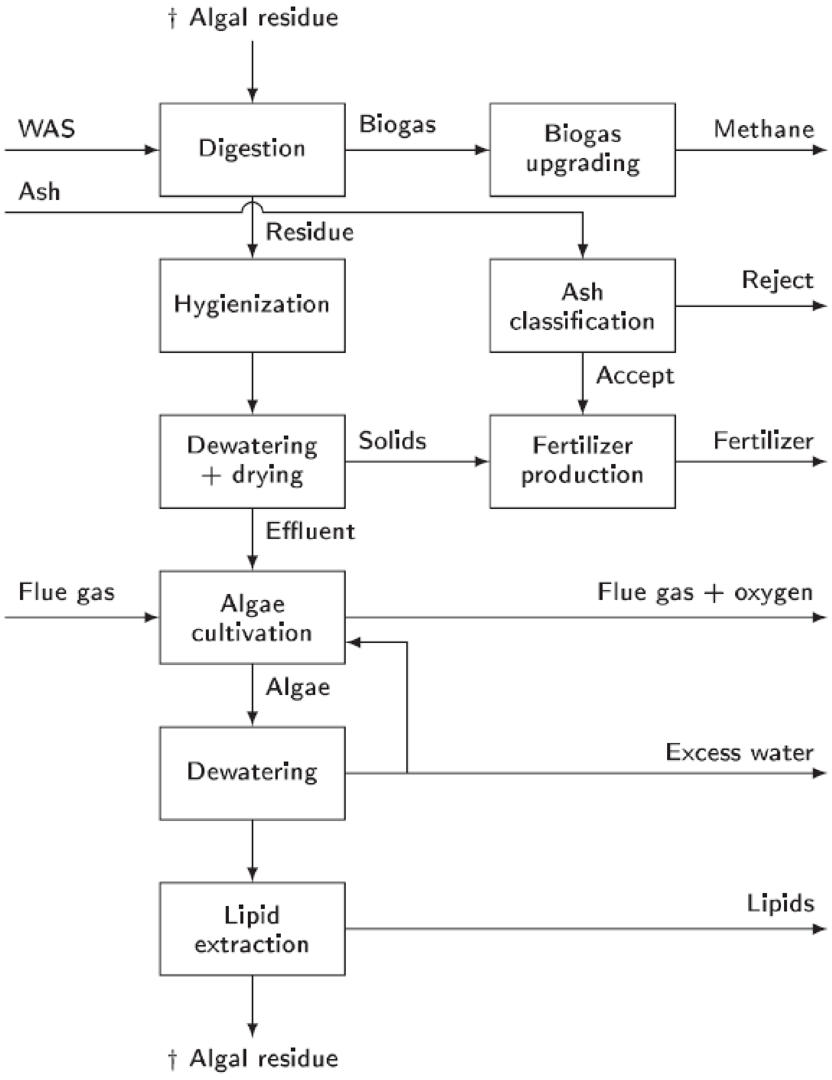

Figure 5. Evaluated biorefinery configuration: waste activated sludge (WAS), ash and flue gas from a pulp and paper mill are consumed in lipid, methane and fertilizer production while utilizing algae in the conversion process (Kouhia et al., 2015. Figure by courtesy of Algal Research, License ID 4053471308391).

Polishchuk et al. (2015) cultivated the EPA containing alga *Nannochloropsis oculata* in a mixture of waters containing 25 % effluent from anaerobic digestion of excess activated sludge of a plant treating wastewaters of a pulp and paper integrate and a municipality, and 75 % of the final effluent of the same wastewater treatment plant. The EPA content of *N. oculata* remained high even if the algae were grown in the wastewater mix (Polishchuk et al., 2015). The factory and the processes are the same as Kouhia et al. (2015) used as the basis of calculations. The findings of Polishchuk et al. (2015) suggest that biosludge from a pulp/paper industry wastewater treatment plant could be utilized first for production of methane by anaerobic digestion and thereafter for growing *N. oculata.* The production of EPA or other long-chain fatty acids for human consumption in wastewater media may, however, not be economically feasible.

Plans for the industrial use of algae tend to choose photobioreactors instead of open ponds in order to prevent contamination and to improve controllability. Oxygen produced by photosynthesis could in principle be utilized, but the amount of oxygen produced by a photosynthetic system is always relatively small. In spite of the small overall amount, oxygen inhibits photosynthesis and is therefore a problem in a closed photobioreactor. Therefore oxygen has to be flushed out of the reactor. Excess heat produced by the pulp and paper industries can be used to heat a glasshouse containing a photobioreactor (Kouhia et al., 2015).

Algal growth is sensitive to light and nutrient availability, and therefore the analysis of feedstocks and optimization of the algal strain are crucial in order to meet requirements set by the algae and the desired end products. Seasonal variation in irradiance results in remarkable changes in algal growth (Kouhia et al., 2015).

5. Algal and cyanobacterial species and strains

5.1. Growth in general

Screening of microalgal strains for various industrial purposes is in its infancy, and most studies have been done with a very few species. Even less is known about the conditions like nutrient requirements and about tolerances of temperature, salinity, pollutants and biotic factors that allow growth of particular strains (Ghosh et al., 2016). Optimization of growth conditions for a particular algal strain requires consideration of a large number of abiotic factors, including temperature, light intensity, spectrum, light-dark periods, nutrients, salinity, quality of the water (e.g. color) and oxygen content. Carbon dioxide concentration is usually regulated simply by adjusting the pH of the medium with addition of CO_2. In addition, biotic factors like the ability of the algae to tolerate mechanical stress during cultivation, and the density of the culture need to be optimized (Razzak et al., 2013). The ability of the cultivated strain to compete with heterotrophic bacteria and other organisms is highly important, as wastewaters are never sterile.

There are significant differences in biomass and lipid productivities between various species (Mata et al., 2010). When reading literature, one should be cautious about the interpretation of productivity values if the conditions of the measurement have not been properly standardized. For example, light intensity, carbon dioxide concentration and optical thickness of the culture have strong effects on volumetric productivity, and nitrogen status and the history of the culture affect lipid productivity.

5.2. Metal tolerance of algae

Algae and cyanobacteria can be used for bioremediation, i.e. removal of toxic substances like heavy metals from water. Heavy metals may be harvested by algae either by biosorption of the metal to the cell wall or by uptake of the metals to the algal cells. The efficiency of biosorption of heavy metals may be affected by metal concentrations and algal species, temperature, pH and other metals (Zeraatkar et al., 2016). The uptake of metals to algal cells occurs either via active transport through the cell membrane or by endocytosis assisted by metal chelator proteins. The molecular basis of heavy metal toxicity is not quite clear and different metals certainly have different toxicity mechanisms. One general reason for the toxicity of heavy metals is their ability to bind to sulphydryl groups of proteins. Furthermore, heavy metals may disrupt protein structure or even displace metal cofactors. Heavy metals tend to induce antioxidant enzymes (superoxide dismutase, glutathione peroxidase, ascorbate peroxidase), suggesting that the metals affect the redox balance of the algal cell. Algal tolerance to heavy metal that enters the cell depends on the efficiency of oxidative defense (Arunakumara and Xuecheng, 2008).

5.3. Edible species

Microalgal and cyanobacterial biomass might be usable as food or feed because it often has high protein content and may contain long-chain polyunsaturated fatty acids. Some algae have traditionally been used as food, especially in East Asia, South Africa and Mexico. Red, green and brown macroalgae from different genera including *Porphyra*, *Ulva* and *Sargassum* are harvested as foodstuff (Koller et al., 2014).

Only few microalgae or cyanobacteria are currently used for human nutrition. The taxa include the cyanobacterium *Spirulina* (*Arthrospira*), green algae *Chlorella* and *Dunaliella*, and (to some extent) cyanobacteria *Nostoc* and *Aphanizomenon* (Pulz et al., 2004). The latter two cyanobacterial families are also known of their poisonous members. The green algae *Chlorella* and *Tetraselmis*, the haptophyte *Isochrysis*, the dinoflagellate *Pavlova*, the eustigmatophyte *Nannochloropsis* and the diatoms *Chaetoceros, Skeletonema*, *Thalassiosira* and *Phaeodactylum* are used as animimal feed (Spolaore et al., 2006). *Isochrysis* is cultivated for bivalve feed.

Microalgae are usually dried and pressed to capsules, tablets, or sold as a suspension. Microalgae are available also as ingredients of pasta, snacks, candies and beverages (Spolaore et al., 2006). The most crucial barrier for algal consumption for food is the unpleasant taste of algae and cyanobacteria.

5.4. Potential biofuel strains

Many microalgae switch from normal growth to production of triacylglycerols (TAGs), or storage fat, if carbon skeletons are produced (by photosynthesis) but nitrogen is scarcely available. Other adverse environmental conditions may affect in the same way as nitrogen depletion. The fatty acids of TAGs are produced in the chloroplast but the regulation of the rate of fatty acid synthesis in microalgae is not very well understood (Hu et al., 2008).

Lipid yields from microalgae can be very high compared to land plants. Even 10 to 20 times higher microalgal lipid yields have been calculated when compared to oil palm oil (Chisti, 2007). However, figures for algal oil yield are usually based on laboratory experiments at the time when there were very high expectations on algal lipid yield. Later these expectations were proved to be unrealistic, and the algal biofuels have not yet reached economic feasibility.

Due to the biodiesel hype, research on algae has largely focused on lipids. In some species (e.g. *Nannochloropsis* spp.and *Botryococcus braunii*) and in particular conditions (usually achieved by nitrogen deprivation), lipids may comprise even 80 % of dry biomass (Larkum et al., 2012). *Chlorella* sp. have also been suggested as biodiesel algae. Talebi et al. (2013) evaluated the suitability of microalgae strains as biodiesel feedstock. *Chlorella vulgaris* was shown to have a high biomass and high volumetric lipid productivity. According to this study, *C. vulgaris*, *C. emersonii*, *C. protothecoides* and *Dunaliella salina* could be regarded as the best candidates for large scale cultivation for biodiesel production.

Li et al. (2015) isolated and identified 37 microalgal strains belonging to genera *Scenedesmus*, *Chlorella*, *Stichococcus*, *Nannochloropsis*, *Tetraselmis*, *Isochrysis*, *Phaeodactylum* and *Cylindrotheca* and compared their lipid content, growth rate and biomass production. The marine algal strains *Nannochloropsis maritima* strain IOAC710S and *Isochrysis galbana* strains IOAC683S and IOAC724S were reported as promising candidates for biodiesel production (Li et al., 2015). Comparison of *Botryococcus braunii*, *Chlorella vulgaris* and *Scenedesmus* sp. showed that *Scenedesmus* had the best growth rate of the three whereas *B. braunii* was the best lipid producer (Yoo et al., 2010). Taleb et al. (2016) developed a screening procedure for biodiesel production and found *Nannochloropsis gaditana* CCMP527 and *Parachlorella kessleri* UTEX2229 to be the most promising marine and freshwater strain, respectively.

5.5. Algae naturally producing PUFA

Many algae contain special, often polyunsaturated, fatty acids (PUFAs) with modest to high market values, such as eicosapentaneoic acid (EPA), docosahexaneoic acid (DHA), gamma-linolenic acid (GLA) or arachidonic acid (AA) (Koller et al, 2014). Microalgal PUFAs have been recognized as promising candidates the biotechnological market, as purified PUFAs are added to infant milk, and PUFA-containing microalgae can also be used as chicken food to increase the PUFA content of eggs. However, the non-photosynthetic dinoflagellates *Schizochrytrium* and *Cryptecodinium* dominate the market of chicken food for the production of "OMEGA" eggs. These applications have proved to be profitable (Pulz et al., 2004) but the use of photosynthetic algae has not been tested in the market.

EPA and DHA by the non-photosynthetic dinoflagellate *Crypthecodinium* has been considered innovative (Pulz et al., 2004). However, production of PUFAs is economically feasible only in non-photosynthetic heterotrophic algae like *Schizochytrium* sp., and *Aurantiochytrium* sp. (Yen et al., 2013).

The EPA-rich prymnesiophyte alga *Pavlova viridis* is widely used in China in marine aquaculture as bivalve feed (Hu et al., 2008). EPA occurs in many other microalgae including *Phaeodactylum tricornutum*, *Monodus subteraneus, Nitzschia laevis* and *Nannochloropsis* sp. (Harun et al., 2010; Polishchuk et al., 2015).

5.6. Algae producing pigments

Due to the low productivities and high product recovery costs, production of pigments with microalgae is still in its infancy if compared to the chemosynthetic production of the same compounds or their isolation from other natural sources (Koller et al., 2014).

5.7. Genetic engineering of algae

Production of sufficient amounts of known high-value compounds, and especially production of novel compounds that wild-type algae do not produce, cannot be achieved without improvements to the natural strains. Natural wild-type algae do not contain high value products that could be competitively produced in Finland, although wild-type strains may be robust against biotic stress (grazers, infections, etc.) (Benemann et al, 2013). Potential risks and impacts of each transgenic alga on health and environment should be evaluated before industrial use (Ghosh et al., 2016). New genomes are sequenced at high rate, and 27 species of green algae have been completely sequenced at the moment (13.10.2016) (https://www.ncbi.nlm.nih.gov/genome/browse/). In addition to the manipulation of the production of compounds that occurring naturally in algae, genetic modification can be used to make algae or cyanobacteria that produce industrially interesting chemicals like hydrocarbons (Radakovits et al., 2010).

Genetic engineering of microalgae requires the ability to do stable nuclear or chloroplast transformation. Nuclear transformation procedures have been established for several algae, including *Chlamydomonas*, *Dunaliella*, *Haematococcus*, *Nannochloropsis* and *Phaeodactylum* (Radakovits et al., 2010). Genetic modification of microalgae has by now targeted either lipid biosynthesis, photosynthesis or carotenoid biosynthesis. *Chlamydomonas reinhardtii* is the best studied alga for genetic engineering, and *Chlamydomonas* can be relatively easily transformed. However, genetic tools functioning in one alga may not function in other algae (Zeng et al., 2011). Furthermore, both in *Phaeodactylum* and *Chlamydomonas*, two algae with a long history of genetic engineering, the genetic tools best function in mutants with a specific structure of the cell wall (wall-less strain of *Chlamydomonas* or silicon-deficient culture of *Phaeodactylum*). The biolistic method and electroporation are the most common transformation methods for algae.

Chlorella pyrenoidosa is an extensively investigated species that has been used as a commercial microalgal feedstock of protein and lipids. In the study of Run et al. (2016), the electroporation method was optimized for *C. pyrenoidosa*. The result showed that the transformants expressed the foreign genes stably (Run et al., 2016).

Due to the focus of alga research on lipids, one of the prime targets of genetic engineering of algae has been to direct the algal metabolism toward storage fat production without the application of nitrogen depletion, e.g. during exponential growth or during the stationary growth phase (Zeng et al., 2011).

Engineering of eukaryotic nuclear genes has been recently revolutionized by the CRISPR/Cas9 method that can be described as a genome editing tool. In principle, the CRISPR/Cas9 allows knocking out a specific gene and even production of site-specific mutations in nuclear genes (Shabbir et al., 2016). However, even use of the CRISPR-cas9 method requires a functional transformation method for each organism. Although the CRISPR/Cas9 presently only functions for mammalian cell cultures, there is no particular reason why it would not function in any organism that can be transformed.

6. Cultivation of communities of aquatic microorganisms

Natural phytoplankton communities may in some cases be more productive than monocultures of one species, probably because different organisms prefer somewhat different resources (Stockenreiter et al., 2012). Therefore, consortia of phytoplankton may be usable also for removing nutrients from wastewaters. Communities consisting of cyanobacteria and/or microalgae and bacteria may function particularly efficiently in bioremediation. In consortia of heterotrophs and autotrophs, the cyanobacterial/algal photosynthesis provides oxygen to the heterotrophic organisms, usually bacteria. Bacteria, in turn, provide carbon dioxide and may provide vitamins for the photoautotrophic partners of the consortium (Subashchabdrabose et al., 2011).

Bacteria that are associated with microalgae (MGPB, microalgal growth promoting bacteria) are related to bacteria that promote the growth of plants. The best studied example of a close interaction between algae and bacteria is the relationship between the anoxygenic phototrophic bacterium *Roseobacter* and marine algae, especially the haptophyte *Emiliania huxleyi*. During an algal bloom, the bacteria provide both vitamins and protection from other bacteria, and the bacteria turn to pathogens during the decline of the bloom (Ramanan et al. 2016). Some bacteria like *Mesorhizobium* and *Azospirillum* are known to be important for algae because of the ability of the bacteria to fix nitrogen. In industrial cultivation of algae, bacteria are often considered contaminants. Bacteria may also help in flocculation, which reduces the use of chemicals in the harvesting step (Ramanan et al., 2016).

Possibilities for combining microalgae with other microorganisms are particularly interesting for wastewater treatment (Assemany et al., 2015). A consortium of *Chlorella vulgaris* or *C. sorokiniana* and *Azospirillum brasiliense* strain Cd) co-immobilized in alginate beads has been developed for removal of N and P from municipal wastewater. The bacterium enhanced the growth and removal of nutrients by *Chlorella* in wastewater (de Bashan et al., 2004).

Establishment of a stable association between microalgae and bacteria can be challenging because bacteria may easily outgrow the algae. Furthermore, algae should produce enough oxygen for the bacteria. Therefore algal-bacterial consortia function best if a rapidly growing alga with a high oxygen production rate is used (Muñoz and Guieysse, 2006).

7. Algal products

Algae can be used for both energy and non-energy products. Energy products from algae are biodiesel, biogas and bioethanol. In addition to biofuels, animal feed, fish feed, and pharma- and nutraceuticals can be produced. The possibility to use the nutrients for production of high value side products may improve the economy of the algal option (Trivedi et al, 2015). For example, some algae contain interesting nutraceutical compounds like long-chain polyunsaturated fatty acids (Polishchuk et al. 2015).

Many of the potential algal products are based on lipids. Phospho and glycolipids are the primarily membrane lipids whereas neutral tri, di- and monoacylglycerides function as energy storage (Halim et al., 2012). Glycolipids are typical of the thylakoid membranes of chloroplasts and cyanobacteria.

The lipid content may vary a lot depending on the culture conditions even within one species (D'Alessandro and Filho, 2016). Different lipid products occur in different lipid classes and the polyunsaturated fatty acids, in particular, occur mainly in the chloroplast glycolipids.

7.1. Biodiesel

Biodiesel production is the most explored technology in development for utilization of algal lipids. Under optimal cultivation conditions, several species especially belonging to the genera *Botryococcus*, *Chlorella*, *Nannochloropsis*, *Neochloris*, *Nitzschia*, *Scenedesmus*, and *Dunaliella* may contain high amounts of lipids (Koller et al., 2014). Algal biodiesel is always produced from neutral lipids. The fatty acids of triacylglycerols are transesterified to methanol to produce diesel fuel that is less viscous than the triacylglycerols (Trivedi et al., 2015). Non-photosynthetic algae like *Schizochytrium* can, of course, not be used for ecologically sustainable production of fuels although the culture may contain a large volumetric concentration of lipids. High-lipid algae are usually unicellular (D'Alessandro and Filho, 2016).

7.2. Bioethanol

After algal harvest and recovery of marketable products, residual biomass can be converted into bioethanol. Bioethanol production requires starch-rich algal biomass or their hydrolysates that can be fermented with yeasts under anaerobic conditions. Starch and starch-like polysaccharides constitute an alternative class of energy storage compounds, in addition to fat, in several species among the genera of *Chlorophyta*, *Rhodophyta*, *Cryptophyta* and *Pyrrophyta* (Koller et al., 2014). Also *Chlorococcum* and *Chlorella vulgaris* have shown good conversion rates (Trivedi et al., 2015). The cyanobacterium *Synechococcus* sp. PCC 7002, cultivated under nitrogen depletion, may contain up to 60 % of fermentable carbohydrates per dry weight (Möllers et al. 2014). However, the low yield of yeast-based anaerobic ethanol production may hinder the large-scale production of ethanol for fuel in this way (Koller et al., 2014).

7.3. Biogas

Most types of wet biomass can be anaerobically digested to produce biogas, and algal/cyanobacterial biomass is not an exception. Biogas is a mixture of CO_2 and CH_4, and a complex consortium of bacteria and archaea is needed for the various steps of the digestion of biomass and methanogenesis (Trivedi et al., 2015). The digestibility, required pretreatment and biogas yield depend on the species and other characteristics of the biomass.

Production of biomass for biogas production alone is not economically feasible, but after extraction of a high-value primary product, the residual algal biomass can be used for anaerobic digestion. An economical advantage of anaerobic processing, in comparison to use of the biomass for heat production by burning, is that there is no need for drying of the algal biomass. Biogas is a low-value energy product like biodiesel but the generation of biogas from algal biomass is technically simpler than extraction of oil and transesterification of the fatty acids. Furthermore, the solid residue of anaerobic digestion can be used for production of fertilizers because of its high content of ammonium, potassium, phosphate and other mineral nutrients. The origin of the digestate is highly important, as the heavy metal content of the digestate may prevent its use as a fertilizer for food plants. It also appears reasonable to apply the liquid part of the digestate as nutrient supply for algae (Koller et al., 2014). Diluted liquid digestate from anaerobic treatment of the biosludge of pulp and paper production was found suitable for growing algae (Polishchuk et al., 2015).

7.4. Microalgal pigments

Algae, like all photosynthetic organisms, contain carotenoids and chlorophylls as their photosynthetic pigments. Carotenoids are grouped as carotenes that do not contain oxygen and to oxygen-containing xanthophylls, and they are yellow, orange, reddish or brown. All chlorophylls (*a* to *f*) are green pigments. Cyanobacteria and red algae (Rhodophyta) as well as glaucocystophytes and some cryptophytes also contain the blue or red phycobilins. Carotenoids and chlorophylls are lipophilic molecules that can be extracted as free pigments but phycobilins are covalently bound to the protein and their color is therefore lost by treatments (e.g. cooking) that denature the protein. The phycobiliproteins are water-soluble.

Due to the low productivities and high product recovery costs, microalgal pigments have not really come to the market yet. Furthermore, microalgal pigments have to compete with inexpensive identical pigments made by organic synthesis. If algal biomass is fractionated to oil fraction and a pigment-containing fraction that contains the chloroplast membranes, then the latter can possibly be marketed as nutrient supply because it always contains β-carotene (provitamin A) and α-tocopherol (vitamin E). Use in cosmetics is also possible (Koller et al., 2014).

7.4.1. Carotenoids

Carotenoids function as antioxidants in all organisms. Carotenoids specifically quench the harmful singlet form of oxygen and may also become oxidized by free oxygen radicals, thereby protecting cells against damaging action of several reactive oxygen species. This antioxidant capability makes carotenoids important constituents of "functional food". Approximately 1000 different carotenoids are known from the nature.

Two carotenoids are of particular importance. Firstly, β-carotene (provitamin A), a constituent of virtually all types of photosynthetic biomass, is particularly important for the biosynthesis of rhodopsin needed for the retina, and the lack of β-carotene in the diet is one of the most important causes of blindness in areas where rice is the most important food. The halophilic green alga *Dunaliella salina* can have an intracellular β-carotene content of 14% (Brányiková et al., 2011) and is therefore cultivated for provitamin A production. The second highly important algal carotenoid is astaxanthin that is considered an extremely powerful natural antioxidant. The main astaxanthin-producing alga is the green alga *Haematococcus pluvialis*, and astaxanthin production is done in industrial scale e.g. in China. In human metabolism, astaxanthin is important in protection against damage caused by UV radiation, in antibody production, and has been used for prevention of cancer and in anti-tumor therapy (Koller et al., 2014). Astaxanthin, or whole *H. pluvialis* biomass, is also added to salmon feed to color the fish flesh. Astaxanthin can also be used as a colorant in food industry (Wu et al., 2007).

7.4.2. Phycobilins

Phycobiliproteins are mainly used for specialty applications as chemical tags in biochemical research and as food colorants and in cosmetics (Koller et al., 2014; Arad and Yaron, 1992).

7.4.3. Chlorophylls

Most algae and cyanobacteria contain the same chlorophylls (*a* and *b*) as plants, and all chlorophylls are chemically relatively similar. Isolated chlorophylls have some uses in food coloring and even in cosmetics (Koller et al., 2014), but chlorophylls *a* and *b* are probably cheaper if isolated from grass and vegetables rather than from algae. Special algal or cyanobacterial chlorophylls (*d* to *f*) might have further, yet unexplored uses.

7.5. Microalgal lipids

Microalgal lipids have been of interest due to the possibility to use triacylglycerols for biodiesel production and due to the polyunsaturated fatty acids (PUFAs) of the chloroplast membrane lipids of many algae. Triacylglycerols usually do not contain PUFAs and therefore an increase in the oil content of the algae has little effect on the PUFA content (Polishchuk et al., 2015). The most important algal PUFAs are gamma-linolenic acid (GLA), eicosapentaenoic acid (EPA), docosahexaneoic acid (DHA) and arachidonic acid (ARA). PUFAs sold as pharmaceuticals have much higher prices than algal oil (Koller et al., 2014). ARA, DHA, EPA and GLA are important in human metabolism as precursors of the signaling molecules called collectively as eicosanoids. Prostaglandins are the best known eicosanoids. Eicosanoids are important in a large number of mammalian signaling systems in immune response, inflammation, allergy, cell growth and regulation of blood pressure (Karmali, 1996). Human metabolism produces all precursors of eicosanoids but their amounts can be sub-optimal. Fish and fish oil are traditionally used as specialty sources of these fatty acids in human nutrition.

7.5.1. EPA

The ω-3 fatty acid EPA is marketed as a food supplement and in aquaculture for fish-farming. The rationale of the use of EPA in fish feed is the possibility to boost the EPA content of the fish. *Nannochloropsis* sp. and *Phaeodactylum tricornutum* are EPA producing algae that have been tested for industrial use (Koller et al., 2014; Polishchuk et al., 2015). The EPA content of *Phaeodactylum tricornutum* is very low.

7.5.2. DHA

DHA is particularly important for the normal development of brain and eye (Koller et al., 2014), has anti-inflammatory effects and is important for the development of the fetus and for the production of breast milk (Kelley et al., 2009). In addition, DHA functions against colon cancer (Kato et al., 2007) and breast cancer (Trappmann and Hawk, 2011). DHA is marketed as dietary supplement.

DHA is a constituent of fish oil but is also produced commercially by the heterotrophic dinoflagellates *C.cohnii* and *Schizochytrium.* Some algae contain DHA, including *Pavlova lutheri,* but due to the relatively low price of sugar used to grow the heterotrophic organisms and the high density at which these organisms can be cultivated, it is unlikely that photosynthetic production of DHA could be economically viable.

7.5.3. ARA

ARA is a four-fold unsaturated ω-6 fatty acid that acts as a vasodilator and has anti-inflammatory effects and is necessary for the growth of skeletal muscles (Koller et al., 2014). ARA is also a component of membrane phospholipids. ARA is marketed as a food supplement.

7.5.4. GLA

GLA is a C18, ω-6 unsaturated fatty acid with anti-inflammatory effects and effects in autoimmune diseases and in suppression of tumor growth and metastasis (Koller et al., 2014), in skin allergies, diabetes, obesity, rheumatoid arthritis, regulation of blood pressure, premenstrual syndrome, multiple sclerosis, and in neurological diseases (Fan and Chapkin, 1998). In addition to the 18C GLA, a 20C version (DGLA) has importance in human metabolism.

GLA is found in cyanobacteria like *Arthrospira* (Mendes et al., 2006).

7.6. Carbohydrates

Microalgae may accumulate starch in the plastids in the same way as plants. Furthermore, chlorophytes may have cellulosic cell walls like plants. Cyanobacteria, in turn, synthesize glycogen or polyhydroxybutyrate as storage carbohydrate. The accumulation of storage carbohydrates can often be enhanced by similar conditions as used for enhancement of storage oil synthesis, e.g. nitrogen depletion or high carbon dioxide concentration. In addition to the storage carbohydrates that are long polymers, both algae and cyanobacteria may contain di or monosaccharides (Trivedi et al., 2015). The red alga *Porphyridium* contains sulfated polysaccharides that have pharmacological uses (Trivedi et al., 2015), and it is possible that new, yet unknown carbohydrate compounds with industrial use may be found from algae and cyanobacteria.

7.7. Fertilizers

Microalgal or cyanobacterial biomass could be used as a fertilizer as such, and extraction of oil or carbohydrates would not remove nitrogen, phosphorus and potassium that are the key ingredients of fertilizers. Unfortunately, it is not known how well algal biomass, as such or after extraction of carbonaceous compounds, fits as a fertilizer and how expensive the production would be (Maurya et al, 2016). Use of the remains from anaerobic digestion of algal biomass for fertilizer production was discussed already above.

7.8. Fish feed

Feeds make 30-60 % of the running costs of fish farms and the feed costs 1.5-2 EUR/kg. Microalgae may be used to supply part of the protein content of the feed but more importantly, the PUFAs present in some algae will find their way from the feed to the fish, thus improving the nutritional value of the fish.

The nutritional value and digestibility of each algal biomass must be tested before use as an ingredient of fish feed (Burr et al., 2011). A test of inclusion of *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* or *Isochrysis galbana* in the feed of Atlantic salmon showed that 36 %, 80 % and 19 % of the proteins were digested from the three species, respectively (Chauton et al., 2015).

8. Conclusions

The climate and the weather conditions set restrictions for outdoor cultivation of algae in Finnish conditions, and therefore a closed photobioreactor with (partly) artificial illumination would the easiest cultivation method to adopt. Greenhouses or underground tunnels with constant temperature make a possible environment for algal growth. Combining cultivation of algae with industry offers the access to excess heat streams and possibly to inexpensive electricity. Algae offer possibilities to capture nutrients, particularly nitrogen and phosphorus, from various wastewater streams.

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