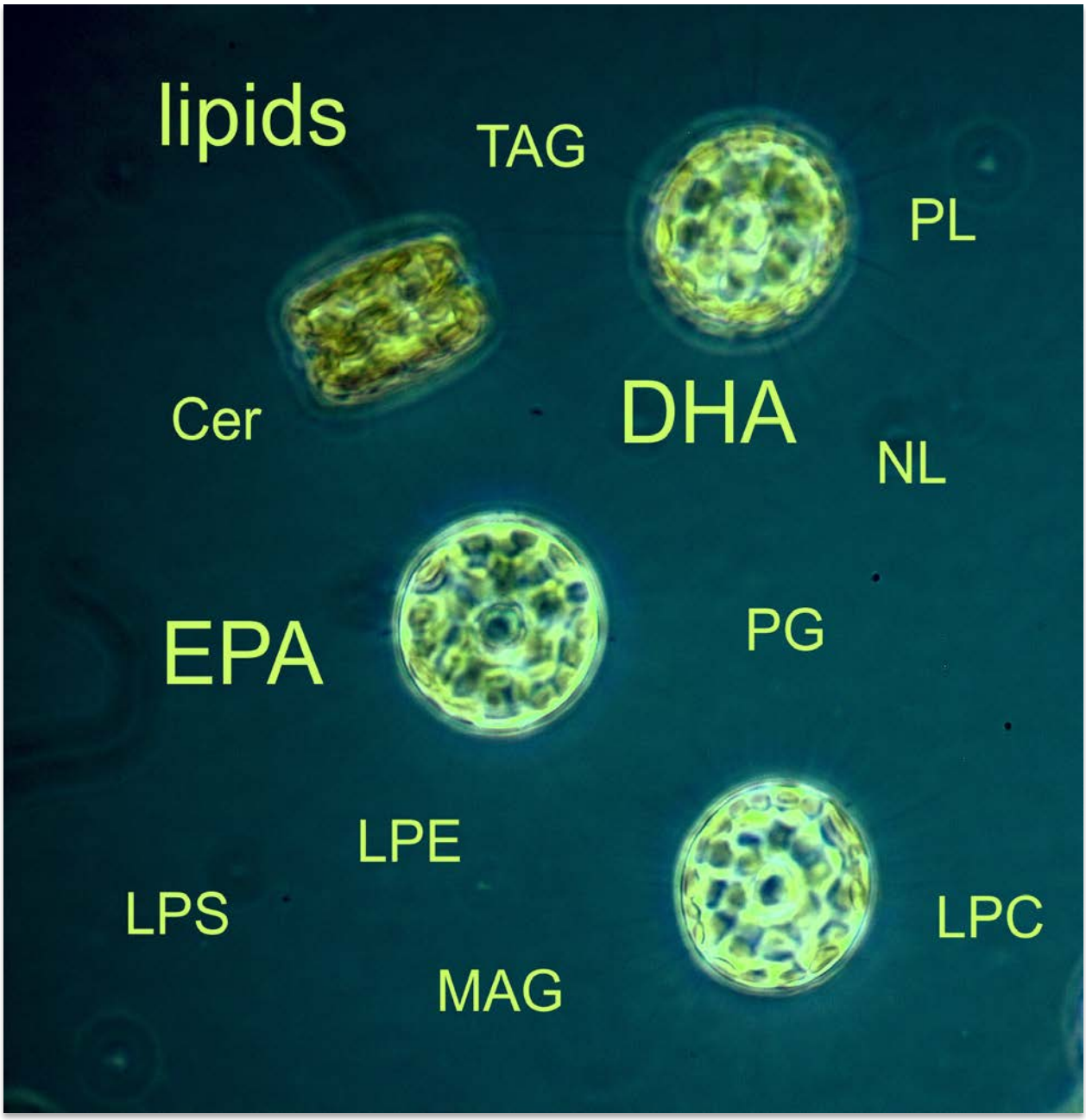
UiT
THE ARCTIC UNIVERSITY OF NORWAY

Faculty of biosciences, fisheries and economics
The Norwegian College of Fishery Science

Mass cultivation of some common coldwater diatoms (Bacillariophyceae): lipids vs. growth conditions

Elena Artamonova

A dissertation for the degree of Philosophiae Doctor – February 2017



UNIVERSITY OF TROMSØ

Abstract

The present study was aimed at investigating northern diatoms as sustainable sources of omega-3 fatty acids widely used and now much needed in salmon aquaculture as well as ingredients to be used in nutraceuticals in the human diet.

As known, fish oil is currently the main source of physiologically requisite fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). Due to the rapid increase of aquaculture production volumes and the world population in the recent decades, the problem of omega-3 lipid deficiency has attracted increased attention the last years. An increase in wild fish catches cannot be considered as the optimal solution to this problem, this since the anthropogenic pressure on the fish populations nowadays is already too high.

In the light of the universally accepted concept of sustainable development (Sustainable development paradigm), it is therefore essential to find completely new, environmentally friendly sources of the omega-3 fatty acids. Northern cold-water microalgae of the diatom group (Bacillariophyceae) are, in this context, potential candidates here due to their high growth rates and phylum- characteristic high content of omega-3 fatty acids, particularly EPA. However, knowledge on diatom lipidome is scarse, even less is known on how different abiotic parameters influence lipogenesis processes in a diatom cell.

Therefore, in the present study the northen cold-water diatoms were investigated in terms of their lipid and fatty acid composition. Besides, the effects of different cultivation parameters (light, temperature, CO2/pH) on lipogenesis were studied.

The results of the current research demonstrated that temperature decrease together with moderate light intensities may trigger accumulation of the polyunsaturated fatty acids (including physiologically requisite EPA) in diatom species. Besides, total lipid content and production of certain PUFAs in a diatom cell may be enhanced by means of CO_2 aeration. However, it should be emphasized that the metabolic responses demonstrated by diatoms were highly variable and species - specific. Thus, for the purpose of mass cultivation every single species (and even strain) should be investigated individually.

Abstract (summary in Russian)

Настоящее исследование посвящено поиску альтернативных источников омега – 3 жирных кислот, широко применяемых в аквакультуре и в качестве пищевых добавок в рационе человека. Как известно, в настоящее время рыбий жир является главным источником таких физиологически важных жирных кислот, как эйкозапентаеновая кислота (ЭПК) и докозагексаеновая кислота (ДГК). В связи со стремительным увеличением объёмов мировой аквакультуры в последние десятилетия, а также ростом населения Земли, проблема дефицита незаменимых жирных кислот стоит особенно остро. Увеличение объёмов вылова дикой рыбы не является оптимальным решением данной проблемы, так как в настоящее время антропогенный пресс на популяции промысловых рыб итак слишком велик. Чтобы следовать повсеместно принятой концепции устойчивого развития (Sustainable development paradigm), необходимо найти принципиально новые экологичные источники незаменимых жирных кислот.

В качестве таких альтернативных источников были рассмотрены обитающие в северных морях микроводоросли группы диатомовых (Bacyllariophyceae), которые имеют характерное для своего филума высокое содержание омега – 3 жирных кислот, в частности ЭПК. Микроводоросли выращивались в фотобиореакторах с регулируемыми параметрами культивации (свет, температура, pH и др.). Было рассмотрено влияние абиотических факторов, таких как свет, температура, аэрация углекислым газом, на рост и липогенезис водорослевых культур в целях определения оптимальных параметров культивации.

Результаты данного исследования показали, что при определенном сочетании параметров культивации, строго индивидуальных для каждого тестируемого вида микроводорослей, возможно существенно увеличить продукцию липидов, а также содержание незаменимых жирных кислот в культурах. Кроме того, была выявлена возможность применения водорослевых культур для секвестирования производственных выбросов углекислого газа.

List of Papers

Paper I E.Y. Artamonova, J.B. Svenning, T. Vasskog, E. Hansen, H.C. Eilertsen. Analysis of phospholipids and neutral lipids in three common northern cold-water diatoms: *Coscinodiscus concinnus, Porosira glacialis* and *Chaetoceros socialis* by ultra-high performance liquid chromatography-mass spectrometry (accepted for publication in *Journal of applied phycology*).

Paper II E.Y. Artamonova, H.C. Eilertsen, T. Vasskog. Lipid content and fatty acid composition of *Phaeocystis pouchetii*, *Chaetoceros furcellatus* and *Thalassiosira nordenskioeldii* cultivated at varying light and temperature conditions (under second revision in *Open Life Science*).

Paper III E.Y. Artamonova, T. Vasskog, H.C. Eilertsen. Lipid content and fatty acid composition of *Porosira glacialis* and *Attheya longicornis* in response to carbon dioxide (CO_2) aeration (under revision in *Microbial cell factories*).

I look forward with great optimism. I think that we undergo not only a historical, but a planetary change as well. We live in a transition to the noosphere."

- V. I. Vernadsky ("The Biosphere and the Noosphere", 1945)

Introduction

Cold water marine diatoms and their potential for production of high quality lipids in mass cultures

The contemporary world is facing numerous ecological and environmental problems due to the exponentially growing population, the associated increase of industrialization and the anthropogenic pressure imposed upon wild nature. That is why the concept of *sustainable* development is a cornerstone of successful integration with nature, which in the longer run can secure the survival of humanity as a biological species. *Vladimir Ivanovich Vernadsky*, a famous Russian scientist and philosopher, stated that the human mind or cognition is a powerful geological force analogous to that of all the living organisms that in eons has changed the face of the Earth by creating a "*bio-sphere".* According to Vernadsky, the next evolutional stage of the planet's (biosphere) development is its transition to a "*noo-sphere"* (*Greek: "*nous – mind"), when humanity using its intellectual potential will create a unique planetary guise, where the human interests are perfectly balanced with nature (Vernadsky 1945). It is therefore a task of the science, in the service of the humankind, to meet this global challenge.

The PhD study was meant as a contribution to the establishment of more sustainable environmental practices, thereby minimizing the pressure put on the wild nature today.

One central issue that today's lipid research is focused on is finding sustainable alternative sources of fish oils (omega – 3 lipids) widely used in aquaculture and as supplements for human nutrition. As recent studies have shown, the global lack of fish oil supply will be a huge problem within a few years (Ward and Singh 2005). Beside this, the pressure put on the wild fish stocks nowadays is severe, and this can, if continued, lead to a collapse (Pauly et al. 2002).

The aquaculture today hence is “struggling” with fish oil deficiency, and this is “compensated” by partial or total substitution of fish oils by fats originating from terrestrial plants. This practice has been shown not to be optimal due to unbeneficial fatty acid composition of the higher plants (high n-6/n-3 ratio) that is claimed to cause numerous health pathologies in farmed fish (Izquierdo et al. 2005; Montero et al. 2003; Morkore 2006; Seierstad et al. 2005). Therefore, sources of lipids with high omega-3 content is constantly sought for, and here the microalgae of the diatom group may be promising candidates due to their beneficial, omega - 3 rich lipid content (Kates and Volcani 1966; Levitan et al. 2014).

Diatoms (Bacillariophyceae) are unicellular autotrophic organisms that have a characteristic structure – frustules, comprising two *thecas* i.e. silica cell walls. Depending on the shape of the frustule, the diatoms are subdivided into two major orders – Centrales and Pennales. The Centrales are radially symmetric and the Pennales have bilateral symmetry (Hasle and Syvertsen 1997). The diatoms are a highly diverse taxon, comprising 100 000 - 200 000 species (Mann and Droop 1996). Being exceptionally productive (40-45 % of primary oceanic production), diatoms form the basis for sustaining of large fisheries. In fact, diatom productivity surpasses that of the world tropical rainforests. Diatoms also play an important part in the biogeochemical cycling of C and Si (Field et al. 1998). The ecological success of this microalgal group is usually associated with its high genomic plasticity and ability to adapt to highly variable habitats (Armbrust et al. 2004).

In the high northern latitudes, cold water diatom species are an important part of the marine ecosystems since they accumulate solar energy in form of energy-rich molecules, i.e. fats that are transferred up the food chains. This is why northern marine organisms are highly dependent upon diatoms, especially during the winter darkness periods (Sargent et al. 1985). Microalgae, particularly diatoms, synthesizes *de novo* all fats in the marine ecosystems, implying that all other organisms obtain their fats directly or indirectly from the diatoms (Guschina and Harwood 2009).

Though the diatom lipidome is in general similar to that of higher plants, some important differences exist. In contrast to higher plants’ lipid profile, which has linoleic acid as the dominating polysaturated fatty acid (PUFA), the most abundant diatom fatty

acids are EPA and palmitoleic acid (Kates and Volcani 1966). This is in addition to phospholipids, nonpolar glycerolipids, glycolipids and betaine lipids commonly present in microalgae (Guschina and Harwood 2009). Neutral lipids in the form of triacylglycerols (TAGs), diacylglycerols (DAGs) and monoacylglycerols (MAGs) can amount to more than 60 % of total lipids in diatoms (Chen 2012). Due to their high productivity, elevated lipid content and beneficial fatty acid composition, diatoms are often perceived as promising candidates for production of food supplements, fish feed and biodiesel (Adarme-Vega et al. 2012; d'Ippolito 2015; Levitan et al. 2014; Ryckebosch et al. 2014; Spolaore et al. 2006).

The idea to use microalgae as "biofactories" for lipid production is not a novel one. The attempts to "tame" microalgae dates back to the early 50's, when alternative protein sources were intensively sought for. The major interest to commercial cultivation of microalgae was demonstrated by Asian countries where large microalgae industries (based mainly on *Chlorella*) emerged. Since then, the cultivation biotechnology has increased significantly in terms of scale and geographic coverage. Besides, many new species have been introduced (Spolaore et al. 2006). The main species dominating microalgae mass production initiatives nowadays are from genera *Arthrospira, Chlorella, Dunaliella* and *Aphanizomenon* (Spolaore et al. 2006), while diatoms are still underexploited (Levitan et al. 2014).

Cultivation of microalgae may help to cope with another important environmental challenge, i.e. CO_2 emissions from power plants and heavy industries. Carbon dioxide, one of the main atmospheric greenhouse gases, constitutes around 0.04 %, and its volume is claimed to increase due to the increasing industrialization rates (Ramanathan 1988). The Kyoto Protocol (1997) aimed to reduce the emissions of the greenhouse gases by 5.2 % was ratified by more than 170 countries. There are two major groups of methods that were investigated to reduce the emissions of CO_2: a) chemical reaction-based; b) biological mitigation (Wang et al. 2008). The first group of methods is rather costly since expensive chemicals are needed, while the other is more economically beneficial and environmentally friendly. This since mitigation of CO_2 from power plants and industries can be combined with production of algal biomass (Wang et al. 2008), and its further conversion to valuable products like biodiesel, fish feed, food supplements, etc.

Attempts to use microalgae to mitigate CO_2 has also been made earlier (de Morais and Costa 2007b; Gomez-Villa et al. 2005; Scragg et al. 2002), however cold water diatoms have not been studied in terms of their tolerance to elevated CO_2 levels and/or as CO_2 sequestrators.

It should be noticed that diatom species were shown to be highly diverse either genetically (Lundholm et al. 2006; Sarno et al. 2005) or in terms of chemical composition (Huseby et al. 2013). This is likely to be the result of their complex evolutionary history and the fact that they were proved to encompass genes of both green and red algal origin (Moustafa et al. 2009). Some data have shown as well the presence of bacterial genes in diatoms (Bowler et al. 2008).

Mapping of the diatom lipidome. Effects of light, temperature and CO_2 aeration on lipogenesis in diatoms

Though diatom lipid profiles have been reported frequently (Chen 2012; de Castro Araújo and Garcia 2005; Duerksen et al. 2014; Rampen et al. 2010), the diatoms living at low ambient temperatures have rarely been studied. Therefore, the current study has its focus on commonly occurring northern diatom bloom species cultivated at the ambient in the area temperatures.

The mass cultivation of diatoms is a challenging task, since algae are grown at unnaturally high densities. Here a lot of density associated problems may emerge, such as self-shadowing, nutrient deficiency, bacterial contamination, etc. (Sheehan 1998). Besides, abiotic parameters, i.e. light (intensity/duration), CO_2 aeration, temperature, pH, salinity, nutrients, etc. should be tested for the optimal microalgae growth and the desired lipid composition. The influence of three main cultivation parameters (light, temperature and CO_2 aeration) on the lipogenesis in diatoms were therefore studied (*Papers II* & *III*). Besides, the diatom lipidome was mapped in terms of lipid - and fatty acid composition of two major lipid groups: phospholipids and neutral lipids (Paper I).

Though there is a large number of abiotic factors effecting the growth and chemical composition of microalgae, the above mentioned parameters were chosen firstly due to their importance for the microalgal physiology and, secondly, due to the relative easiness of their control and adjustment under mass cultivation conditions.

There is a large amount of information on how light and temperature can influence lipid and fatty acid composition of microalgae, e.g. (Jiang and Gao 2004; Khoeyi et al. 2012; Roleda et al. 2013; Thompson et al. 1990), see Table 1. As it can be noticed, the metabolic responses to these variables are highly variable and species-specific, though some important common trends exist.

Table 1. Influence of light, temperature and CO_2 aeration on the lipogenesis in microalgae.

Cultivation parameter	The observed physiological effect	Species of microalgae	Reference
Light	The content of 20:5 (n-3) and some other polyunsaturated fatty acids decreased at higher light intensities (1100-1200 µE m-2s-1). The concentration of 22:6 (in *Isochrysis* sp), on the contrary, increased.	*Isochrysis sp., Nannochloropsis oculata*	(Renaud et al. 1991)
	The concentration of PUFA (n-3) increased with increasing light intensity (ranging from 83 to 1395 µE $m^{-2}s^{-1}$).	*Chaetoceros gracilis*	(Mortensen et al. 1988)
	At the lowest light intensity (9 Wm^{-2}) the highest content of EPA and DHA was found.	*Pavlova lutheri*	(Guedes et al. 2010)
	The amount of EPA and the MGDG : DGDG ratio increased with the decreasing (2 µE $m^{-2}s^{-1}$) light intensity.	*Navicula gelida, Fragilariopsis curta, Nitzschia medioconstricta (mixed culture)*	(Mock and Kroon 2002)
	The increasing light intensity (from 37.5 to 100µE m-2s-1) and duration caused decrease in PUFAs and monoens.	*Chlorella vulgaris*	(Khoeyi et al. 2012)
	EPA concentration increased in lower light intensities, while DHA content, on the contrary, decreased. The concentration of 16:0 was positively correlated with light intensity.	*Chaetoceros calcitrans,Thalassiosira pseudonana, Chaetoceros simplex, Chaetoceros gracilis, Phaeodactylum tricornutum, Dunaliella tertiolecta, Pavlova lutheri, Isochrysis gabana*	(Thompson et al. 1990)
	Total lipid content increased with increase of light intensity (up to 150 µmol/m2/s)	*Isochrysis galbana LB987, Nannochloropsis oculata CCAP849/1, and Dunaliella salina,*	(Gim et al. 2016)
	Total lipid content increased from 24.8% to 37.5% with increasing light intensity.	*Chlorella protothecoides*	(Krzemińska et al. 2015)
Temperature	The total omega -3 PUFAs of Chlorella strain increased with the temperature decrease from 30 ^{0}C to 15 ^{0}C. In *Nannochloropsis*, the total omega -3 PUFAs increased with the temperature increase up to 25 ^{0}C with further decline (up to 35 ^{0}C).	*Chlorella* strain MFD-1 *Nannochloropsis* strain MFD-2	(James et al. 1989)
	The ratio of n-3/n-6 and the content of unsaturated fatty acid increased at the lowest cultivation temperature (18 ^{0}C).	*Chaetoceros gracilis*	(Mortensen et al. 1988)
	The concentrations of EPA and total fatty acid (TFA) increased with the temperature decrease from 26 ^{0}C to 17 ^{0}C.	*Nannochloropsis salina*	(Hoffmann et al. 2010)
	The content of EPA and PUFAs increased with the temperature decrease from 25 ^{0}C to 10 ^{0}C.	*Phaeodactylum tricornutum*	(Jiang and Gao 2004)
	The concentration of EPA increased with the temperature decrease from 40 ^{0}C to 24 ^{0}C.	*Phaeodactylum tricornutum, Chaetoceros muelleri*	(Rousch et al. 2003)

	The content of PUFAs in *Chaetoceros* sp. increased with the temperature decrease from 35 ^{0}C to 25 ^{0}C. The total lipid content, EPA and DHA increased at the lowest cultivation temperature (25 ^{0}C). *Rodomonas* sp., *Cryptomonas* sp. and *Isochrysis* sp. had highest lipid content at 27-30 ^{0}C (medium temperature range).	*Chaetoceros sp.,Rodomonas sp., Cryptomonas sp.,Isochrysis sp.*	(Renaud et al. 2002)
	PUFA concentrations decreased with the increasing temperature (from 10 to 35 ^{0}C) in *N. paleacea*, *Isochrysis* sp. and *Nitzschia closterium*	*Isochrysis sp. Nitzschia closterium, N. paleacea*	(Renaud et al. 1995)
	Decrease of temperature from 30 ^{0}C to 20 ^{0}C triggered synthesis of longer fatty acid chains with more unsaturations (including EPA) in MGDG and DGDG.	*Haslea ostrearia, Phaeodactylum tricornutum*	(Dodson et al. 2014)
	The total lipid concentration increased with the temperature decrease (from 30 ^{0}C to 25/20 ^{0}C).	*Chaetoceros* cf. *wighamii*	(de Castro Araújo and Garcia 2005)
	Temperature decrease induced significant lipid accumulation in all species but *Nannochloropsis oculata*. In *N. oculata* the increase of total lipid content was <2 %.	*Thalassiosira pseudonana, Odontella aurita, Nannochloropsis oculata, Isochrysis galbana*	(Roleda et al. 2013)
CO_2 aeration	High CO_2 levels (30-50 %) enhanced the total lipid and PUFA accumulation.	*Scenedesmus obliquus, Chlorella pyrenoidosa*	(Tang et al. 2011)
	CO_2 supply increased lipid productivity of the culture.	*Chlamydomonas* sp. JSC4	(Nakanishi et al. 2014)
	CO_2 aeration enhanced lipid productivity in *Scenedesmus* sp., *Botryococcus braunii.*	*Scenedesmus* sp., *Botryococcus braunii, Chlorella vulgaris*	(Yoo et al. 2010)
	CO_2 supply increased lipid productivity but decreased the amount of PUFAs.	*Pavlova lutheri*	(Carvalho and Malcata 2005)
	High CO_2 levels (10 -30 %) induced lipid accumulation in the culture.	*Chaetoceros muelleri*	(Wang et al. 2014)
	CO_2 supply increased lipid productivity of the culture.	*Nannochloropsis oculata*	(Chiu et al. 2009)
	CO2 supply did not influence lipid content of the culture	*Chaetoceros* cf. *wighamii*	(de Castro Araújo and Garcia 2005)

Thus, temperature decrease was shown to enhance PUFAs accumulation in microalgae by a number of publications e.g. (Jiang and Gao 2004; Renaud et al. 1991; Roleda et al. 2013), this due to necessity to maintain membrane fluidity at low temperatures (Harwood 1988). Since the northern diatoms live at low ambient temperatures, their lipid profile is likely to be naturally rich in polyunsaturates (Leu et al. 2006). Illumination is another important parameter that was proven to affect lipid composition of microalgae. However, the effect of light on the lipid content and fatty acid composition seems to be complicated and highly variable between the species (Table 1). In a number of publications the lower light intensities proved to induce PUFAs accumulation (including EPA), but differences between the species should be taken into consideration (e.g. Mortensen, 1988). This effect might be due to photooxidation of PUFAs in the higher light intensities as it was demonstrated by Leu et al. (2006).

CO_2 aeration is a relatively new cultivation parameter that is not properly studied yet. Though, the publications available (Table 1) point out the positive effect of CO_2 on lipid

productivity and PUFAs accumulation (e.g. Tang et al., 2011; Nakanishi et al., 2014). The important issue here is to keep the levels of CO_2 supply within the physiological optimum of the species in order to avoid the reduction of growth/production.

There are four main strategies that are used nowadays to utilize the CO_2 constituent of the flue gas coming from the plants (Thomas et al. 2016): 1) CO_2 segregation using adsorbents; 2) CO_2 segregation using adsorbents with consequent regeneration; 3) conversion of flue gas to liquid for direct mitigation by microalgae; 4) direct mitigation of flue gas. Depending on the applied strategy, the CO_2 fixation rate and the biomass productivity may differ (see Table 2).

Table 2. The main production parameters depending on the applied strategy (modified from Thomas et al. (2016)).

Strategy applied	Microalgal species	CO_2 fixation rate	Biomass produced	References
CO_2 segregation using adsorbents with consequent regeneration	*Desmodesmus sp.*	1.5 % optimum utilized		(Brilman and Veneman 2013)
Conversion of flue gas to liquid for direct mitigation by microalgae	*Scenadesmus sp.*	216.4 mg CO_2/L/day	115.7 mg /L/day	(Choi et al. 2012)
Direct mitigation of flue gas	*Spirulina sp.*	37.9 % in the presence of 6 % CO_2(v/v)	0.22 g /L/day	(de Morais and Costa 2007a)

Objectives of the present study

The main objectives of the current study were to:

a) Analyse the lipid composition of northern marine diatoms and assess their suitability for production of high quality lipids (Paper I);

b) Evaluate the influence of abiotic factors (light, temperature, CO_2) on lipid production and growth of the microalgal cultures (Paper II, Paper III);

c) Evaluate the potential of diatoms to CO_2 mitigation (Paper III).

Methods

Species used in the study

All species but one used in the present study are common cold water diatoms belonging to the subdivision Centrales: *Porosira glacialis, Chaetoceros socialis, Chaetoceros furcellatus, Coscinodiscus conncinnus*, *Attheya longicornis, Thalassiosira nordenskioeldii*. One of the species analyzed is the prymnesiophyte *Phaeocystis pouchetii.* These species are typical representatives of the spring bloom assemblages of the northern fjords and the Barents Sea that rarely changes in time and space (Degerlund and Eilertsen 2010).

Diatom cultivation

Monocultures of the species used in the current research - *Porosira glacialis, Chaetoceros socialis, Coscinodiscus conncinnus* (Paper I); *Porosira glacialis, Attheya longicornis* (Paper III) were established from the stocks collected in the Barents Sea (80 ^{o}N) or from the coast of northern Norway (70^{o} N). For Paper II, experimental data (cultivation conditions, growth rates) obtained during the 80-s by Tromsø University personnel and kindly analyzed for lipids by Jim Henderson were used for the analysis. The species analyzed in Paper II - *Phaeocystis pouchetii, Chaetoceros furcellatus, Thalassiosira nordenskioeldii*.

The identification of the investigated species was performed by means of morphological and molecular methods as described by Huseby (2011). The isolation of species was performed manually by transferring a single cell or a colony with a micropipette to 50 mL Nunclon culture flasks filled with f 10 medium (Guillard and Ryther 1962). When the cultures reached sufficiently high densities, they were transferred to sterilized 1.5 L soft drink (PET) bottles that were further used to inoculate large volume (100 - 300L) plexi columns with external illumination in temperature and irradiance controlled rooms (Paper I, III). For the cultivation of the microalgae used for the analysis in Paper II small volume bottles (1.5 L soft drink (PET)) were used. The temperature was kept at 5-7 ^{0}C (close to the ambient in the area) and the irradiance at 66 µmol quanta m^{-2} s^{-1} (Paper I) 33, 2 µmol quanta m^{-2} s^{-1} (Paper III) at photoperiod 14:10 (light : dark). The microalgal cultures used for the analysis in Paper II were cultivated at varying light (76 -, 43-, 20 -, 8 µmol quanta m^{-2} s^{-1}) and temperature (2 and 5 ^{0}C) conditions at photoperiod 16:8 (light : dark) .

Surplus CO_2 supply (Paper III) 20 - 25 % was provided to the microalgae culture three days before harvesting. For this purpose, the ambient air mixed with CO_2 coming from

40 L pressured (200 atm) CO_2 steel tank (AGA, UN 1013, Norway) was provided to the cultures.
The harvesting was performed by means of vacuum filtration through plankton mesh (Sefar Nytal R) and the algal biomass was freeze-dried until further analysis was undertaken.

Biochemical analysis

For the biochemical analysis (Paper I) the freeze-dried microalgal pellets were transported to Prague Institute of Chemical Technology (ICT) and Biolab (Bergen) where they were analyzed for total lipid content (Biolab) and lipid composition (ICT). For the total lipid extraction a modified method of Bligh and Dyer (1959) was applied and then the quantification was performed gravimetrically. This method was originally developed for working with animal (cod) tissues and was shown to be efficient even with low (<2 %) lipid content in the samples (Iverson et al. 2001). Two non-polar solvents, chloroform and methanol in proportion 1:2 are used for the lipid extraction by the method. The sample to solvent proportion is 1:3.

The separation of the lipid classes was done by means of the ultra-high pressure liquid chromatography–mass spectrometry (UHPLC-MS). Shortly, the method unites two different techniques; separation of the matters by means of liquid chromatography and their detection by means of mass spectrometry. First, the analyzed substances are partitioned between the mobile liquid phase and the stationary solid phase that is packed into the chromatographic column. Then the single compounds in the mixture are chromatographically separated and converted into the ions in the gas phase, while the eluent is discarded. The ionization methods that are widely used today include Electrospray ionization (EI) and Atmospheric pressure chemical ionization (APCI). The ions are sorted according to their mass to charge (m/z) ratio (molecular weights) in the mass analyzer. The mass anylizers may be of different types: Time of Flight, Ion Trap, Quadropole and Magnetyic sector (Pitt 2009).

The total lipid analysis for Paper II was performed by means of two methods: a) the acid–dichromate method by Amenta (1964) that uses a nonspecific reaction for quantifying all the lipid species that were previously separated by chromatography; b)

the method by Folch et al. (1957) followed by gravimetric estimation. The method by Folch et al. (1957) uses the same solvent mixture as that of Bligh and Dyer (1959) - chloroform and methanol, but in the proportions 2:1, while the sample : solvent ratio is 1:20.

For the analysis of the fatty acid composition the gas chromatography-mass spectrometry (GC-MS) was used. This method uses some inert gas (e.g. argon) as the mobile phase. The stationary phase is a packed capillary column covered by a polymeric film. The separation is dependent on the polarity and the retention time of the single compounds in the mixture. The operating temperature is usually about 300 ^{0}C that allows individual compounds elute from the GC column and enter the electron ionization detector where the compound are bombarded by electrons in order to fragment them and form ions. The resulting compounds are detected based on their m/z ratio (Sneddon et al. 2007).

For Paper III the total lipid analysis was performed as described by Cequier–Sanchez et al. (2008) with slight modifications. This method uses dichloromethane: methanol (2:1 v/v) as the extractant. The extraction of lipids was performed twice to increase the resulting yield. After centrifugation, the chlorophorm phase containing lipids was damped by nitrogen in EVAP. The lipid content was then quantified gravimetrically. The total fatty acid composition was determined applying a slightly modified method by Stoffel et al. (1959). The method uses modification (esterification) of the analyzed fatty acids prior to GC-MS analysis by means of methanol and sulfuric acid. For the reaction catalization, the reagent mixture was heated to 100 ^{0}C for 1 hour. Then salt water (5 % NaCL) with hexane was added (1:1) to the mixture. The phase containing lipids (hexane) was damped under nitrogen in EVAP and further used for the total fatty acid analysis by means of GC-MS. For the separation and quantification of the methylated fatty acids, the samples were transported to our collaborators Norut Northern Research Institute (Tromsø) where the GC-MS analyses were performed.

Statistics

Descriptive and univariate statistics (Paper I, II, III) was performed by using Excel 2013. For the multivariate statistical analysis of the data (Paper III) R version 3.0.2 was used.

Summary of results and discussion

Influence of cultivation conditions on growth and total lipid content in diatoms

The results obtained by the current study (Papers I - III) support the previously suggested assertion that diatoms are promising candidates for mass cultivation (Levitan et al. 2014; Mata et al. 2010). The species examined - *Porosira glacialis, Chaetoceros socialis, Coscinodiscus conncinnus, Attheya longicornis, Chaetoceros furcellatus, Thalassiosira nordenskioeldii* and also the haptophycean *Phaeocystis pouchetii* demonstrated reasonable amounts of lipids, though highly variable dependent on the cultivation conditions and the species examined (Papers II, III). Thus, when cultivated at 2 °C, the lipid content of *C. furcellatus* varied from ca 2 % to 20 % depending on the light intensity it was cultivated at (Paper II). Similarly, *T. nordenskioeldii* demonstrated variation in lipid content from ca 3 to 30 % at different light intensities when cultured at 5 °C. At 2 °C this variation was not that tremendous (from ca 3 to 7 %). The lipid content of haptophycean *P. pouchetii* also differed a lot depending on the light intensities it was cultivated at (from ca 4 to 27 % at 5 °C and from ca 6 to 12 % at 2 °C). Previous studies (Gim et al. 2016; Krzemińska et al. 2015) demonstrated positive effect of the light intensity on the total lipid content in microalgae. In contrast, our study did not demonstrate any consistent trend in terms of lipid content variation as response to the light variable. However, the light – associated effects were not possible to test in terms of statistical significance in this study and thus the results should be interpreted with precaution.

The temperature decrease from 5 to 2 °C did not show any statistically significant ($p > 0.05$) effect on the total lipid content in either of species. This is opposing results of Roleda et al. (2013) and de Castro Araújo and Garcia (2005) that demonstarted negative correlation between total lipid content and temperature. Most likely, such decrease (Δ 3 °C) was not significant enough to trigger changes in lipid accumulation reactions of the investigated species.

CO_2 supply (Paper III) was shown to increase the total lipid content in *Porosira glasialis*. Thus, the lipid content of the CO_2 aerated culture constituted 10.57 % if compared to 8.91 % in control. This result is in accordance with e.g. findings of Tang et al. (2011) (Wang et al. 2014) that showed positive effect of CO_2 aeration on the total lipid content in *Scenedesmus obliquus*, *Chlorella pyrenoidosa* and *Chaetoceros muelleri.*

However, *Attheya longicornis* did not show any statistically significant ($p > 0.05$) increase in terms of total lipid abundance when subjected to CO_2 aeration. Similarly, de Castro Araújo and Garcia (2005) did not find any significant difference in lipid content of Chaetoceros cf. wighamii when it was subjected to CO_2 aeration. Thus, species-specific differences in response to this variable were obvious.

The growth of the microalgae was proved to be dependent on the cultivation temperature for all species but *T. nordenskioeldii,* that did not demonstrate any statistically significant ($p > 0.05$) difference of the growth rates between the investigated temperatures. Thus, temperature increase from 2 to 5 ^{0}C positively influenced the growth of *C. furcellatus* and *P. pouchetii*, which is in coincidence with the Eppley (1972) model.

Additionally, the linear regression analysis did not show any significant (at 95 % level) correlation between the growth rate and the total lipid content in any of the investigated diatoms.

The light also influenced growth of the examined species (that is biologically reasonable), though the statistical significance of this effect was not possible to test. Thus, *C. furcellatus* demonstrated increase in the growth rate with the reduction of the light intensity from 76 to 20 μE $m^{-2}s^{-1}$. Further decrease of the light intensity (from 20 μE $m^{-2}s^{-1}$to 8 μE $m^{-2}s^{-1}$) resulted in the decrease of the growth rate in this species. In contrast, *T. nordenskioeldii* demonstrated increase of the growth rate with the increasing light intensity at both temperatures.

CO_2 aeration was shown to have an effect on the growth of the microalgal cultures, but similarly with other variables its influence was species-specific. Thus, the microalgal cultures demonstrated different grade of tolerance to the high levels (20 - 25 %) of CO_2 supply: while the growth rates of *P. glacialis* stayed almost unchanged ($p > 0.05$) if compared to the control, the cultures of *A. longicornis* are likely to undergo reduction of the growth rates (stress reaction). Multiple studies e.g. (Tang et al. 2011; Wang et al. 2014; Yoo et al. 2010) demonstrated positive effect of CO_2 aeration on microalgae growth, this since the tested aeration levels were within the tolerance borders of the investigated species.

Lipid and fatty acid composition (EPA and DHA) of diatoms and its response to the abiotic variables

Qualitative analysis of the lipid content in the diatom species revealed their beneficial lipid composition with prevailing amounts of omega-3 fatty acids (Papers I - III), especially when the physiologically requisite EPA was considered. However, the variations between the species and the cultivation conditions were evident. Another important omega-3 fatty acid, DHA was either absent or present in significantly lower amounts than those of EPA, dependent on the species examined. Thus, in *C. socialis* no DHA was found, while *P. glacialis* demonstrated 6.3 % of DHA in its phospholipid profile. The haptophycean *P. pouchetii* showed naturally high content of this fatty acid (up to 9.8 %), though extremely variable depending on the cultivation conditions (Paper II).

In addition to the above mentioned omega-3 fatty acids, diatoms showed large amounts of palmitic, palmitoleic and myristic fatty acids (Papers II, III), that is typical for the microalgae of the diatom group (Kates and Volcani 1966).

Neutral lipids (TAGs, DAGs, MAGs and FFA) were shown to be the most abundant lipid group, followed by phospholipids (Paper I). Among phospholipids, PC, PG, and PI were the dominating lipid species. The fatty acid composition of both lipid groups demonstrated that both neutral lipids and phospholipids had high amounts of EPA, while DHA was mostly concentrated in phospholipids.

The fatty acid composition of the investigated diatoms was proven to be a function of cultivation variables and the species examined (Papers II, III). Thus, the principle correspondence analysis (PCA) revealed that the polyunsaturated fatty acids (including EPA) were most abundant in the species grown at low/moderate light intensities (mostly equal to 20 $\mu E\ m^{-2}s^{-1}$). Previous studies e.g. (Khoeyi et al. 2012; Renaud et al. 1991) demonstrated negative correlation between light intensity and PUFAs content in microalgae. This since polyunsaturated fatty acids are prone to photooxidative damage when subjected to high light intensities as it was shown by study of Leu et al. (2006).

The temperature variations also influenced the fatty acids distribution in the investigated species. Thus, temperature decrease resulted in an expected increase in the concentrations of polyunsaturated fatty acids in both *P. pouchetii* and *T. nordenskioeldii,* while the amount of saturates in these species correspondently decreased. This pattern of metabolic response to temperature lowering is well documented for both diatom and non - diatom species e.g. (Hoffmann et al. 2010; James et al. 1989; Jiang and Gao 2004; Renaud et al. 1995).

In contrast, *C. furcellatus* did not show any statistically significant ($p > 0.05$) differences in the fatty acid composition between the temperatures. That is in coincidence with a study by Renaud et al. (2002) which did not demonstrate any significant change in polyunsaturates abundances of Chaetoceros sp. when subjected to temperature decrease.

CO_2 supply was also shown to influence the fatty acid composition of the microalgal cultures, but similarly with other variables, this influence was species-specific. Thus, EPA content of *P. glacialis* decreased from ca 27 to 23 % in CO_2 aerated cultures, while in *A. longicornis* the concentration of this PUFA did not show any statistically significant variations.

DHA concentration, on the contrary, significantly ($p > 0.05$) increased in *P. glacialis* cultures (from ca 4 to 6 %) when subjected to CO_2, while in *A. longicornis* DHA content stayed almost unchanged.

The concentration of total PUFAs demonstrated a slight increase (from 48.63 to 49.26 %) in CO_2-aerated cultures of *A. longicornis*, while in *P. glacialis* such effect was not statistically obvious ($p > 0.05$). These results are partly in coincidence with a study by Tang et al. (2011) that demonstrated elevated PUFAs content in CO_2 aerated cultures of *Scenedesmus obliquus* and *Chlorella pyrenoidosa.* However, Carvalho and Malcata (2005) showed opposing results for *Pavlova lutheri*.

Conclusion

The current study has demonstrated that northern cold-water diatom microalgae have a great potential to become a sustainable source of high quality omega-3 lipids in the future. Besides, the results of the present research suggest that northern diatoms may be successfully used for industrial CO_2 mitigation if species – specific tolerance thresholds are considered. However, we are just at the beginning of the path and a substantial research work has still to be done in order to find the optimum parameters for the diatom cultivation and investigate the diatoms ability for CO_2 sequestration (here especially species-specific CO_2 tolerance thresholds should be focused on).

It should be pointed out that the species examined have a very low temperature optimum coupled with more than a moderate light demand due to the genetically comprised adaptation to the severe environments of the northern seas. These

outstanding physiological features of the northern diatoms makes possible their cultivation in the ambient in the area water temperatures i.e. without any substantial energy supply. This implies that the cost of the microalgae cultivation can be drastically reduced (given the increasing production volumes). Though we do not provide any economically based estimations in the frames of the current study, it looks reasonable to couple the future biologically oriented research with the economic advice.

Acknowledgements

I would like to thank my family, especially my father Yuri Aleksandrovich Artamonov, who gave me an example of unprecedented courage and optimism, when he was fighting with kidney cancer without saying a single word of complaint to his colleagues and relatives. Until the last days of his life he attended his work at the Murmansk Technical University (where he taught future captains the basis of Global Maritime Distress and Safety System (GMDSS)), though every single step he made was causing him pain due to cancerous metastasis in the thighbone. I hope that his nature of the optimistic and never - giving up fighter I have proudly inherited.

I would like to sincerely thank my father –in- science Hans Christian Eilertsen that introduced me into the field of marine biology research, helped me to develop my researcher's skills by patiently correcting hundreds of raw versions of my papers. With his help, I managed to strengthen my scientific writing abilities. I appreciate his optimistic nature, his enthusiasm and that even in the difficult period of his life he continued to help me when I was struggling with the publications. Thank you very much! I also would like to thank my co-supervisors/co-authors Terje Vasskog, Espen Hansen and Trond Jørgensen for the valuable piece of advice on my papers! Special thanks go to Jim Henderson who substantially contributed for data collecting and analyzing for my second paper!

I would like to thank all my colleagues – Martina Uradnikova, Jon Brage Svenning, Nerea Aalto, Andrea Gerecht, Gunilla Eriksen, Tien Huang, Renata Døving, Richard Ingebrigtsen that helped me with my research and paper writing! Thank you for assisting me in many ways – you are fantastic colleagues!

References

Adarme-Vega T, Lim DK, Timmins M, Vernen F, Li Y, Schenk P (2012) Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microbial Cell Factories 11:96

Armbrust EV et al. (2004) The genome of the diatom Thalassiosira pseudonana: Ecology, evolution, and metabolism. Science 306:79-86 doi:DOI 10.1126/science.1101156

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Phys 37:911-917 doi:10.1139/o59-099

Bowler C et al. (2008) The Phaeodactylum genome reveals the evolutionary history of diatom genomes. Nature 456:239-244 doi:10.1038/nature07410

Brilman DWF, Veneman R (2013) Capturing atmospheric CO2 using supported amine sorbents. Ghgt-11 37:6070-6078 doi:10.1016/j.egypro.2013.06.536

Carvalho AP, Malcata FX (2005) Optimization of omega-3 fatty acid production by microalgae: Crossover effects of CO2 and light intensity under batch and continuous cultivation modes. Mar Biotechnol 7:381-388 doi:10.1007/s10126-004-4047-4

Chen Y-C (2012) The biomass and total lipid content and composition of twelve species of marine diatoms cultured under various environments. Food Chem 131:211-219 doi:10.1016/j.foodchem.2011.08.062

Chiu SY, Kao CY, Tsai MT, Ong SC, Chen CH, Lin CS (2009) Lipid accumulation and CO2 utilization of Nannochloropsis oculata in response to CO2 aeration. Bioresour Technol 100:833-838 doi:10.1016/j.biortech.2008.06.061

Choi W, Kim G, Lee K (2012) Influence of the CO2 absorbent monoethanolamine on growth and carbon fixation by the green alga Scenedesmus sp. Bioresour Technol 120:295-299 doi:10.1016/j.biortech.2012.06.010

d'Ippolito GS, A. Paris, D. Vella, F. M. Adelfi, M. G. Botte, P. Gallo, C. Fontana, A. (2015) Potential of lipid metabolism in marine diatoms for biofuel production. Biotechnol Biofuels 8 doi:10.1186/s13068-015-0212-4

de Castro Araújo S, Garcia VMT (2005) Growth and biochemical composition of the diatom Chaetoceros cf. wighamii brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. Aquaculture 246:405-412 doi:10.1016/j.aquaculture.2005.02.051

de Morais MG, Costa JA (2007a) Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. J Biotechnol 129:439-445 doi:10.1016/j.jbiotec.2007.01.009

de Morais MG, Costa JAV (2007b) Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energ Convers Manage 48:2169-2173 doi:10.1016/j.enconman.2006.12.011

Degerlund M, Eilertsen HC (2010) Main species characteristics of phytoplankton spring blooms in NE Atlantic and Arctic waters (68-80A degrees N). Estuar Coast 33:242-269 doi:10.1007/s12237-009-9167-7

Dodson VJ, Mouget JL, Dahmen JL, Leblond JD (2014) The long and short of it: temperature-dependent modifications of fatty acid chain length and unsaturation in the galactolipid profiles of the diatoms Haslea ostrearia and Phaeodactylum tricornutum. Hydrobiologia 727:95-107 doi:10.1007/s10750-013-1790-4

Duerksen SW, Thiemann GW, Budge SM, Poulin M, Niemi A, Michel C (2014) Large, omega-3 rich, pelagic diatoms under Arctic sea ice: sources and implications for food webs. Plos One 9 doi:10.1371/journal.pone.0114070

Eppley RW (1972) Temperature and Phytoplankton Growth in Sea. Fish Bull 70:1063-1085

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237-240

Folch J, Lees M, Stanley GHS (1957) A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. J Biol Chem 226:497-509

Gim GH, Ryu J, Kim MJ, Kim PI, Kim SW (2016) Effects of carbon source and light intensity on the growth and total lipid production of three microalgae under different culture conditions. J Ind Microbiol Biotechnol 43:605-616 doi:10.1007/s10295-016-1741-y

Gomez-Villa H, Voltolina D, Nieves M, Pina P (2005) Biomass production and nutrient budget in outdoor cultures of scenedesmus obliquus (chlorophyceae) in artificial wastewater, under the winter and summer conditions of Mazatlan, Sinaloa, Mexico. Vie Et Milieu-Life and Environment 55:121-126

Guedes AC, Meireles LA, Amaro HM, Malcata FX (2010) Changes in Lipid Class and Fatty Acid Composition of Cultures of Pavlova lutheri, in Response to Light Intensity. Journal of the American Oil Chemists Society 87:791-801 doi:10.1007/s11746-010-1559-0

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms .1. Cyclotella Nana Hustedt, and Detonula Confervacea (Cleve) Gran. Can J Microbiol 8:229

Guschina I, Harwood J (2009) Algal lipids and the effect of the environment on their biochemistry. In: Arts M, Brett M, Kainz M (eds) Lipids in aquatic ecosystems. Springer, LLC 2009, New-York, USA, pp 1-25

Harwood JL (1988) Fatty-acid metabolism. Annu Rev Plant Physiol Plant Mol Biol 39:101-138 doi: 10.1146/annurev.arplant.39.1.101

Hasle GR, Syvertsen EE (1997) Chapter 2 - Marine Diatoms A2 - Tomas, Carmelo R. In: Identifying Marine Phytoplankton. Academic Press, San Diego, pp 5-385. doi:10.1016/B978-012693018-4/50004-5

Hoffmann M, Marxen K, Schulz R, Vanselow KH (2010) TFA and EPA Productivities of Nannochloropsis salina Influenced by Temperature and Nitrate Stimuli in Turbidostatic Controlled Experiments. Mar Drugs 8:2526

Huseby S (2011) Metabolic fingeprinting applied in diatom taxonomy. Dissertation

The Arctic University of Norway

Huseby S, Degerlund M, Eriksen GK, Ingebrigtsen RA, Eilertsen HC, Hansen E (2013) Chemical Diversity as a Function of Temperature in Six Northern Diatom Species. Mar Drugs 11:4232-4245 doi:10.3390/md11114232

Iverson SJ, Lang SL, Cooper MH (2001) Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 36:1283-1287

Izquierdo MS, Montero D, Robaina L, Caballero MJ, Rosenlund G, Gines R (2005) Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (Sparus aurata) fed vegetable oils for a long terin period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 250:431-444 doi:10.1016/j.aquaculture.2004.12.001

James CM, Al-Hinty S, Salman AE (1989) Growth and ω3 fatty acid and amino acid composition of microalgae under different temperature regimes. Aquaculture 77:337-351 doi:10.1016/0044-8486(89)90218-4

Jiang H, Gao K (2004) Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom Phaeodactylum tricornutum (Bacillariophyceae). J Phycol 40:651-654 doi:10.1111/j.1529-8817.2004.03112.x

Kates M, Volcani BE (1966) Lipid components of diatoms. Biochimica Et Biophysica Acta 116:264-& doi:10.1016/0005-2760(66)90009-9

Khoeyi ZA, Seyfabadi J, Ramezanpour Z (2012) Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, Chlorella vulgaris. Aquacult Int 20:41-49 doi:10.1007/s10499-011-9440-1

Krzemińska I, Piasecka A, Nosalewicz A, Simionato D, Wawrzykowski J (2015) Alterations of the lipid content and fatty acid profile of Chlorella protothecoides under different light intensities. Bioresour Technol 196:72-77 doi:10.1016/j.biortech.2015.07.043

Leu E, Falk-Petersen S, Kwasniewski S, Wulff A, Edvardsen K, Hessen DO (2006) Fatty acid dynamics during the spring bloom in a High Arctic fjord: importance of abiotic factors versus community changes. Can J Fish Aquat Sci 63:2760-2779 doi:10.1139/F06-159

Levitan O, Dinamarca J, Hochman G, Falkowski PG (2014) Diatoms: a fossil fuel of the future. Trends Biotechnol 32:117-124 doi:10.1016/j.tibtech.2014.01.004

Lundholm N, Moestrup O, Kotaki Y, Hoef-Emden K, Scholin C, Miller P (2006) Inter- and intraspecific variation of the Pseudo-nitzschia delicatissima complex (Bacillariophyceae) illustrated by rRNA probes, morphological data and phylogenetic analyses. J Phycol 42:464-481 doi:10.1111/j.1529-8817.2006.00211.x

Mann DG, Droop SJM (1996) Biodiversity, biogeography and conservation of diatoms. Hydrobiologia 336:19-32 doi:Doi 10.1007/Bf00010816

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: A review. Renew Sust Energ Rev 14:217-232 doi:10.1016/j.rser.2009.07.020

Mock T, Kroon BMA (2002) Photosynthetic energy conversion under extreme conditions - I: important role of lipids as structural modulators and energy sink under N-limited growth in Antarctic sea ice diatoms. Phytochemistry 61:41-51 doi: 10.1016/S0031-9422(02)00216-9

Montero D, Kalinowski T, Obach A, Robaina L, Tort L, Caballero MJ, Izquierdo MS (2003) Vegetable lipid sources for gilthead seabream (Sparus aurata): effects on fish health. Aquaculture 225:353-370 doi:10.1016/S0044-8486(03)00301-6

Morkore T (2006) Relevance of dietary oil source for contraction and quality of pre-rigor filleted Atlantic cod, Gadus morhua. Aquaculture 251:56-65 doi:10.1016/j.aquaculture.2005.05.016

Mortensen SH, Børsheim KY, Rainuzzo J, Knutsen G (1988) Fatty acid and elemental composition of the marine diatom Chaetoceros gracilis Schütt. Effects of silicate deprivation, temperature and light intensity. J Exp Mar Biol Ecol 122:173-185 doi:10.1016/0022-0981(88)90183-9

Moustafa A, Beszteri B, Maier UG, Bowler C, Valentin K, Bhattacharya D (2009) Genomic Footprints of a Cryptic Plastid Endosymbiosis in Diatoms. Science 324:1724-1726 doi:10.1126/science.1172983

Nakanishi A, Aikawa S, Ho SH, Chen CY, Chang JS, Hasunuma T, Kondo A (2014) Development of lipid productivities under different CO2 conditions of marine microalgae Chlamydomonas sp JSC4. Bioresour Technol 152:247-252 doi:10.1016/j.biortech.2013.11.009

Pauly D et al. (2002) Towards sustainability in world fisheries. Nature 418:689-695 doi:10.1038/nature01017
Pitt JJ (2009) Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. Clin Biochem Rev 30:19-34
Ramanathan V (1988) The Greenhouse Theory of Climate Change - a Test by an Inadvertent Global Experiment. Science 240:293-299 doi:DOI 10.1126/science.240.4850.293
Rampen SW, Abbas BA, Schouten S, Damste JSS (2010) A comprehensive study of sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom productivity. Limnol Oceanogr 55:91-105 doi: 10.4319/lo.2010.55.1.0091
Renaud SM, Parry DL, Thinh L-V, Kuo C, Padovan A, Sammy N (1991) Effect of light intensity on the proximate biochemical and fatty acid composition of Isochrysis sp. and Nannochloropsis oculata for use in tropical aquaculture. J Appl Phycol 3:43-53 doi:10.1007/BF00003918
Renaud SM, Thinh L-V, Lambrinidis G, Parry DL (2002) Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211:195-214 doi:10.1016/S0044-8486(01)00875-4
Renaud SM, Zhou HC, Parry DL, Thinh L-V, Woo KC (1995) Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae Isochrysis sp., Nitzschia closterium, Nitzschia paleacea, and commercial species Isochrysis sp. (clone T.ISO). J Appl Phycol 7:595-602 doi:10.1007/BF00003948
Roleda MY, Slocombe SP, Leakey RJG, Day JG, Bell EM, Stanley MS (2013) Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. Bioresour Technol 129:439-449 doi:10.1016/j.biortech.2012.11.043
Rousch JM, Bingham SE, Sommerfeld MR (2003) Changes in fatty acid profiles of thermo-intolerant and thermo-tolerant marine diatoms during temperature stress. J Exp Mar Biol Ecol 295:145-156 doi: 10.1016/S0022-0981(03)00293-4
Ryckebosch E, Bruneel C, Termote-Verhalle R, Goiris K, Muylaert K, Foubert I (2014) Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. Food Chem 160:393-400 doi:10.1016/j.foodchem.2014.03.087
Sargent JR, Eilertsen HC, Falkpetersen S, Taasen JP (1985) Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. Mar Biol 85:109-116 doi: 10.1007/Bf00397428
Sarno D, Kooistra WHCF, Medlin LK, Percopo I, Zingone A (2005) Diversity in the genus Skeletonema (Bacillariophyceae). II. An assessment of the taxonomy of S-costatum-like species with the description of four new species. J Phycol 41:151-176 doi:10.1111/j.1529-8817.2005.04067.x
Scragg AH, Illman AM, Carden A, Shales SW (2002) Growth of microalgae with increased calorific values in a tubular bioreactor. Biomass Bioenerg 23:67-73 doi: 10.1016/S0961-9534(02)00028-4
Seierstad SL, Poppe TT, Koppang EO, Svindland A, Rosenlund G, Froyland L, Larsen S (2005) Influence of dietary lipid composition on cardiac pathology in farmed Atlantic salmon, Salmo salar L. J Fish Dis 28:677-690 doi:10.1111/j.1365-2761.2005.00671.x

Sheehan JD, T. Benemann, J. Roessler P. (1998) A look back at the U.S. Department of Energy's aquatic species program: biodiesel from algae. NREL/TP – 580 – 24190 (National Renewable Energy Laboratory),

Sneddon J, Masuram S, Richert JC (2007) Gas chromatography-mass spectrometry-basic principles, instrumentation and selected applications for detection of organic compounds. Anal Lett 40:1003-1012 doi:10.1080/00032710701300648

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87-96 doi:10.1263/jbb.101.87

Stoffel W, Chu F, Ahrens EH (1959) Analysis of long-chain fatty acids by gas-liquid chromatography - micromethod for preparation of methyl esters. Anal Chem 31:307-308 doi:DOI 10.1021/ac60146a047

Tang DH, Han W, Li PL, Miao XL, Zhong JJ (2011) CO2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO2 levels. Bioresour Technol 102:3071-3076 doi:10.1016/j.biortech.2010.10.047

Thomas DM, Mechery J, Paulose SV (2016) Carbon dioxide capture strategies from flue gas using microalgae: a review. Environ Sci Pollut R 23:16926-16940 doi:10.1007/s11356-016-7158-3

Thompson PA, Harrison PJ, Whyte JNC (1990) Influence of irradiance on the fatty acid composition of phytoplankton. J Phycol 26:278-288 doi:10.1111/j.0022-3646.1990.00278.x

Vernadsky VI (1945) The Biosphere and the Noosphere. Sci Am 33:1-12

Wang B, Li YQ, Wu N, Lan CQ (2008) CO(2) bio-mitigation using microalgae. Appl Microbiol Biotechnol 79:707-718 doi:10.1007/s00253-008-1518-y

Wang X-W, Liang J-R, Luo C-S, Chen C-P, Gao Y-H (2014) Biomass, total lipid production, and fatty acid composition of the marine diatom Chaetoceros muelleri in response to different CO2 levels. Bioresour Technol 161:124-130 doi:10.1016/j.biortech.2014.03.012

Ward OP, Singh A (2005) Omega-3/6 fatty acids: Alternative sources of production. Process Biochem 40:3627-3652 doi:10.1016/j.procbio.2005.02.020

Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM (2010) Selection of microalgae for lipid production under high levels carbon dioxide. Bioresour Technol 101:S71-S74 doi:10.1016/j.biortech.2009.03.030