ProAlgae

Industrial production of marine microalgae
as a source of EPA and DHA rich raw material in fish feed
– Basis, knowledge status and possibilities

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Preface

Fish oil has been an important ingredient in aquafeed for decades, by adding functionality and health benefits to the farmed salmon. However, the price of fish oil is expected to increase significantly in the next coming years due to an upcoming shortage of fish oils. The growing salmon market will directly affect the aquafeed industry. In the long run, new sustainable omega-3 sources must be developed to ensure that the Norwegian salmon industry still will support the market with healthy farmed salmon in the future.

Therefore, the Norwegian Seafood Research Fund (FHF) initiated the development of study report describing the international knowledge status and industrial production of marine microalgae as a raw material in aquafeed. The project "*ProAlgae2012 Industrial production of marine microalgae as an EPA- and DHA-source for use in fish feed"* was started up by Uni Research and SINTEF Fisheries and Aquaculture in May 2012 with the following aims:

1. Develop a "State-of-the-art" report of the international status of knowledge on industrial production of marine microalgae.
2. Describe the possibilities to produce EPA and DHA in microalgae for use in feed at an economically viable cost.

To fulfil these aims several industry visits and extensive scientific meetings and discussions, as well as two workshops with high level researchers have been conducted and a reference group has been established to complete the "State of the art" report.

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Executive Summary

Production of aquafeed requires significant amounts of fish oil or meal, and the omega-3 fatty acids EPA and DHA are essential. As the fish resources become scarce, prices rise and are expected to rise further in the near future. It has become clear that new solutions must be found and searches for alternative sources of EPA and DHA are initiated. Fish by-products/trimmings, krill, as well as genetically modified microorganisms and plants are considered. Marine microalgae are primary producers of EPA and DHA. Due to the high productivity and sustainable production possibilities, microalgae are considered as a promising future alternative.

Most microalgae are *photoautotrophs*, meaning that they use light energy to produce chemical energy and convert inorganic carbon (CO_2) into sugars and organic compounds. Another group, called *heterotrophs*, grows without light and use organic carbon compounds as both the energy and carbon source. The important parameter for commercial scale cultivation is the *productivity*, given as the biomass produced per volume over time. Photoautotroph microalgae can produce 15-30% EPA of total fatty acids, while heterotrophic thraustochythrids can produce biomass with 55% DHA of total fatty acids. The EPA/DHA yield can be optimized by increased biomass production, and/or increased lipid productivity. By systematic investigation of the biodiversity, novel productive strains with high EPA and DHA levels can be identified. The production pathways for EPA and DHA are known, and subject to improvement. Three main strategies to actively increase the yield is described: by exploitation of physiological potential, by strain selection and breeding of promising candidates, or by genetic modification. There is an ongoing development of molecular tools to increase the photosynthetic efficiency, and the EPA and DHA content in the cell. The combination of natural selected strains and improvement strategies is expected to increase in biomass productivity and lipid yield by 2-4 fold (or more) within the coming 5-8 years.

Cultivation of microalgae is conducted in either open pond systems or closed bioreactor systems. Improved biological productivity has the most impact on production economics, and several initiatives are made to increase the photosynthetic efficiency and to adapt better to production conditions. While the photosynthetic microalgae industry is in development, the heterotrophic production of DHA are based on mature technology and is currently in commercial use for high value products. Phototrophic production is considered as sustainable due to factors such as use of renewable resources (CO_2 and sunlight, waste water and animal wastes), use of non-arable land and high productivity.

The harvested microalgae biomass needs to be processed by the most cost efficient methods ensuring high digestibility of the EPA and DHA in the algae when used in aquafeeds. There is an ongoing technology development – driven by the biofuel industry - towards more cost-efficient harvesting systems. To ensure high stability and best use in fish feed the biomass must be dried and suitably processed for use in aquafeed.

Species/strains of microalgae have been suggested to have a great potential to provide protein, lipids, vitamins, carotenoids and energy in feed for carnivorous fish species. Several microalgae/microorganisms have been tested for use in fish feed and the results shows high digestibility and positive growth effects up to a certain addition level. Because microalgae are a novel resource in aquafeed production, little work has been done on the effects of adding algae oil into fish feed in terms of nutrition but also on the technological challenges in feed production

Traditionally, the commercial microalgae industry is directed towards high-value products and low-volume, specialty markets, such as nutraceuticals, cosmetics and food products. However, the political will to develop sustainable algal biofuels in the US have been the key driver of the industrial technology development to make controlled microalgae production more operational, more scalable and more cost-efficient in general. Through strategic and consistent political consistent support over decades, there has been accelerated development the recent years. The algae biofuel industry has just recently entered commercialization of algae biofuels based on both heterotrophic (Solazyme) and phototrophic (Sapphire) production, and is currently scaling up production facilities. There is a clear trend among biofuel companies to explore synergetic opportunities to market co-products while at the same time developing larger scale production to meet the commodity market in the future. Because the production process steps are similar, the technology developments and research advances related to phototrophic biofuels will directly benefit the development of low-cost EPA/DHA.

A SWOT analysis was conducted for the phototrophic production of microalgae based EPA and DHA. The most important strengths: good sustainability at lowest trophic level, original source of EPA/DHA with very high productivity. Among the indicated weaknesses: currently high CAPEX for closed systems, high OPEX formixing, and that a technology development is required to reduce processing costs. The major opportunities are that the technological development will decrease CAPEX and OPEX, and that strain improvement efforts are underway to increase productivity driven by the big biofuel industry. The identified threats are a possible increased production of EPA/DHA in transgenic land plants, yeast, bacteria; the lack of strategic R&D perspective and funding, and contamination by grazers and disease organisms.

A techno-economic analysis was performed to evaluate three different technologies in two different locations. The input data are based on prior research and technological know-how. Among the three technologies, the innovative flat panel reactors show highest production cost efficiency at locations in Spain due to higher irradiation levels, and also cheaper land costs.

At present the estimated production cost for the phototrophic production of EPA and DHA is 39 USD/kg EPA&DHA eq, when using flat panel reactors in high irradiance regions. A future optimization of productivity, and reduction of production costs, which are realistic in a 5 year perspective have been described in the techno-economic analysis. Based on these projections, the production cost may be further reduced to 11.9 USD/kg EPA&DHA eq. At present, the price projections made for the heterotrophic production of DHA (19 USD/kg DHA eq) are competitive with the price levels of DHA equivalents in refined or concentrated fish oil. The production cost may be further reduced to 11.5 USD/kg DHA eq, based on a foreseeable productivity increase in the next 5 years. Microalgae production of EPA and DHA has the potential to develop into a sustainable alternative to fish oil for use in aquafeed. This potential can be realized by establishing a *fit-for-purpose* research and development pipeline with integrated research along the value chain. In light of the recent price development and the future fish oil price projections, this seems to be a viable strategy for accessing novel EPA/DHA sources.

Summary in Norwegian

ProAlgae-prosjektet ble initiert av FHF på bakgrunn av den globale nedgangen i fiskeressurser til produksjon av fiskefôr. Noe av fiskeoljen og melet erstattes med raps, soya o.l., men samtidig har innholdet av essensielle flerumettede omega-3 fettsyrer (EPA og DHA) i laksen gått ned. Regulering av anchoveta-fisket har gitt en stabilisering av bestandene, og økende bruk av hele fiskeråstoffet (i stedet for å prosessere frem mel og olje) kan sørge for at tilgangen ikke blir ytterligere redusert i de nærmeste årene. Fiskeolje med høyt omega-3 innhold selges i dag for 3000 USD/t i Peru, og en 25 % økning i prisen er forventet. Det er også økende konkurranse fra andre markeder som helsekost og næringsmidler, og disse markedene kan kjøpe råstoffet til en høyere pris. Analyser viser at behovet for fiskeolje vil være betydelig større enn de 1 000 000 tonn pr år som er tilgjengelige i dag, og man ser derfor etter alternative kilder til EPA/DHA. Landplanter, zooplankton, genmodifiserte sopp el bakterier har vært vurdert, og nå rettes fokus mot mikroalger, som er produsenter av omega-3 fettsyrer og et naturlig råstoff å ta i bruk til produksjon av fiskefôr.

Mikroalger er en organismegruppe med stor diversitet (og omfatter også thraustochytrider og blågrønnbakterier i mange sammenhenger), og de fleste algene er fototrofe og bruker sol- eller lysenergi til å omdanne CO2 til kjemisk organiske karbonforbindelser. Noen alger er heterotrofe og kan dyrkes uten tilførsel av lysenergi, og de tar opp enkle karbonforbindelser som sukker o.l. Mikroalgene kan syntetisere langkjedete fettsyrer med høy grad av umettethet, og innholdet av EPA/DHA varierer fra art til art. Det er også variasjoner i veksthastighet, biomasseproduktivitet og utbytte av fettsyrer i forhold til ressurstilgang og dyrkingsmetoder.

En viktig oppgave er å finne de rette mikroalgene for industriell produksjon, og optimalisere den biologiske produktiviteten av biomasse/fettsyrer ved hjelp av kunnskap om metabolske prosesser og moderne verktøy som genteknologi. En annen utfordring er å produsere nok biomasse til å utvinne betydelige mengder EPA/DHA, og for tiden jobbes det mye både med forskning og teknologiutvikling for å løse utfordringene med å produsere mikroalger i stor skala. Autotrofe mikroalger dyrkes i åpne dam-systemer eller i lukkede fotobioreaktor-systemer, og formålet med utforming/plassering er maksimal utnyttelse av innstrålt lysenergi. Lys som ressurs er ikke uproblematisk, for det kan bli begrensende for biomasseproduksjon både fordi cellene skygger for hverandre eller hvis det er for sterkt lys fordi cellene setter i verk beskyttelsesmekanismer som reduserer utnyttelsen av lyset. Åpne systemer er rimelige, men det er stor risiko for kontaminering og vanskelig å kontrollere temperatur o.l. I tillegg er biomasseproduktiviteten ofte lav (< 0.2 g tørrvekt/l). I lukkede systemer har man bedre kontroll og oppnår ofte høyere produktivitet (2-4 g tørrvekt/l), men de er mer kostbare i etablering og drift. Teknoøkonomiske analyser favoriserer åpne systemer, men i praksis er det oftest nødvendig å etablere et lukket system for å kunne produsere mikroalger med høyt innhold av EPA/DHA. De teknologiske utfordringene omhandler valg av rimelige materialer og reaktordesign for optimal lysenergiutnyttelse, og reduksjon av energiforbruk til omrøring og pumping av medium.

Heterotrofe organismer dyrkes i lukkede systemer kalt fermentorer, og det finnes allerede en etablert storskala industri på dette området. I heterotrof produksjon representerer imidlertid karbonkilden (ofte glukose eller sukrose) en kostnad, og det fokuseres på å finne rimelige råmaterialer. De produksjonssystemene som finnes er allerede optimalisert for biomasseproduksjon, men man jobber med å øke lipidutbyttet i biomassen. Det rapporteres om biomasseproduksjon på > 160 g/l med 70 % lipider, og dersom man kan optimalisere

DHA-innholdet i lipidene så kan heterotrof produksjon av DHA gi betydelige mengder råstoff til fiskefôrproduksjonen.

Høsting og prosesseringskostnader er andre viktige elementer i teknoøkonomisk analyse av mikroalgeproduksjon. Produksjon av ren EPA/DHA-olje krever høy grad av prosessering og medfører høye kostnader. Rent teknologisk kan man benytte allerede etablerte høstingsmestoder som sedimentering, flokkulering (kjemisk eller biologisk), membranfiltrering eller sentrifugering, eller prosessteknologier som fordampning, oppvarming og ekstrahering (mekanisk eller kjemisk). En utfordring i mikroalgeproduksjon i industriell skala er fjerning av vann, først store mengder dyrkingsmedium (som kan resirkuleres for bedre utnyttelse av næringsstoffer o.l.) og deretter avvanning for å fjerne vann knyttet til biomassen (rundt og inne i cellene). Frossent materiale med restvann kan ikke oppbevares særlig lenge før kvaliteten forringes, og fjerne resten av vannet fra cellene vha tørking (frysetørking el varmetørking) er energikrevende og kostbart. Hvis man skal ekstrahere oljer er det også nødvendig å fjerne mest mulig vann, for å minimere kjemikaliebruken. Det foregår imidlertid utvikling av prosesseringsteknologier for å øke effektiviteten og redusere kostnadene.

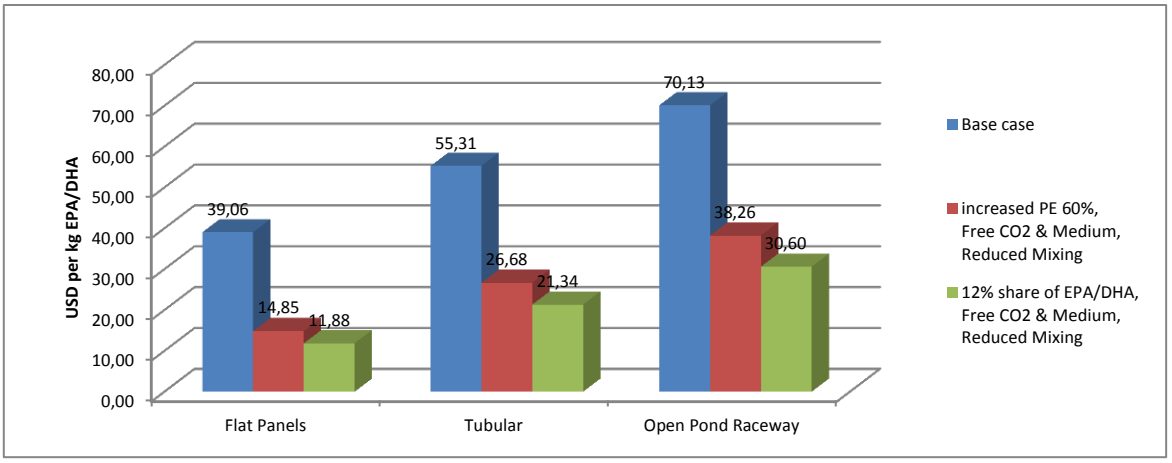
En måte å redusere kostnadene ved bruk av mikroalger i fiskefôr er å minimere prosesseringsgraden. Mikroalger består i hovedsak av proteiner, karbohydrater og lipider, i tillegg til mineraler, vitaminer og andre sporstoffer, og det er i utgangspunktet ingenting i veien for å benytte hele biomassen som en råvare. Det er imidlertid artsspesifikke forskjeller i oppbygging av cellevegg og lokalisering av karbohydrater/lipider, men forskning har vist at visse alger fordøyes godt av fisken og man kan bruke mikroalgebiomassen direkte i fôret. Forskningsutfordringer vil være å finne ernæringsmessig gunstige alger som kan produseres i stor skala, og deretter vurdere i hvilken grad fôrproduksjonen påvirkes og fôrkvaliteten beholdes ved tilsetting av algebiomasse direkte.

Dagens mikroalgeindustri er i stor grad rettet mot høykostmarkeder som kosmetikk, helsekost eller andre næringsmidler, eller "commodity"-markeder som biodrivstoff (der man trenger store mengder råvarer til lavere pris). Det brukes både åpne damsystemer og lukkede reaktorer, og produksjon foregår i solrike områder over hele verden. Fermentorteknologien finnes allerede, og teknologiutviklingen når det gjelder lysstyrt produksjon drives fremover i forbindelse med produksjon av biodrivstoff. Denne teknologien vil være den samme for produksjon av mikroalger til fôr o.l., uten behov for tilpasninger. Lukkede dyrkingssystemer plasseres ofte i veksthus for bedre regulering av lys og temperatur, og man ser klare synergieffekter i områder der man har utviklet veksthusteknologien eller har etablert infrastrukturen. Man kan også utnytte overskuddsenergi som spillvarme/restvarme, CO_2-overskudd fra andre prosesser, for dermed å redusere noen av kostnadene. Det er også en trend mot "joint venture"-aktiviteter, der algeprodusenter innlemmes i et stort selskap med sterk markedsprofil og dermed kan basere seg på mer investeringsmidler og et apparat for videre håndtering/markedsføring. Produksjonsteknologien varierer ikke særlig fra høykost til "commodity"-produksjon, men råvarer som produseres for menneskelig konsum er underlagt strenge regler for kontroll og produksjon i lukkede systemer kan være nødvendig. Dersom det åpnes for bruk av GM-alger så vil det også fremme bruken av lukkede systemer fremfor åpne dammer (og dermed øke kostnadene).

Industriell produksjon av mikroalger foregår over hele verden, spesielt i solrike områder, og det er hovedsakelig to markeder for biomassen: biodrivstoff (karakterisert ved store volum/lav

pris) eller "høy-kost markeder" som helsekost/kosmetikk (men mindre volumer). Den teknologiske utviklingen drives i stor grad av biodrivstoff-aktiviteter, og det forventes at det man oppnår der kan overføres til produksjon av mikroalger for andre formål, inkl. fôr. De ledende produsentene av Spirulina (Earthrise) og heterotroft produsert DHA (DSM- Martek) finnes i USA, og det er også i USA man finner de største selskapene som driver med fotoautotrof produksjon(e.g. Sapphire Energy). På forskningssiden er det flere store EU-prosjekter som fokuserer på mikroalger og biomasseproduksjon, og AlgaePARC i Wageningen hvor ulike kostnadseffektive dyrkingssystemer sammenlignes – med mål om å produsere algebiomasse til 0.5 USD/kg i løpet av de neste 5 år.

Rapporten omfatter også en tekno-økonomisk analyse av ulike scenarioer rundt algedyrking, med fokus på hva som er de største kostnadsfaktorene og hvordan de kan utvikles i tiden fremover. I analysen er det tatt utgangspunkt i to lokaliteter, en solrik lokalitet i sør-Spania og en moderat solrik lokalitet i Holland. Det er tatt utgangspunkt i de biologiske parametrene vi kjenner til per i dag, med fotosyntetisk effektivitet som er relevant for store produksjonssystemer (og en eventuell økning av denne med ca 60%) og dobling av EPA/DHA-innhold i biomassen (noe som er realistisk med dagens kjennskap til valg av arter og genmodifisering o.l). Videre er det lagt inn mulighet for innsparing ved f.eks tilgang på CO_2 el næringssalter uten kostnader (gjenbruk av husdyrgjødsel o.l), el reduksjon av energiforbruk ved å nedsette omrøringshastigheten i dyrkingssystemene. Analysen inkluderer også etablerings-, lønns- og driftskostnader, og tar høyde for at algedyrking investeringsmessig er høyrisiko-aktivitet. Med dette som ramme peker analysen på en positiv prisutvikling, der prisen på fremstilt EPA/DHA reduseres fra 39,8 USD/kg ned mot 14,8 USD/kg og videre ned mot 11,88 USD/kg (se figur).



Den største kostnadsreduksjonen fremkommer i scenarioet der produksjonen foregår i et solrikt område, ved bruk av panelreaktorer med mikroalger med som har 12% andel EPA/DHA/tørrvekt biomasse (en dobling av "base case"), gratis CO_2 og næringssalter/medium og redusert omrøringshastighet (og dermed lavere energiforbruk). En noe lavere prisreduksjon fremkommer dersom man oppnår en 60% økning i fotosyntetisk effektivitet, mens biomassen bare inneholder halvparten så mye EPA/DHA (6% av tørrvekt biomasse). De biologiske faktorene har stor betydning for prisestimatet, i tillegg til energikostnadene.

Risikoanalyse (SWOT) av temaet "industriell dyrking av mikroalger" peker på fortrinn som at mikroalger er en naturlig kilde til EPA/DHA i den marine næringskjeden, de har høy

produktivitet og produksjonen er bærekraftig fordi det er et av de laveste trinnene i den trofiske pyramiden. Det er imidlertid utfordringer i forhold til høye investeringskostnader og behov for teknologisk utvikling på prosesseringssiden. Teknologisk utvikling er forventet og vil bidra til at kostnadene reduseres ytterligere. Det vil kunne være svingninger i biomasseproduksjon (artsbestemte forskjeller, dyrkingsbestemte forskjeller, eller årstidsvariasjoner for å nevne noen) men forskning fokusert mot dette vil kunne bidra til å optimalisere biomasseproduksjonen over hele produksjonen. Det finnes også mange arter av mikroalger og bare noen svært få utnyttes kommersielt i dag. Det ligger dermed et potensiale i å lete etter flere arter som har høyt innhold av EPA/DHA, eller ta i bruk moderne verktøy som genteknologi for å øke biomasseproduktivitet og/eller utbyttet av EPA/DHA. Man ser klare synergier fra integrering av ulike prosesser: drivhusteknologi, resirkulering av avløpsvann eller bruk av CO2 som stammer fra andre produksjoner. Mikroalgene inneholder også andre forbindelser som vitaminer, mineraler, antioksidanter som kan benyttes, og dersom man ekstraherer EPA/DHA vil restråstoffet være protein/karbohydratrikt. Utsiktene til å benytte mikroalger for å produsere EPA/DHA vil påvirkes av fall i fiskeoljeprisen, konkurranse fra transgene landplanter, gjær eller bakterier, eller mangel på strategiske forskningsmidler og/eller investeringsmidler.

Denne rapporten viser status mht kunnskap om mikroalgedyrking (fototrof og heterotrof) og eksempler på industriell utnyttelse av algebiomasse. Rapporter om global tilgang på fiskeolje/mel viser en tydelig nedgang (som forventes å vare) i tilgang, sammen med en klar prisøkning. Per i dag er prisen på EPA/DHA-rik fiskeolje steget til 2300 USD/tonn, mens heterotroft produsert olje (DHA) har en pris på 1800-2200 USD/tonn. En teknoøkonomisk analyse gjort her viser at man – ved gitte betingelser – kan produsere EPA/DHA-rik olje fra mikroalger til en pris av 11.9 USD/kg (i løpet av de neste 5 år). Noen av de viktigste oppgavene for å nå dette målet er å sette i gang strategiske forskningsprosjekter for å løse utfordringer knyttet til biologi og prosessering, og etablere pilotskala-anlegg der man kan teste nye løsninger i realistisk skala. Det er også viktig å utnytte erfaringer fra biodrivstoff-bransjen, i tillegg til eventuelle synergier med for eksempel overskuddsvarme/vann fra andre prosesser, CO_2-overskudd, resirkulering av næringsstoffer og lignende tiltak som kan redusere kostnadene i mikroalgeproduksjonen.

PART I
Introduction

1 Introduction

Chapter summary box

Production of aquafeed requires significant amounts of fish oil or meal, and omega-3 fatty acids EPA and DHA are essential. There has been a global decline in fish oil resources over some time, partly due to climatic factors but also due to overexploitation of natural fish stocks. As the fish resources become scarce, prices rise and are expected to rise further in the near future. Soy and rape seed has partially substituted some of the fish oil/meal, but these plants do not have the optimal fatty acid profile. It has become clear that new solutions must be found and searches for alternative sources of EPA and DHA are initiated. Fish by-products/trimmings, krill, as well as genetically modified microorganisms and plants are considered. The marine microalgae are the primary producers of EPA and DHA. Due to the high productivity and sustainable production possibilities, microalgae are considered a promising future alternative This report elaborate the potential of using marine microalgae (including thraustocytrids and cyanobacteria) to provide essential fatty acids for aquafeed production.

Fish oil has been an important ingredient in aquafeed for the recent decade by adding functionality and health benefits to the farmed salmon. Consumers perceive farmed salmon as a healthy food product much because of the high levels of the healthy long chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that has been added through the salmon feed. However, the inclusion level of EPA and DHA in the aquafeed is declining, due to a global shortage of fish oils and emerging omega-3 applications leading to significantly increased price levels of fish oil in the recent years. Thus, EPA and DHA in fish oil is the only limiting raw material for the growing production of feed for salmonid fish.

1.1 Global fish oil supply

The production of fish oil is based on pelagic feed fisheries, where the global landings has been stable over the recent years around 20-25 mill tons per annum. Based on this, the global supply of fish oil is close to the maximum annual supply levels and has stabilized at around 1 000 000 t per annum. About 70% of the globally available fish oil is used for aquaculture feed and production of salmonids in particular (IFFO, 2013). The global production of aquafeed was 2 750 000 tons in 2011, demanding > 400 000 t fish oil/year for the *salmon feed alone*. However, even if fed aquaculture industry increase annually by 6%, the use of fishmeal and fish oil in aquaculture appears to have slightly decreased the recent years (Figure 1). This is because the aquafeed industry recognized ten years ago that the supply of fishmeal and fish oil could be limiting factors for the expanding aquaculture industry, and developed strategies on how to manage a limited supply. The relative demand of fishmeal and fish oil in aquafeed has thus been reduced, by gradually using these valuable ingredients more efficiently and strategically during critical stages in the life cycle. In addition, both marine ingredients have been increasingly substituted with alternative, vegetable ingredients such as soy bean meal and rapeseed oil over the last decade. While this has been successful in managing the fish oil supply situation, the levels of the healthy omega-3 fatty acids EPA and DHA have decreased in salmon fillets and the omega-6 levels from vegetable oils have increased. Over time, this may affect the consumer perception of the health benefits claimed for farmed salmon.

Limited global supply increase fish oil price levels

The pelagic supply of fish oil has remained constant over the last decade, but the available volume can fluctuate significantly from year to year based on fluctuations in the fishing

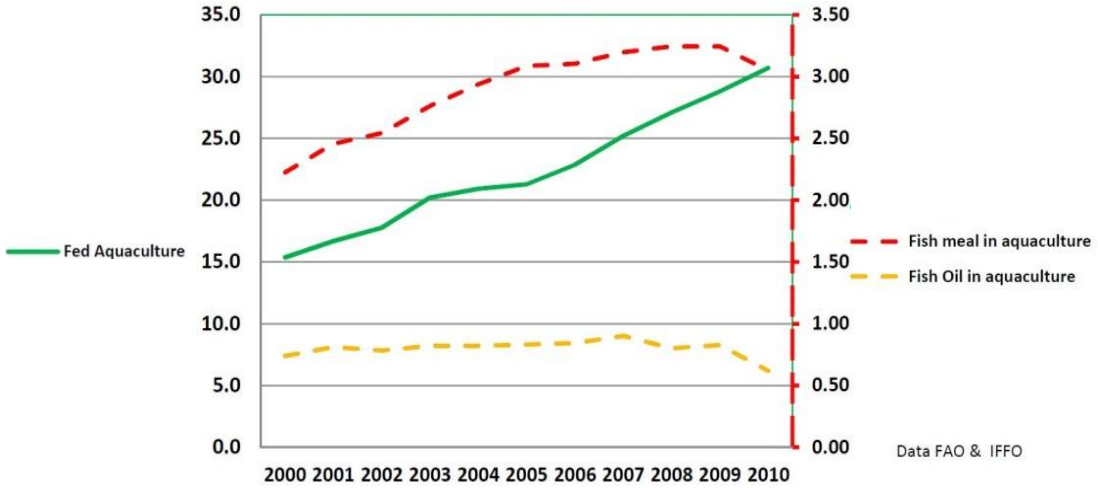


Fig.1 Annual global aquaculture production 2000-2010 (million tons) compared to volumes of fishmeal and fish oil used in aquaculture. *While the global aquaculture industry is expanding, the use of fishmeal and fish oil in aquafeed has been reduced the recent years. As the sustainable production has a ceiling of about 5 million tons fishmeal and 1 million ton fish oil annually, these resources have recently been used more efficiently, and partly substituted by alternative vegetable ingredients. Source: IFFO Positional Statement, Feb 2013.*

seasons, landings and oil yields. The anchoveta from Peru and Chile is the most important source of fish oil to the global market, accounting for almost 70% of the fish oil produced. Over the last decades, the anchoveta fishery has undergone both collapses and recoveries influenced by an extremely variable environment of currents and occasional upwelling events. As the key piece within the southeastern Pacific ecosystem, more precautionary fishery management is likely to be seen in order to keep the anchoveta stock and the associated environment healthy. Another trend that may reduce the pelagic supply of feed fish, is the increased use of whole fish for food instead of processing for oil and meal (FAO, 2012).

The imbalance between supply and demand contributes to drive prices, and the FAO have estimated a 25% price increase over the next 5 years (Figure 2). However, the sensitivity of the fish oil price levels to pelagic landings have recently been demonstrated from the last fishing season in Peru (Nov-Dec 2012), which was one of the worst witnessed in over 20 years. The Peruvian authority IMARPE had precautionary cut the quota by 68% before the opening, and the landings were on average only 14% that of previous years after the first 14 days. In addition, the oil recovery of the landings was about 2%, less than half of the fish oil level seen in previous fishing seasons (Fish, Oil & Meal world, Report Dec 6th 2012). The poor fish oil season in Peru drastically affected the already inclining fish oil prices, which reached 2300 USD/t selling in Peru at the end of 2012 according to Fish, Oil & Meal world (figure 2). This is also a historically high ration to rapeseed oil, which is the vegetable alternative for the substitution for the replacement of fish oil in aquafeed. The price level has apparently stabilized during the first quarter of 2013, but the price development can continue over the next couple of years if the situation in Peru will continue or repeat with short intervals. However, it appears that “omega-3 grade fish oil” selling in Peru have stabilized at around 3000 USD/t (pers. communication, José Rainuzzo, TASA).

While the limited supply situation is an internal factor that affects the price level of fish oil, this trend is accelerated by the emerging applications of omega-3 and more price competitive higher value markets.

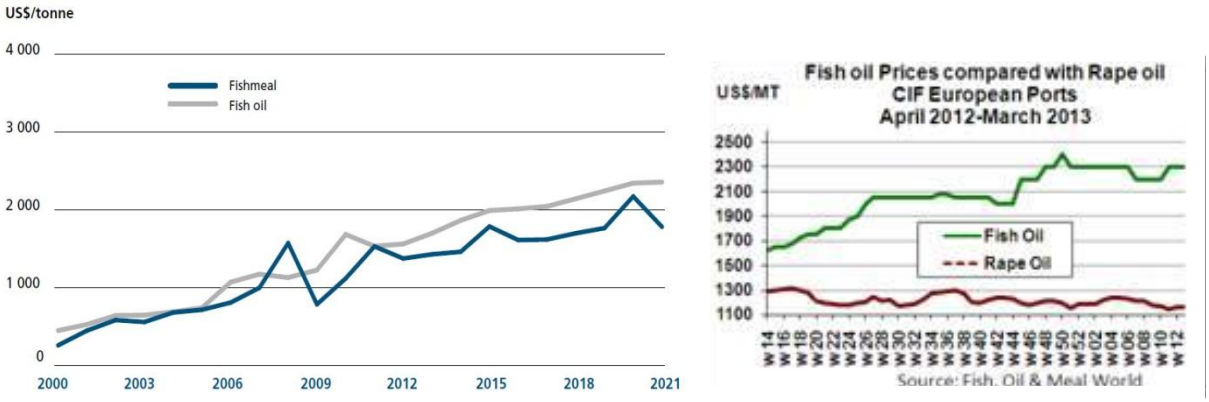


Fig.2 The price development of fish oil. Left: *FAO prediction of price development of fishmeal and fish oil (FAO, 2012).* ***Right:*** *The price level increased from 1600 USD/t to 2300 US/t from April 2012 to March 2013. The price jump at the end of 2012 reflects the precautionary fishery management, low landing volumes and oils yields related to the last fishing season in Peru (Nov-Dec 2012). At the end of 2012, the fish oil/rape oil price ratio reached 1.9, which is a historically high level. Reprinted with permission from Fish, Oil & Meal world.*

1.2 Increased omega-3 demand from emerging markets

The increased awareness of health benefits from EPA and DHA has developed applications and markets for direct human consumption (DHC). Some of the major developers of these applications are BASF, DSM, Vega Nutritionals Ltd, Omega Protein Corp, Horizon Organic, Croda International PLC, and Copeinca ASA (owned by Cermaq). In 2010 the demand of concentrated and refined fish oils for these universal markets reached 24% of the total world fish oil production. In addition, these emerging markets are rapidly increasing (Figure 3). The *average volumetric increase* in overall fish oil demand was 9.9% in 2011 (GOED, 2012), where the most rapidly emerging applications were in food (20.9%), dietary supplements (10.7%) and clinical nutrition (10.2%). Most of these sectors demand EPA and DHA with high purity and concentration. As the production of 1 kg of >90% pure EPA/DHA oil requires >20 kg fish oil, much of the available fish oil is used to produce *pure omega-3 oil* for higher value markets – where the price is 25-30% higher than the aqua-grade fish oil (IFFO, 2012).

According to a recent market report, the global consumer spending on EPA and DHA fortified products was estimated to 25.4 billion USD in 2011, and is estimated to increase to 34.7 billion USD (Packaged Facts, 2012). While North-America accounts for 43% of these consumer sales, the demand from the Asian market is expected to grow significantly.

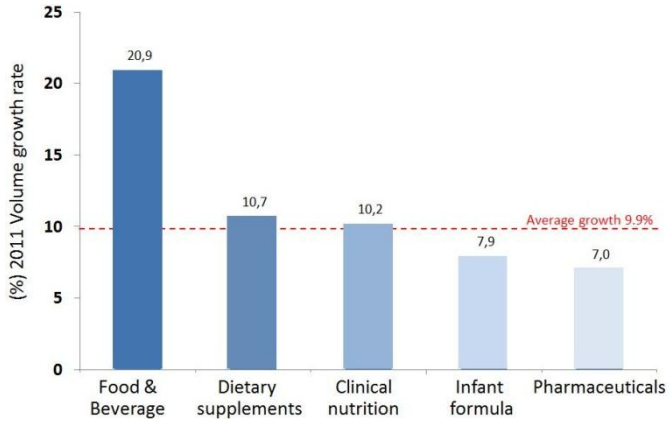


Fig.3. 2011 volume growth rates of EPA and DHA oils by application area*. Emerging markets including food & beverages, dietary supplements, clinical nutrition, infant formulae and pharmaceuticals demand high purity omega-3 oils.Modified from GOED (2012).*

Table 1. Projected volumetric demand (in tonnes) of fish oil in 2017 (grade 30% EPA&DHA) *Source: GOED (A. Ismael, 2012)*		
Market	**Estimated market demand at present**	**Estimated market demand by 2017**
Aquafeed	400 000	600 000
Functional food and Nutraceuticals	120 000 in total	450 000
Pharmaceutical applications		400 000

A recent IFFO projection of future fish oil demands, estimate the volumetric requirements by the major application areas in 2017 (Table 1). This suggests that the annual demand fish oil will be far greater than the current global supply of 1 000 000 t. Thus, the demand from such emerging markets and applications will increase and they are in the position to out-compete the demand from the aquafeed sector. This is also because the omega-3 oil costs for the production of higher value products are relatively low compared to the final products, making the emerging application industry more price competitive than the aquafeed sector.

1.3 Time perspective – when are fish oil alternatives needed?

Of the total global supply of 1 000 000 t fish oil, 70% is already consumed in aqua feed production and the increasing aqua feed production is challenged by the demand of a rapidly expanding direct human consumption (DHC) market in the next 5 years. This rapid development DHC markets has become problematic for the aquafeed industry due to increase competition on the fish oil market.

After a decade of stable fish oil supply for use in aquafeed the recent FHF/NILF report, *"Føre Var i laksenæringen: Tid for kollektiv håndtering av underdekning av fiskeolje* (Steine, Tveterås, Pettersen, 2011), conducted an analysis of how the growing demand of fish oil may affect the aquafeed sector (Figure 4). The growing demand for fish oil in feed has been mitigated by increasing substitution by vegetable oils over the recent years. The current industry norm is to use >10% EPA/DHA in the aquafeed oil fraction, but a further substitution of EPA/DHA with available plant oils can minimize or delay the expected under coverage with a few years. Although the fish welfare may not be affected by the indicated fish oil substitutions, there are several market and consumare acceptance issues related to such a strategy – in particular related to the claimed health benefits by eating salmon regularly.

The worst case scenario (Figure 4, upper graph) is probably the most realistic situation, because the projections of Steine and co-workers (2011) did not take the growing demand from the pharmaceutical industry into account (400 000 t in 2017). This means that the fish oil shortfall for the aquafeed industry is already imminent, and that strategies to address this should be developed for with a short-, medium and long-term perspective.

1.4 Alternative novel sources to EPA and DHA

The emerging imbalance between the global fish oil supply and market demand clearly demonstrate that new and sustainable omega-3 sources must be developed if the salmon industry wants to continue to market farmed salmon as a healthy food product in the future. This was also the topic of the conference *"Novel sources of omega-3 for food and feed"* recently organized by the *European Federation for the Science and Technology of Lipids* in Copenhagen (14-15th Nov 2012). This forum provided a scientific knowledge status about the development projects of novel sources to EPA and DHA fatty acids.

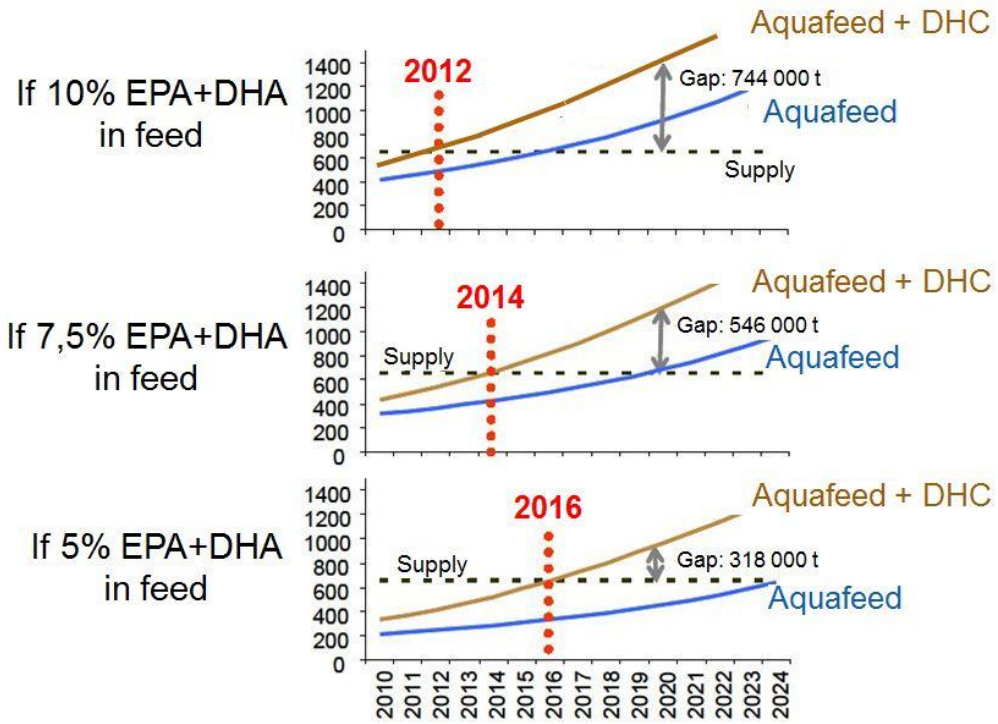


Fig.4. Models of possible scenarios for the globally growing demand for fish oil. *The scenarios describe the fish oil demand (x10^3 t fish oil per year) if the current industry norm at 10% fish oil included in the aquafeed formulation is reduced to 7.5 or 5% fish oil - and the effect on time perspective of the global shortfall. The blue graph shows the consumption of aquafeed production alone, while the brown graph combines the consumption of aquafeed and direct human consumption (DHC). Modified from Steine et al. (2011).*

The industry perspective was also addressed by GOED and IFFO which gave a review of the current fish oil supply and a short overview of the current and future *alternatives* to the pelagic fish oil supply was given. In general, a diverse range of sources to long chain omega-3 fatty acids have the potential to become alternatives or a supplementary to the fish oil from the pelagic fisheries - some that are short-term/low volume and some that are medium-long term/high volumes (Table 2).

Exploitation of trimmings, by-products, by-catch or less exploited species

The optimized use of trimmings and by-products, as well as by-catch and less exploited species with low omega-3 fatty acid levels can be a short- to medium-term source to more fish oil (FAO, 2011). While the supply of aquafeed ingredients from trimmings and by-products has grown to 25% of the total feed supply, this will still only represent a limited amount. By exploiting more of the by-catch and fish discards (7 000 000 t/y according to FAO, 2011), and less exploited species with lower omega-3 levels like tuna, sardines, cod, and squid, there is a huge potential reserve to increase the omega-3 supply. However, the issues related to such fish oil sources are not within the scope of this study.

Zooplankton: Krill and Calanus

Krill (Euphausiacea) is a group of animals within the Crustacea and an interesting resource for fisheries, especially the commercial fisheries of Antarctic krill. Krill is harvested because the high content of EPA and DHA accumulated by feeding on microalgae containing these valuable omega-3 fatty acids. Large scale fisheries started in the 1960-70s by several countries. Increased harvesting of the Southern ocean krill stocks raised concern about the food web, where they perform a very important role (Bostock et al, 2010). The estimated krill

biomass is 60 mill ton in the assigned Antarctic fishing area, and the quota has been set at 620 000 t/y by the Commission for the Conservation of Antartic Marine Living Resources. The annual catch so far has been 250 000 t/y. While the use of omega-3 oil from krill (10-15% EPA, 5-8% DHA from krill) is one of the fastest growing segments, the primary application is in nutraceuticals. The high price levels will restrict krill oil to niche markets also in the future, and is not foreseen as a significant contributor for EPA and DHA into fish feed in the coming years. Expected production of oil is <5000 t in 2017 (IFFO).

Calanus finmarchicus is a commonly found zooplankton species in the subarctic waters of the North Atlantic and is a key species in the Barents Sea. In the Nordic waters it is believed that the annual production of *Calanus finmarchicus* is 100-400 million tonnes. Based on this huge amount of biomass it has been strong interest to look for possible utilization of this resource as a new marine biomass for industry applications. The lipids of *Calanus* contain 3-15% EPA and 2-10% DHA. A Norwegian company, Calanus AS harvest *Calanus* and manufacture the biomass to value-added health- and nutrition products, based on their patent WO 2010077152A1. The current market is nutraceuticals and dietary supplements, and the product profile of Calanus AS aims on high-price segments. It is therefore unlikely that *Calanus* biomass will become a significant resource for fish feed in the near future.

Genetically modified plants: Soybean, rapeseed and false flaxseed

Most of the projects on the transgenic modification of plants to increase EPA or DHA levels are performed in well-established oilseed model species (Petrie et al, 2012). In general, the genetic modification of plant oilseed require complex metabolic engineering where several transgenes must be coordinately expressed in developing seeds, resulting in the accumulation of EPA or DHA fatty acids. As progress has been made the last decade, and the benchmark for

Table 2. Novel sources of EPA and DHA fatty acids

Category	Source	Potential	References
Pelagic Fish	Trimmings and By-products	*Represent 25% of global feed rawmaterial supply, and will continue to grow. Require industrial efforts on logistic*	*IFFO, 2013*
	Less exploited fish species	*Tuna, sardines, squid etc. Require industrial efforts on logistic and amended regulations*	*FAO, 2011*
Zooplankton	Krill	*Large biomass, ecological impact of harvest is disputed. High price - currently not feasible for feed applications. Estimated production in 2017 is 5000 t krill oil*	*Bostock, 2010 IFFO, 2013*
	Calanus	*Large biomass, challenging harvesting methods Uncertain potential and high price level - currently not feasible for feed applications*	*WO2010-077152A1*
Microalgae	Photoautotrophic	*Primary producers, sustainable production using renewables, biological and technological improvements can lead to competitive price level.*	*Norsker, 2011 Draisma,2012*
	Heterotrophic	*Primary producers, mature technology, biological and technological improvements can lead to competitive price*	*US 7732170*
Gene modified organisms	GM fungi	*GM Yerrowia produces 55% EPA by fermentation (DuPont). Commercially used for salmon feed, approved by US FDA*	*US 89619A1*
	GM plants	*GM soybean produce 20-30% SDA in oil (Monsanto) Soymega commercialized, low productivity, not EPA/DHA* *GM Rapeseed produce 12% DHA in oil (CSIRO) Low productivity. Not commercial* *GM False flaxseed produce 20% EPA+DHA in oil (Rothamstead. Low productivity. Not commercial*	*Eckert, 2006* *Petrie, 2012* *Sayanova,2012*

GM oilseed production is now 20% EPA (Cheng et al., 2010). However, plant lipid metabolism has proved significantly more complicated than previously imagined, and there is still significant research efforts required to make advances relevant for the aquafeed industry (Haslam et al, 2013). The most important challenges for the production of EPA or DHA in GM plants are to increase the aerial productivities, which are currently very low (100-200 kg EPA&DHA/ha/y). Furthermore, the transgenic plants may also contain high levels of omega-6 and omega-3 metabolic intermediates that are not found in fish. Some of the most advanced development projects are listed below.

The company Monsanto has developed a *GM soybean* that produces stearidonic acid (SDA) - the first intermediate generated by the Δ6-desaturation of ALA in the biosynthetic pathway to EPA and DHA (Eckert et al, 2006). The further development of EPA or DHA fortified soybean oil is expected to take several years. However, SDA has been shown in animal and human studies to be more effective than its precursor, α-linolenic acid, in enriching membranes with EPA (Harris, 2012). The plant-based SDA soybean oil, branded as Soymega, is recognized as safe (GRAS) by US FDA. The estimated productivity is 100 kg SDA/ha/y.

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia have developed a *GM rapeseed* (*Brassica Napus*) by introducing genes from microalgae like *Micromonas* sp. and *Pavlova* sp. (Petrie et al, 2012). This has resulted in transgenic plants containing 12% DHA in the rapeseed oil, with an estimated productivity of *120 kg/ha/year*

The Rothamstead Research Institute have developed *GM false flaxseed* (*Camelina sativa)* plants containing 3 genes for the biosynthesis of EPA and additional 5-8 genes for the biosynthesis of DHA have been developed (Sayanova et al, 2012). At the moment GM *plants* is producing 20% EPA+DHA (Haslam et al, 2012). The reported productivity is about 150 kg/ha/y. This is still low and need further improvement over the next 5-10 years to come.

Genetically modified microorganisms: fungi

The yeast *Yerrowia lipolytica* is a well-known laboratory "work horse", and DuPont has genetically modify these fungi to so that the yeast triacylglycerides in the oil contains 55% EPA (Darmude *et al.,* US 2011/0089619A1). DuPont has entered a partnership with AquaChile to use the EPA-rich *Yerrowia* biomass as a feed component to raise brand salmon by the Verlasso Company in Chile (www.verlasso.com). Verlasso claims that this may reduce the use of fish oil in feed by 75%, and improve the "fish in-fish out" ratio close to 1:1. Market and consumer acceptance of GM fed salmon is likely to meet a more conservative stand in the EU than the US market. The production cost is not known, but based on the generic production technology the price level should be in the range of heterotrophic microalgae.

Microalgae – the primary producers of EPA and DHA fatty acids

Microalgae are the primary producers of all the EPA and DHA fatty acids which are accumulated along the trophic levels in the marine food web. As microalgae is a highly productive and proven source of omega-3 fatty acids, and as many companies are in commercial operation (and several more are in the late technology readiness levels) they are currently regarded by IFFO as the most promising and sustainable alternative source to EPA and DHA in fish oil (Mallison, 2012).

This report will elaborate on how intensified research on the biological potential and an increased focus on the technological challenges of microalgae cultivation can lead to cost-efficient microalgae production in the next years.

PART II
The knowledge base

2 Microalgae – a source to EPA and DHA fatty acids

Chapter summary box

Microalgae, including thraustochytrids and cyanobacteria, are a natural source of long-chained, polyunsaturated essential fatty acids such as EPA and DHA. Most microalgae are *photoautotrophs*, meaning that they use light energy to produce chemical energy and convert inorganic carbon (CO_2) into sugars and organic compounds. Another group, called *heterotrophs*, can grow without light and instead use organic carbon compounds as both energy and carbon source. Few algae species are used commercially today, also for the industrial production of essential oils. The important parameter for commercial scale cultivation is *productivity*, given as the biomass produced per volume over time. The availability and supply of energy and nutrients affect the biomass productivity and the EPA/DHA content in the cell. Value up to 7% EPA/DHA of the biomass have been reported, but between species. Some species can accumulate > 50% lipids/DW under nitrogen depletion, and the aim is to optimize the fraction of EPA/DHA in these lipids. Photoautotrophic microalgae can produce 15-30% EPA of total fatty acids, while heterotrophic Thraustocythrids can produce biomass with 55% DHA of total fatty acids.

The vast microalgae biodiversity of several hundred thousand species has not yet been fully exploited. A systematic exploration of can lead to discovery of novel, high-productivity strains with high EPA/DHA levels.

Research challenges:

- *Screen the biodiversity to identify novel, productive strains with high EPA and DHA levels.*
- *Establish robust and sustainable strains of the selected algae that can be used in industrial production*

Marine algae are a very large and diverse group of simple, typically photoautotrophic organisms, ranging from unicellular as phytoplankton to multicellular forms, such as the giant kelps that grow to 65 meters in length. Most of the algae are photosynthetic organisms as higher plants. *Microalgae* are microscopic unicellular algae, which exist individually or in chains or groups. Depending on the species, their sizes can range from a few micrometers (µm) to a few hundreds of micrometers. Unlike higher plants, microalgae do not have roots, stems and leaves, and can have ten times more efficient mass-transfer and growth than terrestrial plants.

Many microalgae are *phototrophic* as nearly all algae have photosynthetic machinery (Figure 5). Photosynthesis is conducted by two key processes; the conversion of photosynthetic active radiation (PAR) from the sun into chemical energy (by photosystems I and II), and the fixation of inorganic CO_2 into biomass (by the Calvin cycle). Chlorophyll a is the main photosynthetic pigment for light harvesting, and is characteristic for photoautotrophic microalgae. The fixed carbons in the chloroplast can be used for production of amino acids, fatty acids or carbohydrates, pending on the metabolic status of the cell.

Another group of species can rely totally on organic energy and carbon sources and have limited or no photosynthetic apparatus. Those organisms are called *heterotrophic* organisms.

Some algae species and groups are also *mixotrophic* organisms, with the ability to shift between deriving energy both from photosynthesis and the uptake and use of organic carbon both as energy source and carbon source.

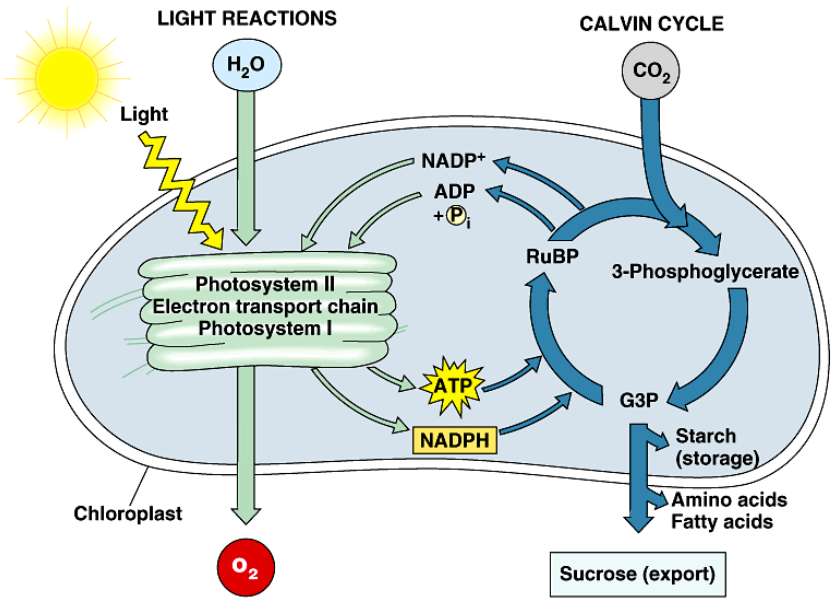


Figure 5. Schematic overview of a typical microalga cell with the overall photosynthetic reaction*. Photosynthesis is conducted by two key processes; photosystems I and II convert solar energy to chemical energy which drives Calvin cycle and fixation of inorganic CO2 into biomass. The fixed carbons in the chloroplast can be used for production of amino acids, fatty acids or carbohydrates, pending on the metabolic status of the cell. Source: http://bioweb.uwlax.edu*

The prokaryotic cyanobacteria are sometimes referred to as blue-green algae, but are now classified as bacteria. The term 'algae' is today mostly restricted to eukaryotic organisms. All true algae therefore have a nucleus enclosed within a membrane and plastids bound in one or more membranes. The Labyrinthulomycetes (slime molds) containing the labyrinthulids and thraustochytrids, are a class of protists that produce a network of filaments or tubes to absorb nutrients. They are mostly marine and in nature and act as parasites on algae, seagrass and invertebrates, or as decomposers on dead plant material. They are a primitive group of heterokonts, and are today regarded as a separate group.

The biodiversity of microalgae is enormous and it has been estimated that hundred thousand species exists. Most of these microalgae species produce unique products like long-chain unsaturated fatty acids, carotenoids and antioxidants. Out of the 35.000 microalgae species which are described, only a very few are commercially produced at the moment: the cyanobacteria *Spirulina* and *Aphanizomenon flos-aquae,* the thraustochytrids *Ulkenia* sp. and *Schizochytrium* sp., and the eukaryote algae *Crypthecodinium cohnii, Chlorella* sp., *Dunaliella salina, Haematococcus pluvialis, Euglena* sp. and *Odontella aurita*. In terms of volume, the three genera *Spirulina*, *Chlorella* and *Cryptecodinium* are contributing to the biggest volumes. About half of microalgae productions are dedicated to products with whole microalgae and the other half to production of extracts.

Biological productivity

Microalgae undergo cell division (sometimes also sexual reproduction via spores). The biomass of an algae culture can be expressed as cell numbers per volume culture (cell density) or as cell dry weight per volume. The growth of the culture can be expressed as the specific growth rate, which describes how fast the cell density increases as a function of time. However, for the production yield of some given compound, e.g. EPA/DHA, the term *productivity* is the preferred parameter. Productivity is expressed as the amount of biomass (or

Table 3. Biomass and lipid productivity, and denominations		
Productivity factor	**Unit**	**Limitations**
Total cell biomass	gram dry weight/liter/day *or* ton dry weight/hectar/year	- Sunlight and ability to convert into energy - CO_2 and nutrients - Efficient circulation for mass transfer
Total lipid fraction in the cell	gram lipid/liter/day	- Metabolic status
EPA or DHA content in the lipid fraction	gram EPA/liter/day gram DHA/liter/day	- Highly specific enzymes involved in the synthesis of EPA and DHA

EPA/DHA) produced per volume of culture over time, e.g. *gram dry weight biomass per liter per day* (Table 3). Mass-cultivation of microalgae cover large land areas to utilize natural sunlight as energy source and the productivity can also be expressed as productivity per area over time (e.g. ton/ hectare/year). The reason for this is that the productivity is strongly dependent of the sunlight irradiance. The productivity of lipids is the amount of lipid produced by a certain biomass or in a given culture volume over time (e.g. mg lipids per gram algae per day), and can range between 10-70% of the dry weight biomass pending on the metabolic status of the cells. The content of EPA and DHA is normally given as the percent of the lipid fraction, but can also be given as the percent of the biomass

EPA and DHA Fatty acid synthesis

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are polyunsaturated fatty acids (PUFAs) of the n-3 family that are essential in animal and human nutrition. PUFAs are chemically characterized as long-chained hydrocarbons with 2 or more double bonds (Figure 6), and they provide e.g. flexibility in membranes or functional properties of the metabolism. Humans acquire PUFAs through the consumption of fish with high fat content, e.g. salmon and mackerel, and plant seed/oils. The fish in turn, acquire PUFAs through their food and depend on a certain amount of PUFAs in the diet.

Animals (including fish and zooplankton) can only produce elongate and synthetize the long chain n-3 fatty acids from precursors (e.g. ALA in Figure 7), while microalgae can synthesize the full array of PUFAs including omega-3 fatty acids EPA and DHA. The basic synthesis pathway is starting from linoleic acid (18:2 (n-6)) that is converted to α-linoleic acid by an enzyme called ω3-Desaturase and from there through a set of desaturation and elongation steps to yield EPA and then DHA (Figure 7), or arachidonic acid (20:3(n-6)) that is converted to EPA by an enzyme called Δ^{17}-Desaturase (Gushina and Harwood 2006, Guedes et al. 2011).

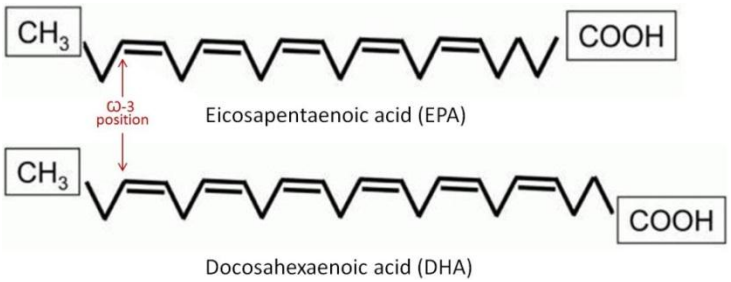


Figure 6. Structures of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)*. EPA and DHA belong to the class of essential fatty acids denominated by the double bond three carbons from the methyl moiety.*

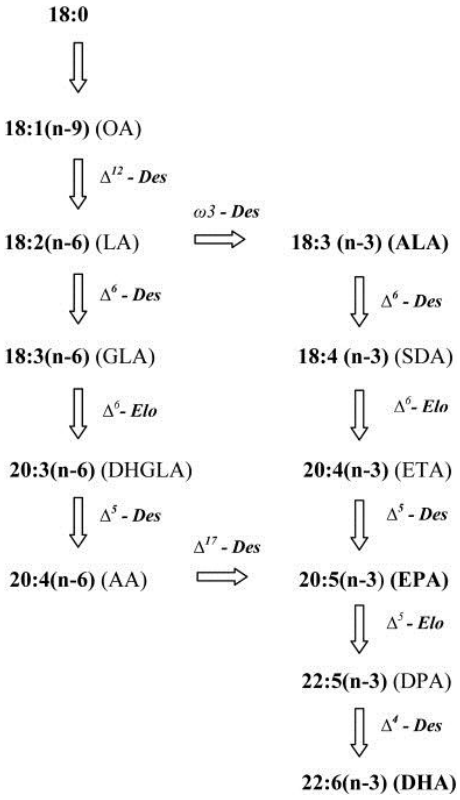


Figure 7. Basic metabolic pathways for synthesis of long-chained polyunsaturated fatty acids (PUFAs) *The starting points are 18–carbon fatty acids via enzymatic steps involving specific desaturases (Des) and elongases (Elo) (from Guedes et al. 2011).*

Although most microalgae have the ability to synthesize PUFAs, there are large species-specific variations in the actual production and storage in each algae species. Environmental conditions such as light and nutrient limitations may induce lipid accumulation in microalgae, and the localization of PUFAs in the algae cell is also of interest (Tonon et al. 2002). PUFAs tend to be included in membranes as polar lipids which make extraction more demanding, while storage lipids triglycerides (TAG) are localized to the cytosol and are more easily accessed via mechanical or chemical extraction. Partitioning of lipids in the cell is often related to cultivation and culture stage and the TAG content is often high in nutrient limited, stationary cell cultures (figure 8).

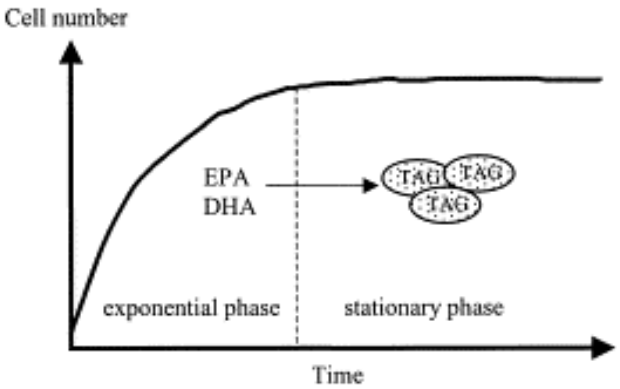


Figure 8. The accumulation of lipids in microalgae*. Partitioning of long-chained polyunsaturated fatty acids (PUFAs) into triacylglycerides (lipid storage molecules) over the cultivation stages from exponential to stationary growth (from Tonon et al. 2002).*

2.1 Microalgae producing EPA and DHA by photosynthesis

Phototrophic microalgae productivity is directly related to the availability of light, CO_2 and nutrients. However, on sunny days the microalgae may reduce or stop the photosynthesis (photoinhibition) due to high irradiance levels. Phototrophic microalgae can in some cases generate 10-20 % LC-3 PUFA (of their biomass) although apparently predominantly at low growth rates (Guedes et al. 2011). Among the many species there are variations both in productivity and content of fatty acids, and some produce mainly EPA or DHA while others may have a certain content of both fatty acids (Adarme-Vega et al. 2012).

Currently, most commercial interest is on the genus *Nannochloropsis* (*N. oculata*, *N. oceanica* or *N. gaditana*) which can produce very high fatty acid contents (>55 % DW) under nitrogen starvation (Table 4). EPA may constitute 5-6 % of the biomass throughout the growth phase, however, during nitrogen starvation the EPA synthesis rate decreases. *Monodus subterraneus* may also be a potential EPA producer, but is less investigated (Hu et al. 1997). Nannochloropsis can be cultivated using either open ponds or closed photobioreactors. Seambiotic in Israel produces *Nannochloropsis* in open ponds with annual productivity averages of 20 g DW m^{-2} day^{-1} at 30% lipids (Ben-Amotz 2008), for a while the first and only source of microalgal biodiesel. This figure is very low by comparison to what can be achieved with a dedicated nitrogen starvation phase where fatty acid contents even reaching 55% DW, but such a regime will normally reduce overall biomass productivity.

Phaeodactylym tricornutum has very similar productivity characteristics as *Nannochloropsis* spp., but cannot be induced to generate quite so high fatty acid contents (about 40% DW). However, *Phaeodactylum* is easier to harvest than *Nannochloropsis*. Other diatoms, such as the genus *Chaetoceros* (including *C.muelleri* and *C.calcitrans* that are widely used in seafood hatcheries) are relatively good EPA producers, with high productivity and lipid content and good growth at high temperatures (35 ^{o}C). Concomitant EPA and DHA production has been demonstrated in *C. muelleri* and *C.gracilis*.

Isochrysis galbana and Pavlova lutheri are also much used in live feed production, and exhibit relatively high DHA values: *I. galbana* 2.2 % (of DW) together with 4.8% EPA. *P. lutheri* contains rather high proportions of DHA, 15-30 % of total fatty acids. Both species can be produced at reasonably high biomass densities (3-10 g DWL^{-1}) with good biomass productivity. *I. galbana* has been produced at high biomass densities in open ponds. Temperature manipulation can change proportions of EPA-DHA and in *P. lutheri*, EPA and under cold conditions the conversion of palmitic acid (C 16:0) to EPA and DHA is increased.

Table 4. Reported EPA or DHA concentrations and phototrophic productivities.
Values are based on cultivation conditions used for each individual study.

Organism	Cell density [g DW/l]	EPA/DHA			Reference
		[% of DW]	[mg DW/l]	[mg/l·d]	
Nannochloropsis sp.	7-8	5-6			Norsker et al. (2011)
N. oculata	0.4-1	4-5	20-50		Reitan, unpublished
Phaeodactylum tricornutum		2.6-3.1		0.148	Sánchez-Mirón *et al.* (2003)
Isochrysis galbana	3-10	6-7			Fradique (2013) Zhang 2003)
Pavlova lutheri	3-10	15-30*		0.29/0.14	Guedes et al. (2011)

** of total fatty acids, TFA.*

Table 5. Reported DHA concentrations and heterotrophic productivities.					
Organism	Cell density [g/l]	DHA			Reference
		[% of TFA]	[g/l]	[g/l·d]	
Thraustochytrid strain 12B	21	50-55	5.6	2.8	Perveen et al., 2006
S. limacinum SR21	59	~65	15.5	3.0	Yaguchi et al., 1997
Aurantiochytrium sp.	90-100	35	14	2.2	Jakobsen et al., 2008
Schizochytrium sp.	160-180	40	40-45	10-12	US 7732170

2.2 Heterotrophic production of DHA by fermentation

A range of heterotrophic microorganisms, including bacteria, yeasts, microorganisms of the family "thraustochytrids" and microalgae are able to accumulate high levels of lipids (triacylglycrols) (>50 % of dw) as storage compound. Of these, only the marine species produce long-chain ω3-PUFA, and only thraustochytrids and the microalgae *Crypthecodinium cohnii* are known to produce LC ω3-PUFA, mainly DHA, as part of their storage lipids. The mechanisms and enzymes involved in accumulation of storage lipids induced by nutrient limitation, are common for photo- and heterotrophic eukaryotes, and similar strategies for optimization can be applied. With respect to EPA/DHA-synthesis, thraustochytrids with high levels of DHA synthesize the DHA via the polyketide synthase enzyme complex (Matsuda, 2012), independent of the general pathway for PUFA-synthesis (Figure 7).

Thraustochytrids are obligate marine, eukaryotic microorganisms, related to the alga *C. cohnii*, and different genera of thraustochytrids (*Schizochytrium*, *Aurantiochytrium*, *Ulkenia*) are currently applied in commercial production processes for human applications (Mendes et al. 2009; Ward & Singh 2005). These organisms can accumulate 50-70% triacylglycerols with 30-40% DHA. Cell densities of 100 g/l have been published for both *C. cohnii* and thraustochytrids (De Swaaf et al. 2003; Jakobsen et al. 2008; US 7732170).

Of the DHA-producing strains, the thraustochytrids have the highest productivities. Maximum published productivities of total fatty acids (TFA) and DHA are 24 and 10-12 g/l·day, respectively, with cell densities of 160-190 g/l and 35-40 g/l DHA (Martek patent, US7732170). The carbon source is glucose or glycerol. Table 5 summarizes published productivities. The major factor contributing to the high productivity achieved by Martek is the high cell density.

2.3 Research challenges - Exploring the biodiversity potential

The ability to explore the full biological potential from the enormous biodiversity of microalgae (> 350 000 species) with regard to increased productivity, photosynthetic efficiency and lipid/EPA/DHA profile, is a significant aspect in the future development of future microalgae industries. Of the naturally occurring algae, only very few species are used in research and industry today (Larkum et al. 2012). As an example, screening of cold-adapted species has shown that some strains have higher content of EPA and DHA than the temperate and sub-tropical species (Jiang and Gao 2004). This EPA/DHA increase has been suggested to be important to maintain cell membrane fluidity in cold adapted species. While few cold-water species are developed for up-scaled production and aquaculture applications, the exploration and discovery of novel strains may be a valuable contribution in the development of microalgae for aquafeed applications. Identification of high productivity strains with elevated levels of EPA and/or DHA can form an optimal the starting point for further optimization of productivity.

3 Strategies to increase the biological productivity of EPA and DHA

Chapter summary box

EPA/DHA yield can be optimized by increased biomass production, and/or increased lipid productivity. By systematic investigation of the biodiversity, or screening of sampled microalgae isolates, novel productive strains with high EPA and DHA levels can be identified. The flux of carbons to lipid synthesis pathways is determined by the metabolic status and specific capability of the algae. The production pathways for EPA and DHA are known, and subject for improvement. Furthermore, there are *three main strategies* to actively increase the yield: by exploitation of physiological potential, by strain selection and breeding of promising candidates, or by genetic modification. There is an ongoing development of molecular tools to allow genetic modification, both by US efforts related to the biofuel industry and through EU FP7 research projects. These efforts focus on increasing the photosynthetic efficiency, and the EPA and DHA content in the cell. The combination of natural selected strains and improvement strategies can also lead to higher productivity. It is estimated that a 2-4 fold (or more) increase in biomass productivity and lipid yield can be obtained.

Research challenges:

- *Develop model systems and molecular tools to allow genetic modification programs.*
- *Combine optimal traits and coordinately channel energy into synthesis of EPA and DHA.*
- *Develop improved strains with 2-4 times higher levels of EPA and DHA.*
- *Develop model systems and molecular tools to allow genetic modification (aimed at light absorption optimizing and directing carbon flow to EPA and DHA production)*

The cost of algae production has been a challenge for the commercial utilization of algal biomass or derived compounds. Based on sensitivity analyses in techno-economic studies, the factor with most influence in driving down the production cost is to increase the *biological productivity* (Davis 2011). The EPA/DHA yield from algae is dependent on two factors; the biomass density that can be achieved per volume or area of culture, and the quantity of relevant lipids that can be obtained per weight unit of algal biomass. For both these aspects the potential for manipulation and optimization is limited for a given strain, which is why strain improvement is an important issue. In principle, three strategies can be described for the increase of biological productivity of EPA and DHA; exploiting the maximized physiological potential by directing the cell metabolism towards lipid/EPA and DHA production, selection and breeding of strains with increased productivity of total biomass or high lipid yield, and genetic modification to fortify the natural productivity of a high productivity strain (Figure 9). The improvement of productivity can be obtained by increasing the EPA/DHA concentration in the culture volume, determined by; *i)* the cell density, *ii)* the lipid content of the cells and *iii)* the EPA/DHA-content of the lipid - and/or by increasing the production rates.

3.1 Optimizing productivity by the physiological potential

The potential for lipid production is first and foremost an organism-specific feature. For the selection of an organism suitable for lipid production, a rapidly growing body of literature is available (e.g. Zhou et al., 2013, Song et al., 2013, Lim et al., 2012). This literature provides detailed insight on crucial parameters like doubling time, maximal cell density, maximal oil content and fatty acid composition of lipids.

In addition to organism-specific characteristics that define the potential for lipid production, physiological variables can be adjusted to induce lipid accumulation and shift lipid composition to enhance desired lipids. Most of these physiological conditions can be applied

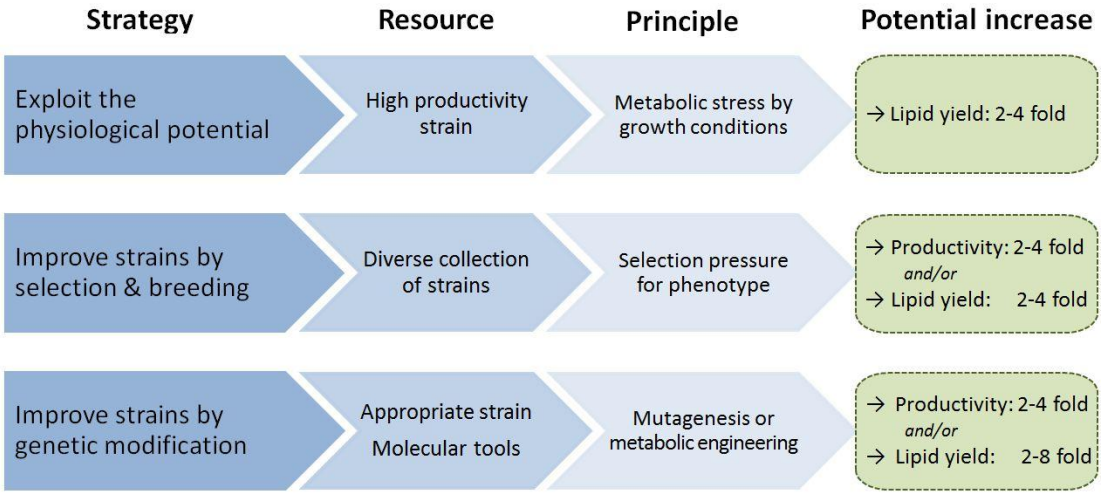


Fig. 9. Three possible pathways to achieve higher biomass productivity and/or higher lipid content. *Exploitation of natural potential by means of metabolic stress to enhance lipid production and storage, strain improvement by inducing directed selection pressure, or strain improvement by means of genetic engineering to alter the genetic apparatus. Potential increase estimates are based on Meireles et al. (2003) and Courchesne et al. (2009).*

across species. Growth restriction due to limited illumination, nitrogen (Lin & Lin 2011; Mujtaba et al. 2012; Longworth et al. 2012), sulphur (Cakmak et al. 2013) and phosphate deprivation (Khozin-Goldberg & Cohen, 2006) are known to induce lipid accumulation and composition changes. Different light qualities (Forján et al. 2011) have also been shown to influence lipid composition and are currently also investigated by the Bones group at NTNU.

In conclusion, a mature body of descriptive work is available that defines lipid producing organisms and suitable growth conditions and harvesting technologies. The main remaining challenge, for any chosen system, is to coordinate biomass accumulation and lipid (quantity and quality) accumulation. Current mechanical harvesting technologies for microalga are mature but have potential for substantial improvement using flocculation

In principle strain improvement can be done in three different ways: Selection of phenotypes, through genetic modification by mutagenesis, or genetic engineering. All three techniques have been utilized successfully in agriculture.

3.2 Strain improvement by selection and breeding (non-GM)

Strains with high lipid content can be obtained by selective breeding of individuals, which are sorted out based on desired characteristics (like high lipid content) or by putting up a selection regime where individuals lacking a desired characteristic experience restricted survival. A combination of flow cytometry and cell sorting has been applied to select for algae with high lipid content using Nile Red staining as an indicator of lipid content (Montero et al. 2011). In this study they were able to select the equivalent of a stable "fat marathon runner" through three sorting events with populations of *Tetraselmis suecica*. A given selection regime may cause genetic drift on the time scale of weeks in microalga (Yoshida et al. 2003). Optimizing EPA/DHA content by strain selection is possible, but requires sophisticated experimental design, as methods for the distinction of EPA/DHA have to be implemented. Vadstein et al. (unpublished) have preliminary data from selection experiments with *Isochrysis* T-iso suggesting that selection through a four week period resulted in a genetic drift where lipid accumulation during stationary phase increased more than a factor three.

3.3 Strain improvement by genetic modification (GM)

Mutagenesis is widely used for creating strains with a desired characteristic for many applications. However, the method is normally not targeted and will therefore involve time intensive screening of mutants to discriminate against the majority of mutants that carry undesired features. Moreover, if an improvement is detected the new strain has to be sequenced to gain insights into the underlying mechanisms. An advantage of traditional mutagenesis is that generated mutants are not considered 'genetically modified', as mutagenesis protocols mimic phenomena that organisms experience in their natural environment. Starchless mutants of *Chlamydomonas reinhardtii* produced 2- to 5-fold more lipids than the wild type, or 2- to 8-fold more TAG per cell (Work et al. 2012). EPA and DHA contents increased more than 30% (of DW) in UV-induced mutants of *Pavlova lutheri* (Meireles et al. 2003).

Metabolic engineering has the promise of providing a targeted method to achieve a desired modification. However, metabolic engineering requires a considerable foundation and understanding of the system that has so far been rarely demonstrated in algal systems. However, high-throughput (Lv et al., 2013) and computational approaches (Chang et al. 2011; Boyle and Morgen, 2009) that will enhance our understanding to achieve targeted manipulation of organism characteristics are rapidly developing. Therefore targeted modifications that result in higher lipid and EPA/DHA yields appear feasible using the current state of metabolic engineering. Enzyme activity increased 2-3 fold-after overexpression of the ACC gene from *Cyclotella cryptica*, but similar results from attempts to increase lipid production in microalgae are few (Courchesne et al. 2009).

In summary, there are ample of opportunities to generate improved lipid-producing strains by selective breeding and traditional mutagenesis techniques. Nevertheless, the *competence and capacity* to produce phototrophic microalgae in a reproducible and controlled system will still be a critical factor to benefit from such biological progress. However, the utilization of advanced genetic engineering techniques for the development of feed resources may affect the consumer acceptance, although the GMO fed salmon is currently approved for human consumption by the US FDA.

3.4 Research challenges on increasing biological productivity

Biological productivity is considered a key driver for the economy of algae industry, and strain improvement of natural strains is considered as an important way to improve algae productivity to produce biofuels and other commodities. Algae strain improvement and progress have been limited by lack of advanced molecular tools for most eukaryotic microalgae. The U.S. Department of Energy report "National Algal Biofuels Technology roadmap" (US DOE, 2010), address the algal biotechnology challenges and point to the need for a genetic toolbox consisting of mutagenesis (spontaneous or targeted), transformation of genes between species, sexual crossing (in individuals with sexual reproduction), homologous recombination and other techniques to either enhance or silence gene expression of selected traits. Biotechnological enhancement of metabolic processes should be aimed at optimizing light absorption, balancing energy input (ATP vs NADPH), and directing carbon flow to EPA and DHA production.

As genetic engineering of microalgae is not yet developed to a large extent, intensive large-scale research efforts on synthetic biology must be employed to advance algal functional genomics and biotechnology.

However, research progress is being made on species that are relevant for lipid production, both through academic research (Radakovits, 2012) and by commercial efforts like that of Aurora Algae (Kilian, 2011) and Synthetic Genomics (www.syntheticgenomics.com). The latter company, led by Craig Venter who is known for developing the first synthetic bacterial cell (Gibson, 2008), has a world class competence in synthetic biology and will engineer algal cells to secret lipids in a continuous manner.
The ongoing EU 7FP project GIAVAP (Genetic Improvement of Algae for Value Added Products, 2011-2015) address the question of whether genetically modified algae are necessary for the development of algaebased bioproducts (http://giavap.eu/home). The current research focus on increasing the lipid content while at the same time maintaining high growth rates, reducing the light-harvesting antenna size to enhance light utilization, ease harvestability and processability, enhancing carbon flow from carbohydrates to lipids and enhanced accumulation of added value compounds. They report research activity including successful transformation on various microalgae: *Chlamydomonas reinhardtii*, *Chlorella* sp., *Phaeodactylum tricornutum, Ostreococcus taurii, Haematococcus pluvialis, Parietochloris incisa, Nannochloropsis oceanica* and *Thalassiosira* sp. The GIAVAP project also emphasize that production and marketing of GMO algae is only achieved at very high costs, so the gain must be significant in order to proceed with GMO projects.

In terms of improving the productivity for EPA and DHA for aquafeed applications, techno-economic analyses show that the lipid content had a stronger impact on the economics than increasing the overall biomass productivity (Davis, 2011). While these findings were made for microalgae producing lipids for biofuel production, this may also be the case for the production of microalgae rich in EPA and DHA for use in aquafeed.

4 Production of microalgae biomass

Chapter summary box

Cultivation of photosynthetic microalgae is conducted in either open pond systems or closed photobioreactor systems. The open ponds are cheap raceway constructions to encompass large volumes, but the productivity is low and high energy costs are used to harvest large volumes of culture media containing 0.1-0.2 g dry weight/l. Closed photobioreactors are more expensive to build, but have much higher productivity and cell densities at 2-4 g dry weight/l, which lower the harvesting costs. R&D efforts are made to *increase productivity* by using the location, reactor design, plant layouts and most productive strains. Improved biological productivity has the most impact on production economics, and several initiatives are made to increase the photosynthetic efficiency and to adapt better to production conditions. Several technology development projects aim to *reduce costs* by cheap up-scaling using hybrid reactor-pond systems, improving circulation systems, and use low-cost materials. While the photosynthetic microalgae industry is in development, the heterotrophic production of DHA are based on mature technology and is already in commercial use for high value products. Phototrophic production is considered as sustainable because of factors such as use of renewable resources (CO_2 and sunlight, waste water and animal wastes), use of non-arable land and high productivity.

Research challenges:

- *Development of low-energy circulation systems for mass transfer*
- *Establish cultivation systems using low-cost materials*
- *Identify novel strains with optimal production characteristics*
- *Ensure sustainability and improve process design through life cycle analysis*
- *Improve process design through techno-economic analyses*

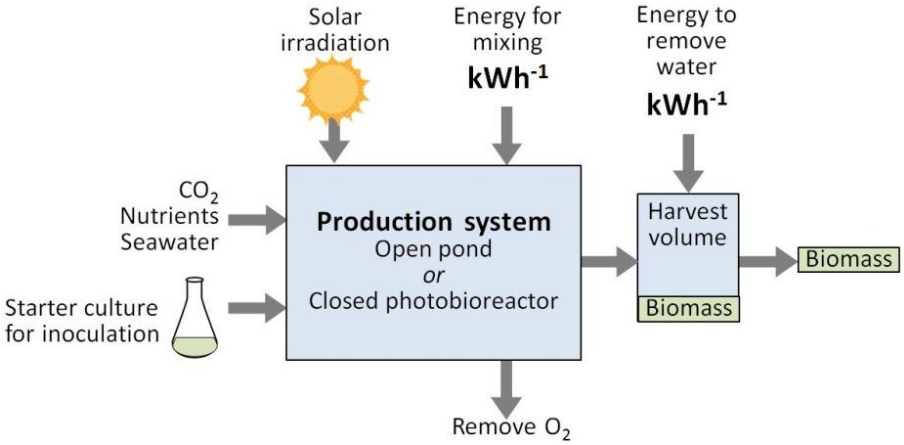


Fig. 10. Schematic overview of phototrophic microalgae production*. The principle requirements are the same for various production system designs; (sun)light, CO_2, nutrients are added to the cultivation medium (seawater) which is inoculated with the appropriate amount of starter culture. Energy is required for proper circulation and for the harvesting/removal of water to recover the microalgae biomass.*

4.1 Autotrophic EPA and DHA microalgae production

Microalgae using sunlight and CO_2 are considered to be one of the most promising feedstocks for sustainable supply of commodities and specialties for both food and non-food products (Draaisma, 2012; Wijffels, 2010; Milledge, 2011), but the key to unlock these opportunities is to optimize the cultivation and production of biomass on a large scale. The use of phototrophic algae cultivated on renewable resources such as sunlight, CO_2, and waste streams, offers the potential for sustainability benefits over fermentation based production that require a organic carbon source.

There are in principle two main methods of phototrophic algae cultivation using either open systems (ponds) or closed systems called photobioreactors (PBR). The critical cultivation parameters are the same for both systems (Figure 10); to maximize the utilization of sunlight and to achieve efficient mass transfer (uptake of CO_2 and nutrients, and removal of O_2). Photosynthetic productivity is directly depending on solar irradiation, and in theory up to 10% of the solar light energy can be converted into chemical energy in the biomass – while the remaining 90% is lost as heat. The properties of the strain and the design of the production system contribute to determine the *photosynthetic efficiency*, which is the parameter that describes how much solar energy from the light that is actually turned into biomass.

There are several design principles to ensure a maximum illumination surface to volume ratio, and minimal use of energy for circulation of the culture medium (Figure 11). In addition, the design of production systems must also take capital investments into consideration, as the use of materials and instrumentation must be highly cost-efficient and sustainable in use.

Open cultivation systems; open ponds

Open ponds are shallow annular, channel system that are recognised for being simple, easy to operate and inexpensive (low capital and operating costs). These are the most common cultivation systems, and have been used for decades for a range of nutraceutical and food products. Extensive experience exists on operation and engineering of raceways in particular related to the efforts of the growing biofuel industry (Chisti et al. 2007).

The main disadvantage of open ponds is that they are not very efficient and generally has low biomass productivity. This is because open systems suffer from a small illumination surface to volume ratio, so that the limited light penetration only reaches the cells near the surface (Ugwu 2008). The cells just a few centimeters below the surface get less sunlight, resulting in low photosynthetic efficiency (1.5%) and low biomass density at 0.1-0.2 g/l (Pulz 2001, Norsker, 2011).

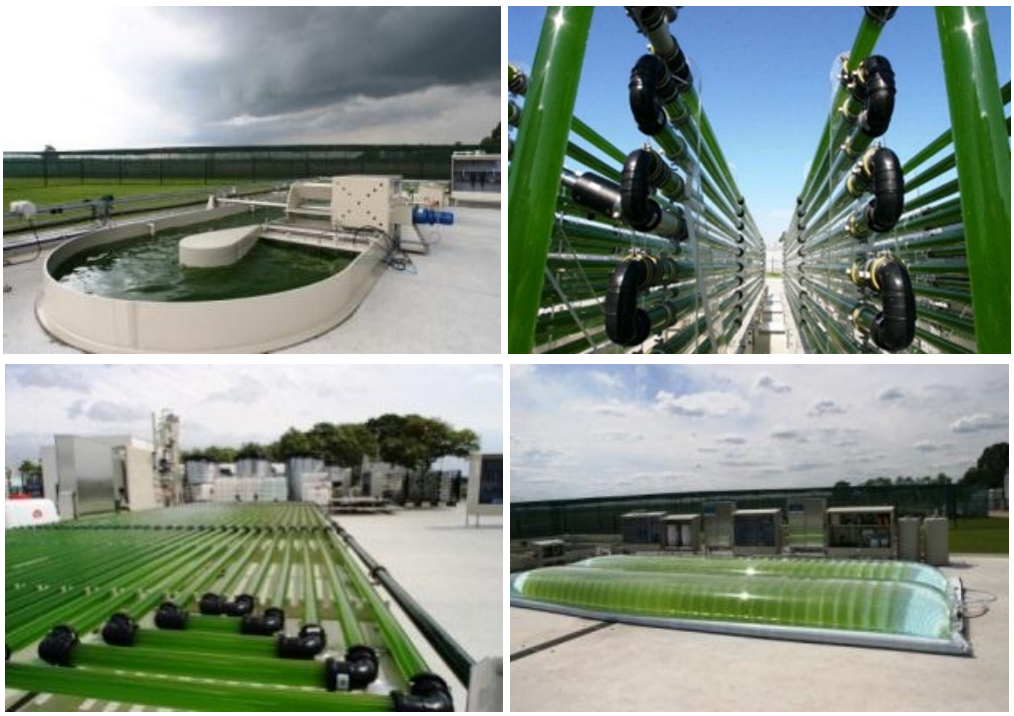


Fig. 11. Pilot production systems of various designs*. Top left: Conceptual model of open ponds system. Top right: Closed vertical (3D) tubular PBR. Bottom left: Closed horizontal (2D) tubular PBR. Bottom right: Innovative design of flat panel PBR, enclosed in a water bag for temperature control. Source: AlgaePARC.*

The low cell density will also add considerable costs of harvesting and dewatering (Figure 10), because large volumes of culture medium must be removed in order to recover the actual biomass (Pulz 2001, Chen et al. 2009).Besides poor productivity, a large ground space is needed for such operations. The expansion of open systems is only possible in 2D, but the pond systems are easily scaled up to several thousand m^2. The loss of CO_2 added to the culture and water by evaporation can be significant (Ugwu et al. 2008). Because the system is open, the loss of CO_2 added to the culture and water by evaporation can be significant (Ugwu et al. 2008) and there is a high contamination risk which can lead to invasion of predators and culture crashes (Waltz 2009). A large amount of algae stock culture is needed to initially inoculate the pond and there is only a low level of control over culture conditions. Algae strain chosen for open systems must be able to cope with extreme temperature conditions and rainfall, and is absolutely weather dependent. *Nannochloropsis* can be cultured continuously and remain virtually unialgal over periods of several months (Boussiba et al. 1988), apparently through suppression of other organisms. Only a few algal varieties will cope with this setup (Pulz 2001).

Closed cultivation system; photobioreactors

Closed photobioreactors are tubular or panel systems that are designed with large *surface to volume ratio* and a short optical path to increase the photosynthetic efficiency (PE). The proper spacing and orientation of flat panels, to maximize the solar irradiation, can also strongly influence the productivity (Chen et al, 2009). Such closed systems make it possible to optimize and control the algal growth more closely than in open systems, so that much higher cell densities and productivities can be achieved. This can result in a much higher biomass yield and density (2-8 g dry weight/l), and the harvesting process will become more efficient due to small volume of fluid and the high concentration of algae (Norsker, 2011). Product standardization is possible because every element of the production can be controlled: CO_2 supply, water supply, temperature, light exposure, culture density, pH, mixing regime. Closed systems offer good control of inputs and helps minimal CO_2 and culture medium loss. Depending on the design, closed systems require less ground area as 3D expansion is possible. There is less contamination and no external predation on the microalgae. Recently developed self-cleaning bead systems allow less fouling than in open and early closed systems and allows continuous production for nearly one year (pers. communication, Salata GmbH). Compared to the open ponds, there is less dependence on the weather which makes it adequate for many algal species. The sufficient amount of sunlight and optimal cultivation temperatures are the most critical factors.

Closed systems are far more complex than open ponds and have therefore higher capital and operating costs than open systems (Waltz, 2009). Elevated cost is the major disadvantages for closed cultivation systems compared to open ponds. Biomass productivity may not always balance the production cost, so the end product usually determines the type of production unit chosen. Continuous production requires very fine tuning of all the elements to prevent a collapse of the culture. In order to achieve better cost-efficient photosynthetic efficiency, the mixing velocity must be finely balanced with the incurred energy costs. The control of gradients of pH, oxygen removal, and the formation of biofilm may be challenging during production, but PBRs are under continuous development to solve those problems. Overall closed PBRs are better systems for high value products, but there is a development towards low-cost closed hybrid systems combining the advantages of classical PBRs and the volumes of pond systems (Alduo by Cellana). If GM microalgae were to be produced, the use of closed systems would be absolutely necessary to protect the environment from GM organisms.

Table 5. Open ponds versus closed photobioreactors		
Parameter	**Open pond**	**Closed PBR**
Illumination Surface-to-area ratio	High	Low
Biomass density	Low: 0,1-0,2 g/l	Moderate: 2-4 g/l
Aerial productivity	10-20 g/m^2/day	35-40 g/m^2/day
Capital costs	Low	Moderate to high
Harvesting costs	High	Low to moderate
Process control	Limited	Possible
CO_2 and water loss	High	Low
Contamination risk	High	Low
Technology base	Mature	In development
Species used	Limited	Multiple

Production systems - open or closed systems?

Although pond cultures per area unit usually come at much lower installation costs than closed photobioreactors (PBR's), they are not necessarily more economic in operation – mainly due to low productivity and high harvesting costs (Table 5). Pond cultures yield a relatively low photosynthetic efficiency, around 1.5% as compared to 3-5% which today is possible for closed PBRr's and produce low biomass density, making dewatering costly. More efficient use of water resources, reduction of evaporative water losses and carbon dioxide losses are other benefits of closed PBR's over open ponds.

A desk study for the comparison of biomass production costs for 3 systems has been performed, evaluating an open pond, a tubular PBR and a flat panel PBR operated at a Dutch location (Table 6). A cost prediction was also made based biological improvements (60% increase in photosynthetic efficiency), technology improvements (modest reduction in mixing energy in the closed PBR's), optimal solar irradiation conditions (Dutch Antilles), and synergies integrating industrial waste streams (application of waste CO_2 and nutrients). The data show that the closed systems are competitive with the low-cost open pond systems for biomass production, and that biological and technological improvements of systems operated at optimal conditions can drive the costs down below 1 euro/kg (see also chapter 8). Most techno-economic studies have evaluated closed systems to be less cost-efficient than open ponds (Benemann, 2009; Davis, 2011; Richardson, 2012), and this is the reason why most algae pilot facilities for biofuel production are based on open pond systems. Most algal species, however, require a closed photobioreactor environment for continuous cultivation including the EPA/DHA producing species (see Chapter 2.1). The decision on which production systems and design to use should be guided by careful analysis of the product and the process involved (Brentner, 2011).

Table 6. Biomass production costs for Dutch production and an optimized, high irradiation site scenario (Norsker *et al*, 2011)			
System	**Open pond**	**Tubular PBR**	**Flat panel PBR**
Base case Biomass production costs, Dutch site (€ / kg DW)	4.91	4.16	5.96
Optimized case Biomass production costs, high irradiation site(€ /kg DW)	1.28	0.70	0.68

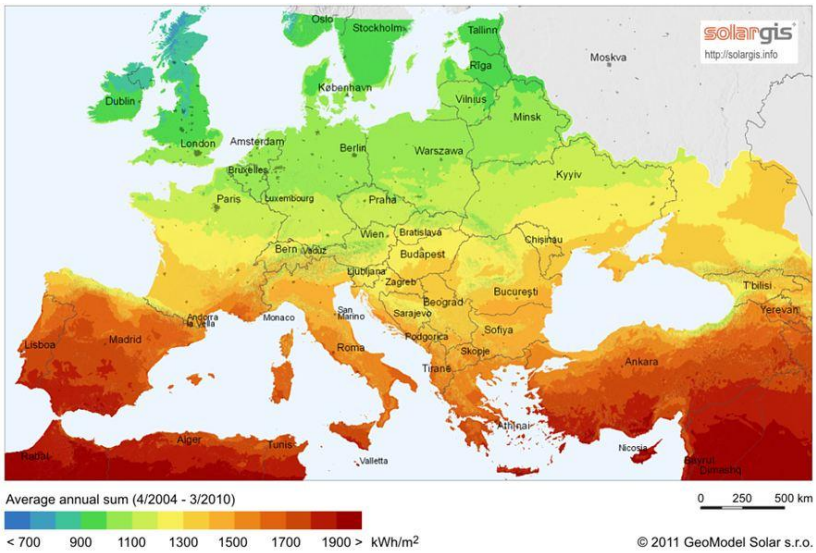


Figure 12. Distribution of global horizontal irradiation in Europe. *The solar energy potential of each region isvisualised.. Source: http://solargis.info*

Potential to increase productivity

The main issues for improving economic viability of photoautotrophic microalgae are to increase productivity of the biomass or compound of interest (Davis, 2011). Potential high-impact improvement factors improvement are discussed below.

Optimal geographical location: Light is the energy source of for phototrophic algae, and the availability and intensity of the photosynthetic active portion of natural light (PAR) is therefore a very important factor for productivity. Increased PAR intensity will directly increase the growth of microalgae until a certain point where maximum productivity occurs.

The difference in biological productivity can be several fold higher under optimal sunlight conditions (Figure 12), but also the temperature, climate, water availability, CO_2 and nutrient availability, infrastructure availability are important factors to consider. Sites in the south of Europe with high irradiation typically are intuitively optimal sites, but are challenged with high temperatures where shading and cooling is required to avoid overheating and too much sunlight where photoinhibition will occur. Coastal zones offer the possibility of using sea water for cooling, but in such areas the cost of land may be high. A GIS-based model for site selection has been developed by US NREL to evaluate the thermal conditions and irradiation/PAR levels, as well as other factors like the land availability, land classification, farm size, CO_2 availability in to a dynamic model to locate suitable production areas.

Hybrid production system: The open ponds and closed PBRs systems both have benefits and weaknesses, and this has led to a development of combined production systems. Continuous photobioreactors are used to produce a high-volume stream of pure biomass that is used to inoculate open ponds for a short batch phase. This cultivation cycle, known as a *hybrid*, benefit from the high-productivity PBRs that produce high enough initial biomass concentration to inoculate larger volumes in open ponds to maximize biomass productivity. The algae production company Cellana has optimized a hybrid cultivation cycle economically for its EPA production at Hawaii (Bai, 2012), using 20 % of the land area for continuous closed photobioreactors and 80 % for open ponds. According to the Cellana patent *(*US 7,770,322) the hybrid system protects a system with fast growing photosynthetic species, both in the closed system and in the open system, with doubling times < 16 hours, till 90% of the

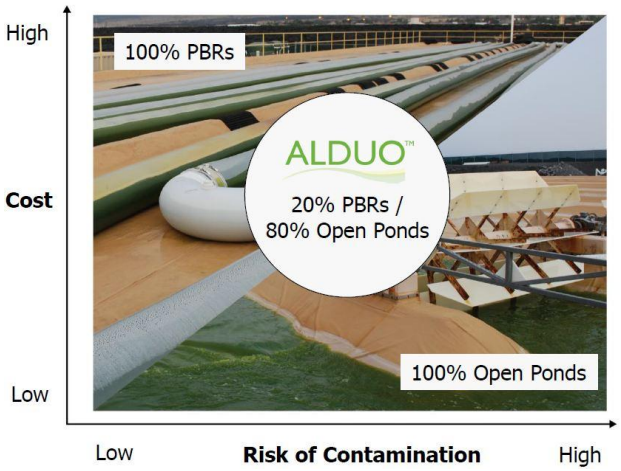


Fig. 13. The Alduo™ system of Cellana is combining closed and open systems. *The systems combines high-productivity at a high cost and low risk, with inoculation of open ponds to obtain larger volumes at lower cost. Source: Sabarsky, 2012.*

"carrying capacity" has been reached but in no more than 5 days in total. From the 90% mark, the production will continue with slower, nutrient limited growth (This contributes to upscale the dense PBR culture to large volume using cheaper production facilities.

Photobioreactor design, orientation and production plant layout: While sites with stable sun light with high irradiation values are most suitable locations, the optimization of design is far from exhausted. An increasing integration of academic competence on microalgae growth with process engineering skills is likely to yield improvements in productivity. Also, panel orientation significantly affects the productivity. Simulation models have shown that north–south oriented PBR panels can produce up to 50% more biomass than east–west oriented panels at higher latitudes (Slegers, 2011). In addition, the height and distance between panels or vertical tubular systems on a production plant can also affect the productivity, and should be designed to accommodate the solar conditions at the specific geographical location (Slegers, 2013).

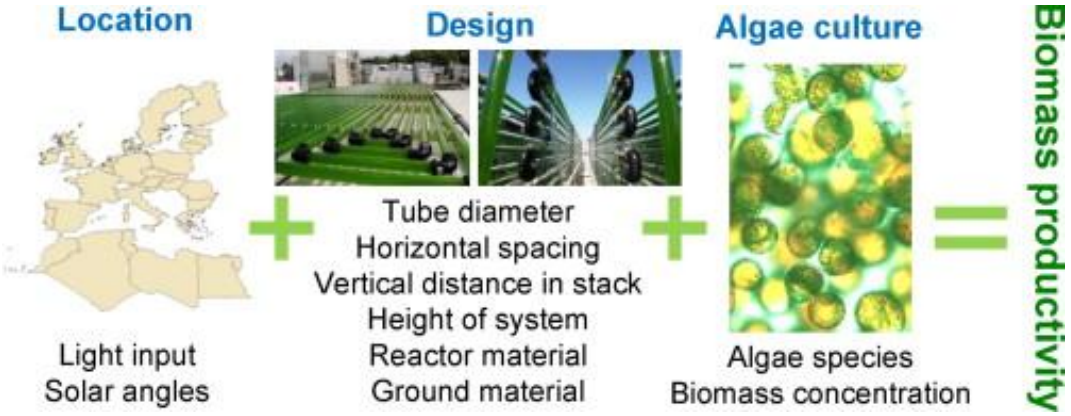


Fig. 14. Variables that affect the microalgae productivity in photobioreactors. *The productivity varies between locations and the varying light input over the day and the year, the reactor design and plant layout (distance between tubes and for vertically stacked systems, the number of horizontal tubes per stack), and the algae species. The optimal combination of these factors will give the highest yield. Figure from Slegers et al. (2013).*

Develop high-productivity strains well-adapted to production conditions: As increased productivity is the key to commercial viability, efforts should be made to ensure the optimal *combination* of all factors – location, design and production strains (Figure 14). The biological productivity can be improved by following various strategies (chapter 3), and by focusing on different targets to increase the photosynthetic efficiency (by reducing antenna size, increasing RuBisCO activity or increase CO_2 concentration in the chloroplast) or to increase lipid formation (direct the carbon flux by up-regulating biosynthetic pathways, down-regulating competing pathways, and knocking out β-oxidation enzymes). Site selection, production design and strain selection should be addressed in parallel to achieve the highest productivity. Sapphire Energy has a dedicated program to develop optimal combinations of algae cultivation systems and strains. A well-adapted and robust strain can double the productivity in outdoor raceways, and contribute to culture stability (Behnke, 2012).

Potential to reduce production costs

Both open and closed systems need to lower production costs by reducing both capital costs and operational costs. Factor that will reduce costs are discussed below.

Low-cost materials: The closed PBR systems are highly productive, but the material costs are high (steel frames and tubes made by glass or polycarbonate). Alternative low-cost materials are explored such as flexible polyethylene tubes, but have a limited life time because of biofilm formation and fouling. However, redesign and development of novel materials may contribute to lower the material cost and enable longer tube life times.

Energy used for mixing: Improvements in simple mechanical engineering are important with respect to the mixing of the culture medium in closed PBRs. Electrical power is used for mechanical mixing in both open and closed photobioreactors and is necessary to keep the algae suspended, to provide sufficient mass transfer which denotes the exchange of oxygen and carbon dioxide, and to obtain a certain level of light integration.

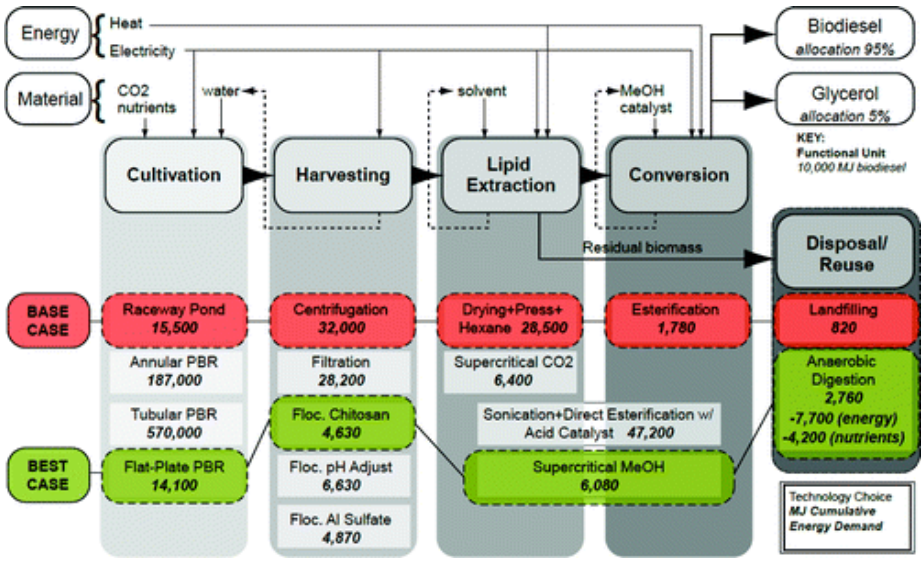


Fig. 15. Example of a system flow-chart used for comparative life cycle assessment. *Various production and processing routes are compared and analysed wrt the environmental impact and energy use. This is common strategy to increase sustainability, but also to improve process design by identifying the most cost-efficient production line. Figure from Brentner et al (2011).*

Most circulation systems use conventional technologies, where energy consumption add a significant cost factors in the operation, but major improvements can be obtained by careful design. The mixing costs for flat panel PBRs consist of the value of the depreciation and of the energy cost of the blowers or compressors delivering the compressed air for sparging. In this case, the pressure at the bottom of the flat panel has a dramatic effect on these factors. For flat panel PBR systems with a 1.5 m tall cultivation vessel, the aeration constituted 52 % of the biomass production costs. However, by reducing the panel height to 0.5 m, a maximum pressure difference of 100 mbar would be required and this would allow the use of low cost, high efficiency blowers for circulation. A redesign would then reduce the production costs from €5.96 (Norsker, 2011) to about 3€ (Norsker et al., 2012). See also chapter 8.

Reduce cost and improve sustainability by optimal process design: The costs and sustainability of microalgae production can be improved by making the right choice of technology for each of the process steps. Comparative life cycle assessments (LCA) are modeling tools that can be used to systematically quantify the impacts of the microalgae production processes with respect to multiple environmental categories, in particular to identify the least energy-demanding and most cost-effective process pathway (Figure 15). In this respect, LCAs are important tools for the industry to make *informed decisions about the process design*, and to address the barriers with most impact on the *environment* or the *cost-efficiency*.

Reduce cost for CO_2 and nutrients by using industrial waste streams: The use of waste CO_2 effluents from industry, as well as waste streams with nitrogen, phosphorus, potassium and trace nutrients for the cultivation of microalgae will reduce the production costs.

4.2 Heterotrophic production by fermentation

Fermentation processes for heterotrophic microorganisms represent well established, large scale technology. Products such as ethanol, citric acid, amino acids, enzymes and antibiotics are produced by fermentation (Figure 16). Organic acids and amino acids represent the largest volumes of products produced in aerobic processes, with existing production plants comprising 10-20 reactors of 300-400 m^3. Production plants for anaerobic processes (e.g. ethanol) employs even larger fermenters.

Figure 16. Different fermenting set-ups from lab-scale, pilot-scale and at the industrial scale (left to right). *Sourcse: SINTEF Materials and Chemistry and www.amyris.com.*

The global fermentation production of amino acids alone amounts to 2.5 mill tonnes annually. Alternative sources of ω3-oils should be able to provide in the order of 100 000 tonnes oil annually in order to be of relevance for the feed industry (Wathne 2011). This corresponds to 25 000 tonnes EPA/DHA, assuming the same content as in fish oil (25 %, see below). Production of 25 000 tonnes microbial DHA will require one or more production plants with a total of 20 fermenters of 350-400 m^3. No technology development is therefore required for heterotrophic production of ω3-PUFA.

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Carbon sources for heterotrophic production

An annual production volume of 100 000 tonne oil will constitute in the order of 2 % of the current global fermentation production (amino acids, organic acids etc), and the additional need for carbon source will be insignificant. Glucose, from hydrolysis of starch, and sucrose are the most common carbon sources for industrial fermentation processes. If the production volume increases beyond 100 000 tonnes, sustainability issues will be of more importance, and waste products, or sources that do not compete with arable land for direct food production, should be applied. Thraustochytrids can utilize glycerol with at least the same productivities as with glucose as carbon source. Glycerol is a waste product from biodiesel production, with a lower price than glucose and sucrose. A considerable research effort is currently directed at development of sustainable feedstocks for biofuel production, in particular lignocellulose, but also on cultivation of seaweed as a carbohydrate source for fermentation. Production of feed ingredients could be part of a lignocellulose or a seaweed biorefinery, where products with higher price than fuels should be a part of the product range.

Potential improvements related to heterotrophic DHA production

Improving the productivity can be obtained by increasing the product concentration, determined by the cell density, lipid content of the cells and the DHA-content of the lipid, and/or by increasing the production rates. The cell density will be limited by the oxygen transfer, viscosity etc, and the lipid content of the cell can probably not be much higher than 70 %. The values reported by Martek for cell density (more than 160 g/l) and lipid content of the cell mass (60-70 %) are therefore probably approaching the maximum achievable.

The highest potential for further improvement of the product concentrations will be by increasing the DHA-fraction of the fatty acids. The DHA-fraction can be manipulated by the cultivation conditions, e.g. oxygen levels (Jakobsen et al., 2008), but significant improvements can probably only be achieved by genetic engineering. As an example, increasing the DHA-fraction to 70 % with the current lipid productivity will increase the DHA-productivity to 17 g/l·d. Such an increase of the DHA-fraction would reduce the DHA-price to 12 USD/kg, corresponding to a fish oil price of 1.9 USD/kg with 10 % DHA (see chapter 8). However, the rate limiting factors for the lipid and the DHA-production is not known, and a better

understanding of metabolic carbon partitioning is required in order to develop strategies based on targeted genetic engineering.

4.3 Mixotrophic production

Some microalgae can grow both phototropic and heterotrophic. It has been suggested that heterotrophic production can be used during the winter season, with poor light availability in Norway. For species that can use light energy and organic carbon sources, this mode of cultivation has been shown to increase both biomass productivity and the levels of EPA in *P. tricornutum* (Fernandez Sevilla, 2004). The practical application of such practice is being discussed, due to the risk of bacterial contamination (outcompeting the microalgae) and the adaptation (reduction of EPA/DHA levels) when phototrophic algae are grown mixotrophically over time. Nevertheless, the commercial production company Cellana is using mixotrophy as a finishing step in their current microalgae production (Bai, 2012).

4.4 Research challenges on cost-efficient production systems

While there is currently a significant gap between the actual production costs and the target production cost, this gap is likely to be closed over the next 5-8 years. There are significant ongoing research efforts invested in algae-to-biofuel projects worldwide, focusing on improving cultivation systems and lowering production costs. In particular, the US biofuel industry has a focus on development of low-cost, high-volume production systems. Aligned with the EU's ambitious renewable energy targets, the EU FP7 call topic on microalgae in 2010 aimed at large scale demonstration of biofuels production from algae with a minimum plantation area of 10 hectares, and *minimum productivity of 90 dry solid tons per hectare per year*. Three large-scale industry-led projects where funded (BIOFAT, INTESUSAL, and All-Gas) with the aim to demonstrate the production of algal biofuels along the whole value chain, covering strain selection to algae cultivation and production, oil extraction, biofuel production and biofuel testing in transportation applications. All three projects will also focus on the development of cost-efficient production technologies.

Another significant initiative is the Algae Production and Research Centre (AlgaePARC) is a 5-year research project established by Wageningen University (NL), where the main focus is to develop knowledge, technology and processing strategies to *scale up* microalgae facilities under industrial settings and optimize biomass productivities. The project aims to demonstrate that microalgae can produce commodities, like biofuel, bulk chemicals and feed products, by reducing the production costs down to 0.5 €/kg biomass. The research focus is on efficient use of sunlight, reduce energy input, use residual nutrients, increased lipid accumulation, strain improvement, up-scaling, systems design and development, and algae biorefinery (www.algae.wur-nl).

There are several technology providers that focus on the development of bioreactor technology. The Institut für Getreideverarbeitung (IGV, Germany) is currently evaluating a new and innovative bioreactor design concept, called the *Horizon*. IGV consider that phototrophic biomass production has a great productivity potential, and this new system is expected to increase the biomass production yield considerably.

To increase the system productivity the following challenges have been identified:

- Selecting the optimal geographical location.
- Further develop cost-efficient hybrid production systems.

- Improve photobioreactor designs for better light utilization, and gain more understanding of orientation and production plant layout.
- Adapt high-productivity strains also to the production condition.

To reduce the production costs the following challenges have been identified.

- Develop PBRs with low-cost materials.
- Reduce energy input used for mixing.
- Improve optimal process design through LCAs, to reduce cost and improve sustainability.
- Explore the use of waste CO_2 sources and nutrient waste streams in microalgae cultivation.
- Explore low-cost carbon sources for hetrotrophic production.

5 Harvesting and processing of microalgae biomass

Chapter summary box

The harvested microalgae biomass needs to be processed by the most cost efficient methods ensuring high digestibility of the EPA and DHA in the algae when used in aquafeeds. The harvested culture volumes need to be separated into algae cells and external culture fluid. There is an ongoing technology development – driven by the biofuel industry - towards more cost-efficient harvesting systems. To ensure high stability and best use in fish feed the biomass must be dried and suitably processed for use in aquafeed. An alternative is to use the dewatered biomass directly without drying, and also include cell disruption to increase the nutrient bioavailability. The most costly processing route is to extract lipids from the algal biomass to produce algal oil.

Research challenges:

- *Development of low-cost dewatering of specific microalgae cultures with high content of EPA and DHA*
- *Development of low-cost drying methods for dewatered microalgae biomass*
- *Develop minimal processing procedure for EPA/DHA-rich microalgae for use in aquafeed*
- *Identify the need for lipid extraction of microalgae biomass*

The goal of this study is to evaluate the use of microalgae as a source for EPA and DHA rich raw material in fish feed. The microalgae raw material can be used in fish feed production as different refined products; being intact algae cells; dried algae powder or extracted algae oil. In contrast to the use of microalgae for biofuel, where a costly extraction of the oil from the cells is crucial, the algae in fish feed production can be used as an unprocessed raw material. However, sufficient preprocessing or processing of the microalgae raw material need to be done in order to ensure optimal digestion of the nutrients from the algae. Figure 17 shows flow scheme of possible processing of microalgae before use in fish feed.

5.1 Harvesting and dewatering the microalgae culture

Cultivation of photo-autotropic microalgae gives lower cell concentration compared to normal heterotrophic production in fermenters. The concentration of microalgae in conventional tubular photobioreactors is reported to reach about 1-7 g/litre. This means that the normal microalgae culture contain less than 0.7 % algae cells.

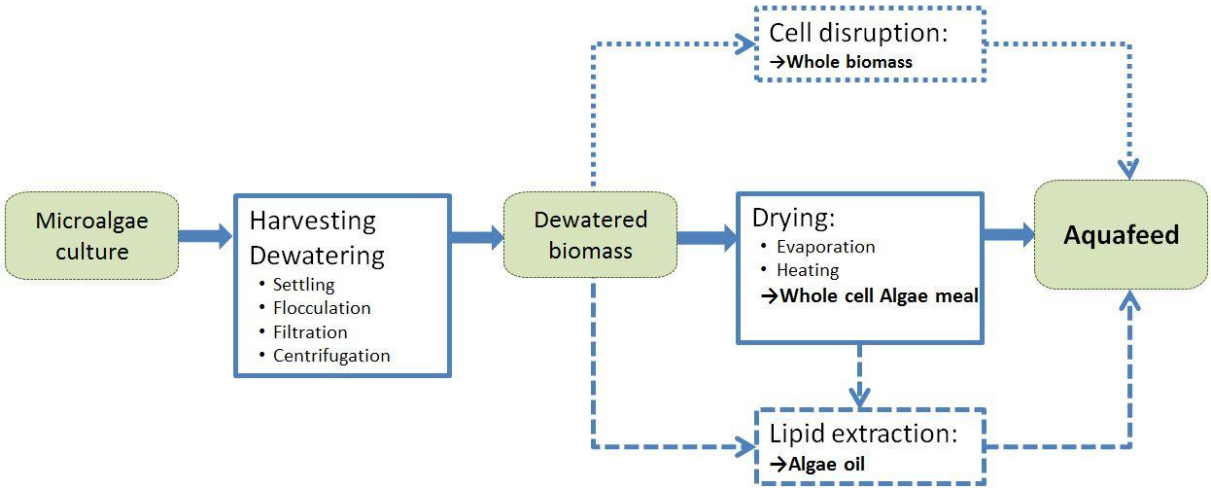

***Fig. 17. Flow scheme showing the processing of microalgae following various routes**. After the initial harvesting process the recovered microalgae biomass can be dried to produce a stabilized ingredient suitable for aquafeed production. An alternative is to use the dewatered biomass directly without drying, and may also include cell disruption to increase the nutrient bioavailability. The most costly processing route is to extract lipids from the algal biomass to produce algal oil rich in omega-3, which can be directly included in the current feed production line.*

At certain biomass density or at regular intervals, parts of the microalgae culture or the whole culture volume need to be harvested. In principle microalgae culture can be run as batch culture or as continuous cultures. Batch cultures are normally grown until they reach the desired cell density and then the whole culture is harvested. Continuous cultures are run over a longer period and a portion of the culture volume is harvested at regular intervals and replaced with new culture medium. At harvesting, a part or the whole culture volume are pumped out of the culturing units, and algae cells in the harvested culture volume must be separated from the culture fluid.

Separating the algae from external water remains a major challenge to industrial scale processing, mainly due to the small size of the algal cells, with unicellular eukaryotic algae typically 3–30 µm. In addition, the relatively dilute cultures of (max 7 g/l) require that large volumes of water need to be processed. The initial harvesting step is not only costly, but also affects any later processes. Conventional techniques that are used today for removing the culture fluid after harvesting involve sedimentation, flocculation and centrifugation. Aggregation of cells by flocculation is an efficient method for harvesting cells. Recent advances in this field utilize natural polymers like chitosans (Beach et al., 2012). The Biopolymer group at NTNU is leading in such chitin modifications and may contribute substantially to improve harvesting of cells in the future. A selection of methods tested or ready for testing is listed (Table 7), some of them as possible pre-concentration steps before centrifugation and be used either separately or in combination. The use of centrifuges involves both high investment and operational costs driven by the energy consumption. In order to reduce dewatering cost, alternative methods for pre-concentration and dewatering methods need to be considered.

Table 7. Different types of dewatering methods used separating microalgae cells and culture fluid.		
Method	Benefits	Disadvantages
Centrifugation Today the most adequate technical solution for concentration of algae cells	*Can be used on several species, size, morphology, density and physiology of the algae cells.*	*Expensive process, the cost of centrifugation for open ponds is calculated to approx. 30% of overall cost (Gudin & Therpenier, 2009).* *High energy requirement at 1-1.4 kWh per m^3 removed (Milledge 2011).*
Settling Sedimentation is the most common harvesting technique for algae biomass in wastewater treatment because of the large volumes treated and the low value of the biomass generated (Brennan, 2010).	*Effective method for large algae (above 70 mm as Spirulina)*	*Cannot be used for small microalgae such as Nannochloropsis*
Chemical flocculation Flocculation is a preparatory step prior to other concentration methods (Molina Grima, 2003; Beach, 2012)	*Addition of different chemicals to bind the algae cells into large and stable «flocs» which can be easily separated.*	*Addition of relevant chemicals is not consistent with the use of algae for food and feed*
Bio-flocculation Addition of an auto -flocculating microalgae to the non-flocculating. The cells aggregate and can be collected, either flotated or settled.	*Increases the intial sedimentation rate and the recovery. Potentially cheap, no chemicals added. Flocculation even at moderate rates reduced the centrifugation energy input from 13.8 to 1.8 MJ kgDW-1 (Salim et al. 2011).*	*Tested on lab scale (Salim et al. 2011), not industrial scale yet.*
Flotation Flotation is use of solved or suspended air (DAF/SAF – Wiley, 2009).	*Effective flotation need chemicals, to form big flocks of algae cells with of finely dispersed air.*	*Cannot be used for food and feed applications.*
Membrane filtering Rotational fine screens or batch filters have limited effect on the majority of microalgae.	*Promising method, cheaper - 0.8 kWh per m^3 (Lemmens, 2012). Reported to be energy efficient as reported by Bhave (2012).*	*New method with limited commercial experience.*

5.2 Drying of marine biomass

After dewatering the microalgae slurry may be stored for some time (up to 1-2 weeks, depending of the species) in the refrigerator or frozen before further use. Cryoprotective agents (e.g. glucose, dimethylsulfoxide) are added to maintain cell integrity during freezing. However, cell disruption and limited shelf-life remain the major disadvantages of long-term preserved algal biomass.

In order to get an algae product that can be stored for later use, drying is an appropriate storage method. However, drying methods are costly and selection of drying technologies will strongly influence the profitability of such industrial process. In the following an overview over different drying technologies and criteria for choosing the technological solutions will be described. The best solution will often be a combination of several drying technologies in a specific order. What solution, and in which order, will typically depend on the design capacity

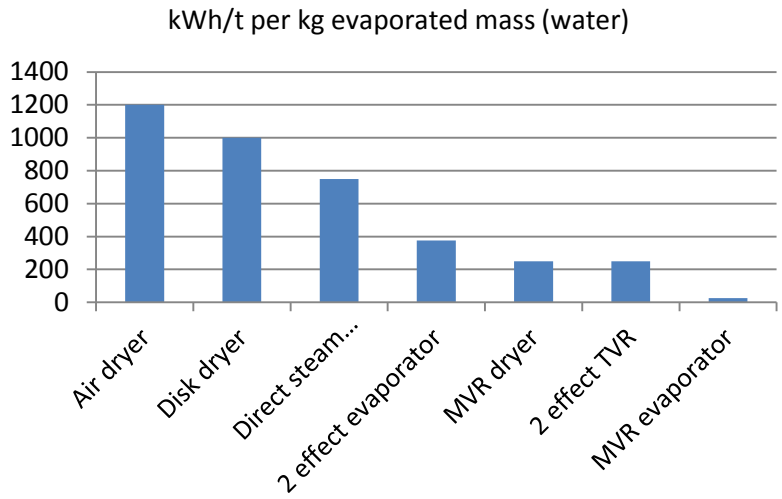

Fig. 18. The energy requirement for different drying methods to evaporate water from biomass. *Source: EPCON evaporation technology.*

of the production plant, the original dry matter content in the liquid and the characteristics in the liquids' with increasing dry substance (and temperature) during the drying process.

Drying technologies

1. Passive drying

Passive dewatering or drainage techniques rely on natural evaporation and drainage to remove moisture. Drainage may occur by gravity or by use of natural temperature.

2. Mechanical drying

These systems require the input of energy to squeeze, press, or draw water from the feed material.

3. Active evaporation and drying.

Evaporation is the most expensive dewatering method, but is extensively used. Normally most of the water will be removed, resulting in a dry matter content of about 90 percent. The most common conventional evaporation process used for waste recycling is agitated thin-film evaporation. This process is capable of handling high-solids content slurries and viscous liquids. It may also be possible to use conventional evaporation equipment commonly found in the chemical-and food-processing industries. These technologies remove water in the form of steam and may also remove volatile contaminants, and the energy consumption varies significantly (Figure 18).

Technologies applied to sludge's that may be applicable to fine-grained sediments include:

a. *Flash dryers*
b. *Rotary dryers*
c. *Modified multiple hearth furnaces*
d. *Heated auger dryers*

Choosing the best technological solutions for separation of microalgae cells and water, and finding a proper drying technology, where intracellular water need to be removed will be important for developing an overall cost efficient process. To ensure applicable industrial solutions it will be necessary to carry out analyses on the liquids and on concentrated raw material (algae concentrates), both in laboratory and in a semi-industrial scale. It is highly recommended that this process involves suppliers of dewatering technologies. These suppliers

have access to semi industrial test units where essential tests for design of industrial water separation equipment can be performed. The results and conclusions from these tests form the basis for the individual design and adaption of each installation.

5.3 Cell disruption and bioavailability

Several methods for disruption of the algae cell wall have been suggested: ultrasonication, bead beating, microwave treatment (at 100°C), osmotic shock (with NaCl), autoclaving (at 121°C) and ball mills (Jeon et al., 2013). The results showed species-specific variation in cell wall resistance. Sonication has the advantage that it works at low temperatures, demanding less energy input to heat the biomass. The methods of microwave and autoclave involve high temperature and can destroy thermo-unstable compounds of the algae. Sonication does not require the addition of beads or chemicals and thus reduce the processing cost. Ultrasonication has been used for cell lysis and homogenization, and could be an effective treatment for breaking up the rigid cell envelopes of microalgae. During ultrasonication, sonic waves are transmitted to the microalgal culture. The waves create a series of "micro-bubble" cavitations which impart kinetic energy into the surface of the cells. The cell wall disruption induce carbohydrate and lipid release from the cell into the exocellular medium (Tiehm et al., 2001). The microwave oven method has been shown to be effective for extraction of lipids. Similar results have been found also for beat-beating. Autoclaving method has been used for *Chlorella* sp. with high efficiency.

Other methods that also have been used are enzymatic methods, detergents, solvents, "cell bomb" method, where high pressure is rapidly released, and high-shear mechanical methods. High-shear mechanical methods for cell disruption work by placing the bulk aqueous media under shear forces that pull the cells apart. These systems are especially useful for larger scale laboratory experiments and offer the option for large-scale production.

5.4 Extraction of lipids from microalgae

Several methods can be used for extraction of lipids from microalgae. They can broadly be divided into two categories: mechanical methods (pressing, homogenization, milling, ultra-sonic assisted extraction, etc.) and chemical methods (hexane solvent extraction, supercritical fluid extraction, Soxhlet extraction, etc). At present, extraction of lipids from algae is mainly carried out by solvent extraction coupled with mechanical disruption (Davis, 2011).

Mechanical extraction methods are widely used in oil in oil extraction from different type of biomasses, though the process design needs to be tailored with regard to algae strain. However, it requires large volumes and biomass residual of the biomass can remain within the pressed oil.

Chemical extraction methods have shown to be effective for extraction of lipids from microalgae paste. The use of solvents is relatively inexpensive and effective, releasing up to 95% of the oil. However, most organic solvents are highly flammable and toxic. Use of supercritical fluid extraction (with use of CO_2) is promising. Extraction and separation are quick, as well as safe for thermally sensitive products. However, the process has challenges in up-scaling. Ultrasonic-assisted lipid extraction and enzyme-assisted extraction are used to break down cell walls and facilitate the liberation of the lipids. This process is safe and environmentally friendly. However, the processes need to be tailor-made for microalgae.

Today extraction of lipids from microalgae is carried out by using solvent extraction (hexane), coupled with mechanical disruption techniques like pressing or bead milling. Most recent

development has been on the use of non-solvent extraction techniques like enzymes, ultrasonic energy, and mechanical disruption. However, little or no testing at pilot or industrial production has been performed.

5.5 Research challenges – minimal processing of algae biomass for aquafeed application

In the project ALGAFEED freeze dried biomass from three different microalgae species were tested as ingredient in aquafeed (Skrede, 2011). Even though the digestibility rates were promising, the conclusion was that a disruption of cell walls before feed formulation may enhance the feed digestibility, by increasing the bioavailability of the nutrients. The method for further processing of algae biomass to aquafeed depends on the algae species, and can just generally be described. Direct drying of an algae concentrate in for instance a spray dryer would be the simplest way assumed that the product get the desired digestibility. Drying is, however, an expensive dewatering method.

If the total algae biomass in the form of a concentrate may be incorporated in the aquafeed mix a great progress is attained. This concentrate has to be stabilized, and to find a practical way of mixing it with dry feed ingredients is critical. According to Meena (2012) the best result is obtained if the cultivation of microalgae take place at the aquaculture plant for direct feeding of living aquafeed. The choice of algae species giving satisfactory digestibility without processing will be an important premise for this option.

One of the most important challenges related to harvesting is to reduce the energy consumption when separating the microalgae from large volumes of water. Most of the techniques that are under development are driven directly or indirectly by the biofuel industry. *Harvesting technique* alternatives to centrifugation were discussed under an LCA study (NREL, 2012), and the membrane technology described by Bhave *et al* (2012) appears to be the best option with an energy requirement at *0.3 kWh/m^3*, compared to centrifugation and dissolved air flotation (1.0 and 0.6 kWh/m^3, respectively). Employing such energy efficient harvesting methods can significantly reduce the most prominent processing cost.

6 Microalgae as resource in aquafeed – status and potential

Chapter summary box

Species/strains of microalgae have been suggested to have a great potential to provide protein, lipids, vitamins, carotenoids and energy in feed for carnivorous fish species. Several microalgae/microorganisms have been tested for use in fish feed and the results shows high digestibility and positive growth effects up to a certain level. Experiments with Atlantic cod and salmon have shown good results up to an inclusion of 6-12% algae dry matter in the feed. The rigid cell wall of some microalgae can be a hinder for their use directly into fish feed, and treatments to disrupt the cell wall need to be done. Because microalgae are a novel resource in aquafeed production, little work has been done on the effects of adding algae oil into fish feed in terms of nutrition but also on the technological challenges in feed production.

Research challenges:

- *Selection of algae strains that have the right nutritional profile and high nutrient digestibility in carnivorous fish*
- *Develop efficient processing method that ensure high digestion of all nutrients in the microalgae*
- *Find optimum inclusion level of microalgae products into fish feed*
- *Study effects of microalgae on physical quality of extruded fish feed*
- *Define optimum feed production technology with use of microalgae as raw material*
- *LCA analysis for using microalgae as fish feed*

6.1 Fish feed composition today

Norwegian aquaculture used more than 1 365 225 tons of extruded feed in 2010. Diets are composed from a blend of ingredients to meet nutrient requirement for support of fast growth, promote high product quality as well as to ensure a good fish health. The chemical composition of the feed varies with development stage of the fish (see Helland, 2007, for review of nutrient requirements for Atlantic salmon). Young fish is fed diets high in protein and relatively low in energy, while energy content and size of pellets increase with growing fish. Chemical composition of diets used in different life stages is shown in Table 68.

Since 1990, the ingredient composition of fish feed used in Norway has switched from marine resources to feed dominated by plant ingredients (Sørensen et al., 2011; Ytrestøyl et al., 2011). The most important protein and lipid ingredients used by the Norwegian fish feed industry in 2010 were: soy protein concentrate, fish meal, wheat gluten, sunflower meal, pea protein concentrate, faba beans, rapeseed oil and fish oil. However, availability of ingredients with high nutritional value is a defined as one major constriction to take out the estimated growth potential of Norwegian aquaculture in 2050 (DKNVS/NTVA 2012).

Table 8. Chemical composition (g per kg dry matter) of diets vary with life stage of fish.

	3-5 mm pellets[1]	**9-12 mm pellets[2]**
Crude protein, g	480 – 500	350-420
Crude lipid, g	250-300	355-400
Starch, g	60-75	56
Energy, MJ	23-24	26

[1] *Used for Atlantic salmon weighing 40-500 g*
[2] *Used for Atlantic salmon weighing 1200 g - Slaughtering*

The major concern in the aquaculture industry is availability and price of marine oils (or sources of the very long-chained poly unsaturated fatty acids EPA and DHA) to maintain a marine lipid profile of the fish feed (Steine et al., 2011). Even though fish has a low requirement for EPA and DHA for optimal growth (approximately 1% of the diet), the content of EPA and DHA in salmon fillet is important for the reputation of salmonids as healthy food for humans. There is a high correlation between content of EPA and DHA in feed and in the fillet of Atlantic salmon.

6.2 Physical quality of feed

Use of novel ingredients may interfere with physical quality of pellets. High-energy pellets (up to 40% lipid), need to withstand stress during conveying, transportation, storing and feeding without forming fines. At the same time, the feed should have a texture and size that facilitate high feed intake and efficient digestion by the fish (Aas et al., 2011).

A review has recently addressed causes for variation in extruded fish feed (Sørensen 2012). Ingredient composition is known to be one of the most important variables affecting physical quality. Care therefore needs to be taken when new recipes are produced and new ingredients are being used. To our knowledge, no studies are published evaluating effects of microalgae on physical quality of extruded fish feed. Two other studies have investigated effect of inclusion level of red yeast and bacterial meal in extruded diets (Aarseth et al., 2006; Øverland et al., 2007). Aarseth et al. (2006) investigated the effect of cell wall disruption (45%, 70% and 97%) on the physical quality of pellets. The authors concluded that pellets produced with highest cell wall disruption had improved hardness, even though the tested products were added in low levels into the test diets, 0.4-0.5%, respectively.

6.3 Microalgae – in aquafeed

Microalgae are natural food resources for fish, as well as for zooplankton in the food chain, and are also extensively used to feed fish larvae, crustacean larvae and mollusks (Shields and Lupatsch, 2012). Based on chemical analysis, different species and strains of microalgae have been suggested to have a great potential to provide protein, lipids, vitamins, carotenoids and energy in feed for salmonids. Brown (2002) reported that microalgae can contain 30-40% protein, 10-20% lipid and 5-15% carbohydrate when grown in the late logarithmic growth phase. Also more valuable nutrients, such as the very long chained polyunsaturated fatty acids vary among species (Patil et al., 2005). During stationary growth phase, the chemical composition and content of valuable nutrients can be manipulated by changing the culture conditions such as temperature, light intensity and adjusting the source and concentration of nitrogen (Brown et al., 2002; Liang et al., 2006; Reitan et al., 1994). Brown et al. (1997) reported that amino acid composition of microalgae species is rather conserved. Therefore, microalgae have a promising nutrient composition, but knowledge about nutrient digestibility need to be obtained for each species. Bioavailability of nutrients and the potential to support growth also need to be explored in long term growth experiments in order to evaluate the potential of microalgae as feed ingredient. The nutritional value of microalgae seem to vary widely among different species (Skrede, et al., 2011). Published literature about use of microalgae in extruded feed for salmonids is still scarce; however some research is carried out with other species.

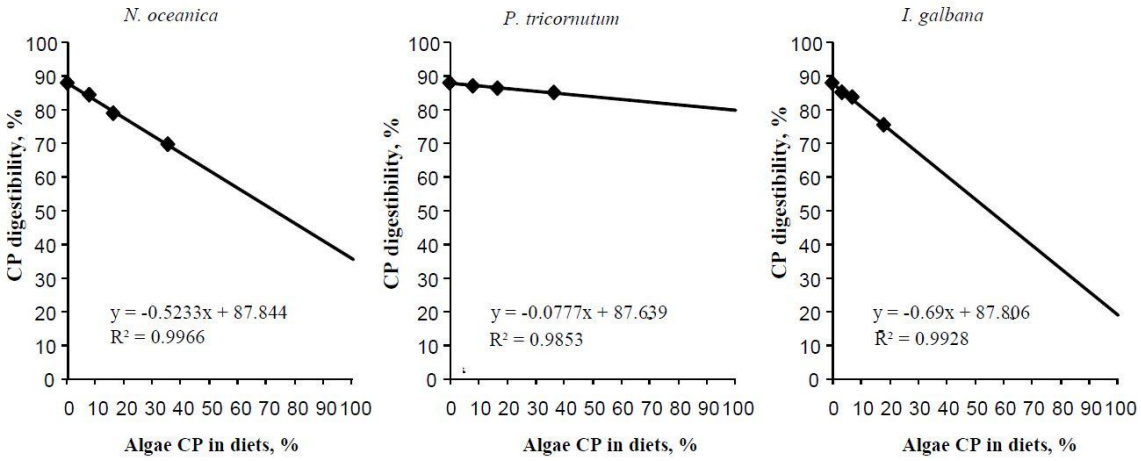

Fig. 19. *Regression lines for apparent digestibility of crude protein (CP) in the three microalgae (y = apparent digestibility of a diet with x% of CP from the algae). From Skrede et al. (2011).*

6.4 Nutrient digestibility

Nutrient digestibilities of the three microalgae species *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana*, were investigated with use mink (Skrede, et al., 2011). A dose-response design was used with the three algae products included in levels of 6, 12 and 24%, respectively. The microalgae were replacing fish meal. The authors found significant linear reduction in crude protein digestibility with increasing inclusion level of all three algae products. *N. oceanica* and *I. galbana* showed negative effects on protein digestibility already at 6% inclusion, while *P. tricornutum* showed negative effects at the highest inclusion level. Based on linear regression, apparent protein digestibility for *N. oceanica*, *P. tricornutum* and *I. galbana*, was estimated to be 36%, 80% and 19%, respectively (Figure 19).

Lipid digestibility was also reduced with increasing level of algae inclusion. Based on results from the latter experiment it was decided to further explore the nutrient digestibility of *P tricornutum* in feeding experiments with Atlantic salmon and Atlantic cod (Reitan et al., 2009). The experiment was designed as a dose-response study with *P. tricornutum* replacing fishmeal, at 0, 3, 6 and 12% level of inclusion. A linear reduction was observed in apparent digestibility of dry matter, lipid and protein for the two species, respectively. Within the same project, another digestibility experiment was carried out, feeding Atlantic salmon the diets with 0, 3% and 6% inclusion of *P. tricornutum*. For this latter experiment, protein digestibility was estimated to 90.1, 89.3 and 89.7 for the three diets, respectively. Lipid digestibility were also high, ranging from 96.0, 95.9 and 95.7, respectively, for the three experimental diets.

No significant differences were observed among the diets for digestibility of dry matter, lipid and protein among the diets. The different results in the two digestibility experiments may be explained by the fact that the highest inclusion level was left out in one of the studies. Based on the two digestibility studies, it can be concluded that *P. tricornutum* can replace up to 6% of the fish meal in diets for Atlantic salmon and Atlantic cod without adverse effects on nutrient digestibility. *Spirulina* may be another microalga with potential for use in feed for salmonids (Burr et al., 2011). The latter authors evaluated apparent protein digestibility for Arctic char and Atlantic salmon fed diets with inclusion level of 30% *Spirulina*. The protein digestibility was estimated to range between 82% and 84.7% for the two species, respectively.

Based on protein digestibility, the presented studies clearly demonstrated that some microalgae have a potential as a fish meal replacer in feeds for salmonids. Nutrient availability and utilization in the diet is, however, highly variable among various genus.

6.5 Growth and feed utilization

Nutrient digestibility does not reveal the full potential of an ingredient to support growth or retained nutrients nutrients in the flesh. Information from digestibility studies need to be combined with long term growth experiments. The nutritional value of *P. tricornutum* was evaluated in a growth experiment with diets containing 0, 3 and 6% microalga, replacing fishmeal (Reitan et al., 2009). The results from this experiment showed no difference in feed intake, weight gain, growth rate, retention of nitrogen and energy among the experimental groups. However, a positive effect of algae addition to the feed was observed on the skin pigmentation of the Atlantic cod (Figure 20).

Other microalgae were explored in a growth experiment with juvenile Atlantic cod (Walker and Berlinsky, 2011). A combination of dried *Nannochloropsis* sp. and *Isochrysis* sp. were used to replace 0, 15, or 30% of dietary fish meal protein. The results showed no significant differences in survival, feed conversion ratios, viscerosomatic indices, and omega-3 and omega-6 fatty acids in the muscle among the treatment groups. However, the authors observed a reduction in feed intake and growth with increasing inclusion level of microalgae that was attributed to palatability problems.

DHA rich oil from another single cell organism, the thraustochytrid *Schizochytrium* sp. has been evaluated in diets fed to Atlantic salmon (Carter et al., 2003) and Atlantic salmon parr (Miller, et al., 2007). Moreover, for Atlantic salmon parr the level of DHA in muscle tissue was increased for the fish fed the thraustochytrid diet.

Fig. 20 *Skin pigmentation of cod that was given microalgae added diets (at the top) and cod that was given control diet (no algae addition, at the bottom). Photo: Elin Kjørsvik, NTNU.*

Carter et al (2003) found no differences in weight gain, feed conversion, organ somatic indices or immune function were observed. However, in a challenge test with *Vibrio anguillarum*, the mortality was significantly lower for fish fed with fish oil.

The cell wall of thraustochytrids is thinner than in other eukaryotic microorganisms (micro-algae, yeast). The chemical composition is poorly characterized, but the cell wall of *Schizo-chytrium aggregatum* have been shown to contain 30-43 % protein and 21-30 % carbohydrate, with L-galactose constituting more than 95 % of the carbohydrate. A *Thraustochytrium* sp. contained galactose and xylose (Darley et al., 1973). From the study by Carter et al. (2003) freeze-dried biomass seemed to be fully digestible, and whether fresh biomass will need any processing to make lipids and protein available is not known.

6.6 Research challenges

As a feed ingredient, microalgae have the advantage that they can be produced under strictly controlled conditions. It is thus possible to produce ingredients without environmental pollutants, one of the major concerns when fish meal and fish oil is used. Compared to fish meal, microalgae have a lower protein content and higher content of carbohydrates, mainly in the poorly digestible cell walls which also impair digestibility of nutrients such as protein and lipid (Skrede et al., 2011). It is shown that the amino acid profile of microalgae is rather similar to fish meal (Skrede et al., 2011). Assuming that technology is developed to improve nutrient bioavailability from microalgae, it is likely to expect that algae lipids and proteins can have a great potential as a aqua feed ingredient. The composition of different algae should be studied in light of fish nutrition requirements, to determine the optimal microalgae (and cultivation conditions) to meet the nutritional specification.

A review has recently addressed causes for variation in extruded fish feed (Sørensen 2012) and ingredient composition is known to be one of the most important variables affecting physical quality. Care therefore needs to be taken when new recipes are produced and novel ingredients are being used. Two other studies have investigated effect of inclusion level of red yeast and bacterial meal in extruded diets (Aarseth et al., 2006; Øverland et al., 2007). Aarseth et al. (2006) investigated the effect of cell wall disruption (45%, 70% and 97%) on the physical quality of pellets. The authors concluded that pellets produced with highest cell wall disruption had improved hardness, even though the tested products were added in low levels into the test diets, 0.4-0.5%, respectively. To our knowledge, no studies are published evaluating effects of microalgae on physical quality of extruded fish feed, and research focus is clearly needed.

PART III
Industrial status and development

7 Industrial microalgae production – status and potential

Chapter summary box

Traditionally, the commercial microalgae industry is directed towards high-value products and low-volume, specialty markets, such as nutraceuticals, cosmetics and food products. The *political will* to develop sustainable algal biofuels in the US have been the key drivers of the industrial technology development to make *controlled* microalgae production more operational, more scalable and more cost-efficient in general. The key element of successful microalgae cultivation lies in the integration of *academic knowledge* with *practical skills* on process engineering and biology. The development is guided by life-cycle assessments (LCA) and techno-economic analyses. Through strategic and consistent political consistent support over decades, there has been accelerated development the recent years. The algae biofuel industry has just recently entered commercialization of algae biofuels based on both heterotrophic (Solazyme) and phototrophic (Sapphire) production, and is currently scaling up production facilities. There is a clear trend among biofuel companies to diversify their product, and explore synergetic opportunities to market co-products while at the same time developing larger scale production to meet the commodity market in the future. Because the production process steps are similar, the technology developments and research advances related to phototrophic biofuels will *directly benefit the development of low-cost EPA/DHA rich biomass* for aquafeed. Heterotrophic production of DHA rich biomass is currently lead by DSM and Alltech.

Industrial challenges:

- *Maximize product value*
- *Develop cost-efficient production lines.*
- *Develop novel value chains*

The microalgae industry today consists of existing production directed towards high-value, low volume markets (cosmetics, nutraceuticals, pharmaceuticals, speciality aquaculture feed etc.), and emerging production for the commodity markets – mainly towards biofuel. There are more than 400 stakeholders in the microalgae business field, and about 75% of them are public or private companies while the others are mainly R&D institutions (BlueBio, 2012). While the algae-to-fuel industry is dominated by American companies, a most of the cultivation for other markets is developed in Asia and Australia.

An important part of the initial project phase was to map relevant international industrial status of microalgal production. Site-visits to relevant commercial and academic microalgae producers (pilot or large scale plants) was conducted, with the intention to learn about these organizations in terms of business structure, production technology and costs, and market. Industrial site visits were conducted at Salata GmbH and NOVAgreen GmbH, both located in Germany (Figure 21), while the pilot plant/academic sector is represented by AlgaePARC (The Netherlands) and IGV (Germany). The companies/organizations are different in many aspects, and it is not possible to compare point by point every topic of interest. There were, however, some important aspects that became clear during the visits and the analyses of business structures and economy, which are common to both the industrial and the academic sector:

- The key element of successful microalgae cultivation lies in the integration of *academic knowledge* with *practical skills* on process engineering and biology.
- Sustainable economy in microalgae production is often based on industrial synergies or available resources such as excess thermal power/heat or CO_2 from other processes.
- Based industrial experiences, start-ups are recommended to focus on high value products from microalgae in the initial phase, and then gradually develop more cost-efficient production that will allow going into commodity products.

Fig. 21. ProAlgae visits at industria production sites. *Left: The pilot production unit at NOVAgreen is a serial V-bag system, where each module holds 4 500 L of continuous culture.* ***Right:*** *The production units at Salata GmbH are glass tube vertical tubual PBR provided and installed by IGV. Photo: H. Kleivdal*

- The microalgae production in North Europe is mainly targeting high-value markets in nutraceuticals and cosmetics, but has an interest in the feed and commodity market.
- There is a clear trend to upscale commercial production in the south of Europe, where A4F in Portugal has installed the World's largest PBR at 1.3 mill m^3.

The ongoing initiatives to commercialize microalgae products are many and diverse (see overview given by Singh *et a*l, 2010). Most of the existing industries are targeting the high-value markets where the operational and economic aspects are quite different from the future production of microalgae as an aquafeed commodity. Therefore, besides a short overview of the high-value markets, this chapter will mainly focus on the emerging biofuel industry, and how this can drive the development of microalgae as a future biomass commodity.

7.1 Industrial Microalgae production of high-value products

About 8000 tons of microalgae are produce annually, mainly for application in cosmetics, nutraceuticals, dietary supplements, pigments (β-carotene, astaxanthin), vitamins and minerals or essential oils (Pulz, 2009). At present, the non-fuel segment includes production of DHAS by traustochytrids and *Schizochytrium* and *Ulkenia*, pigments or biomass extracts from cyanobacteria *Spirulina*, *Nostoc* or *Aphanisomenon*, or fatty acids, pigments and other compounds from microalgae genera such as *Chlorella, Dunaliella, Nannochloropsis, Isochrysis, Phaeodactylum* and others.

Earthrise (California, USA) – the World's leading Spirulina producer

Earthrise® Nutritionals is owned by Dainippon Ink and Chemicals, Inc, which altogether makes the DIC group the largest Spirulina producer in the world. The Spirulina biomass is mostly used for nutraceuticals and health food products.The Earthrise production facility in the Sonoran Desert is the world's largest Spirulina farm, with *open ponds over 15 ha*.

DSM-Martek (USA) – the World's leading heterotrophic producer of DHA

Martek Biosciences, acquired by DSM in 2011, has developed to be a leader in fermentation technology and is an innovator in the research and development of DHA oils derived from *Cryptecodinium* and *Schizochytrium.* Martek has developed and patented the two fermentable strains which produce oils rich in DHA (about 17% DHA). Martek's strains are grown in fermenters that range in size from 80,000 to 260,000 liters, before they are harvested and processed to extract the DHA-rich oil. The product is mainly for application in infant formulas under the brand life's DHA (for cost estimates, see chapter 8). DSM is also

launching novel products with acombination of DHA and EPA (Nutra Ingredients, 2013/02/21). With more than 525 employees worldwide, Martek is headquartered in Maryland with facilities in Colorado, Kentucky and South Carolina. DSM is the major player on the omega-3 nutritional market, and recently confirmed this by the acquirement of the Canadian company Ocean Nutrition.

Salata (South of Germany) – tubular PBR production targeting niche cosmetic markets
The cosmetics industry is the main segment for Salata, and the most important parameters for their customers are relevant volumes and predictability with regards to both delivery and quality. The research focus is novel products within food ingredients, functional foods, omega-3 FA and carotenoids. The phototautotroph production is done in tubular glass photobioreactors (85 000 L total) delivered by IGV (Figure 21), which are placed inside temperature and light controlled greenhouses covering 2 500 $m^{2.}$ This is to keep the production stable and continuous during all seasons. The operation benefits from residual heat from Salata's primary production on processing legumes. The PBRs are suitable for both freshwater and saltwater microalgae, and Salata can routinely produce microalgae like *Nannochloropsis* sp*, Phaeodactylum tricornutum,* and *Chlorella* sp. The total production at Salata is 2-3 tons dw per year - divided into several batches of different strains for different purposes. The main issue to improve cost-efficiency is to increase biological productivity.

NOVAgreen (North of Germany) – scaling up V-bag PBRs based on greentech synergies
The company NOVAgreen established a 1000 m^2 pilot plant close to Bremen in 2004, where the operation is integrated with renewable energy activities. The main product is algae paste for cosmetic applications, but the company also produces microalgae capsules for the health food segment. NOVAgreens patented manufacturing platform based on a unique multi-layer film system, which is implemented with reasonable effort in almost any standard greenhouse (Figure 21). The sophisticated "low-tech V-bag" reactor system is made of hanging polyethylene bags, and can work as a *continuous system* because the culture is circulated through to a common reservoir for degassing and medium replenishment. A maximum density of 3 g/L is achieved with the diatom *Phaeodactylum tricornutum*, and at present NOVAgreen can produce 50 tons dw/ha/year. In collaboration with greentech investors, NOVAgreen is establishing a 3.2 ha production plant in greenhouses with roofs covered with photovoltaic panels to produce energy and provide shade on days of intense solar radiation. The plant is expected to produce up to 150 tons/ha/year of microalgae biomass. The bag system costs 50-60 000 €/ha and is changed approximately once a year.

7.2 Industrial Microalgae production for biofuel

The US drives development of algae production for biofuel
Globally, about 80% of commercial biofuel initiatives are taking place in the US (Singh, 2010). The concept of microalgae lipids as a potential energy source gained momentum during the oil embargo of the early 1970s, when the US Department of Energy (DOE) initiated the *Aquatic Species Program* (25 mill USD total budget during 1978-1996). At present, the US *DOE Biomass program* supports development of algal biofuels by 29 mill USD per year, facilitating technology advancements that accelerate the sustainable production of algal biofuel. This strong focus on domestic energy security has led to increasing budgets for algal research centers, and the development of coherent national strategies on how to unleash the microalgae potential.

In 2010, the US DOE developed the *National Algal Biofuels Technology Roadmap* with the primary objective to highlight the technical challenges and opportunities associated with algal biofuels commercialization (US DOE, 2010). As the largest single user of transportation fuel in the world (300 000 barrels each day), the US Dept of Defense has a goal to replace 50% of the fossil the petroleum (www.nrel.gov/publications).

Driving development of algal biofuels with sound Economics and Sustainability

The *National Renewable Energy Labs* have a strong focus on *sustainability* and e*conomic viability* related to algal biofuels through life-cycle assessments (LCAs) and techno-economic analyses (TEAs). These are important tools for the industry to make *informed decisions to improve the process design*, and to address the barriers with most impact on the *environment* or the *cost-efficiency*. A recent TEA developed by NREL based on conservative base case datasets (Davis, 2011), gave an estimated production cost at 12.14 USD/gal. This is higher than the target market price (3-4 USD/gal), but the study see this target price to be viable for algal fuels based on system improvements.

The biofuel industry is scaling up for commercial production

The potential of microalgal biofuels has led to the investment over 1.5 billion USD into large companies over the recent years, and this has taken large scale microalgae production initiatives very close to commercial deployment for several companies. There are several algaebased companies ranked among the 10 most promising companies in the Biofuels Digest (www.biofuelsdigest.com). Some of the most relevant companies are presented below.

Sapphire Energy – the fastest mover on photoautotrophic biofuel production

Sapphire is an energy company working on algal cultivation, harvesting and extraction of oils from *phototrophic* microalgae. Their "green crude" oil is flexible and can be refined into the three most important liquid fuels used: gasoline, diesel and jet fuel. The green crude is also compatible with existing petroleum infrastructure, all the way to and the retail supply chain. According to Biofuels Digest, Sapphire Energy is the only company that focuses entirely on photosynthetic algae, as most other technology solutions require carbohydrates and sugar in their process, which cannot scale to truly replace petroleum. Sapphire Energy's algae have been bred to tolerate high pH conditions and salty water. According to Singh (2010), Sapphire use proprietary strains that secrete bio-crude oil which rises to the top and can be skimmed. This saves harvesting and processing costs. To further improve biological productivity, Sapphire Energy and Monsanto Company recently announced an agreement to enter a multi-year collaboration, as Monsanto wants access to Sapphire's genetic research technology to use it for its own agricultural development. Sapphire is the fastest mover in the area and is currently in the commercial demonstration phase, and has a test and demonstration sites in Las Cruces and Columbus, New Mexico. The Green Crude Farm in Columbus with a 40 ha open pond facility is now operational (Figure 22), and is expected and produce 1 million gallons of biofuel per year by 2014. The demonstration plant is already producing 2 barrels per day for use by commercial partners, and is currently scaling up. When Sapphire reaches commercial readiness, the plan is to be price competitive with traditional crude oil. Current industry estimates are for algae-based green crude production to result in a 75 – 85 USD/barrel cost at commercial scale. Sapphire has started to deliver 2 barrels per day to Tesoro refining company (www.sapphireenergy.com). Sapphire is listed by *Biofuels Digest* among top 50 companies in bioenergy, and was chosen as one of the top 10 venture-backed, clean tech companies of 2011 by the Wall Street Journal.

Fig. 22 *Sapphire's construction of a 40 ha open pond facility is a major milestone for phototrophic algal biofuel production. Photo: Sapphire*

Solazyme – heterotrophic production of large US NAVY contracts to deliver

Solazyme (San Francisco, CA) is a renewable oil and biochemical production company that transforms a range of low-cost plant-based sugars into high-value oils. Initially, Solazyme is focused on commercializing its products into three target markets: (1) fuels and chemicals, (2) nutrition and (3) skin and personal care. Solazyme use their proprietary biotechnology platform for heterotrophic production, using various carbon feedstocks to produce tailored oils for various applications. Solazyme's lead microalgae strains producing oil for the fuels and chemicals markets have achieved key performance metrics that they believe would allow them to manufacture oils today at a cost below $1,000 per metric ton (0.91 USD per liter) if produced in a built-for-purpose commercial plant. Solazyme has completed production of over 283,000 liters of diesel fuel for the U.S. Navy, in fulfillment of the first phase of its Defense Logistic Agency (DLA) contract that calls for production of up to 550,000 liters in two phases. Furthermore, the company announces contracts with Dow (227 million liters by 2015) and Quantas (200-400 million jet fuel per year). The current production capacity is approximately 8.000 metric tons, but Solazyme expects to be approaching its goal of having 550,000 metric tons of production capacity by 2015. Solazyme is currently listed by *Biofuels Digest* as the most promising company in bioenergy for 2013.

Synthetic Genomics – synthetic biology developments with ExxonMobil at 600 mill USD

Synthetic Genomics Inc. (SGI) and ExxonMobil established in 2009 a multi-year research and development strategic alliance focused on exploring the most efficient and cost effective ways to produce next generation biofuels using photosynthetic algae (www.syntheticgenomics.com). The latter company was established by J. Craig Venter, regarded as one of the leading scientists in genomic research, and known for developing the first synthetic bacterial cell (Gibson, 2008). SGI has world class competence in synthetic biology and will work to discover and develop superior strains of algae to secrete lipids in a continuous manner using cutting edge genomic technologies. ExxonMobil's engineering and scientific expertise will be utilized throughout the program, from the development of systems to increase the scale of algae production through to the through to the manufacturing of finished fuels. The ExxonMobil Algae Biofuels Research and Development Program is a new long term investment focused on biofuel production from photosynthetic algae with a total budget at 600 mill USD over the next 5-6 years, and is still in the first of six research phases.

Strategic industrial research on microalgae production in Europe

There are currently three industry-led EU FP7 projects for large-scale algae biomass production and value-creation: ***BIOFAT*** *(Biofuel from Algae Technologies)* aims for a 10 ha production area in 2015 using PBRs for inoculum and raceways for production; ***ALL-GAS*** *(Sustainable Algae Culture for Biofuels Production)* will use wastewater and a patented "Light enhancement factor" to increase biomass yields in raceway ponds, and produce biodiesel and biogas; ***INTESUSAL*** *(Integrated and sustainable microalgae cultivation with biodiesel validation)* will optimize the production of algae by both heterotrophic and phototrophic routes and integrate technologies to achieve productivities of 90-120 dry ton/ha/year. In addition to the EU projects, the most notable national initiative is the ***AlgaePARC*** project at the Wageningen University Research (NL). In addition to a strong research activity of the University on the application potential of microalgae, the research team will test various cultivation systems and compare them. Based on these results and data obtained from the laboratory, the team will develop a new reactor design for application on commercial scalescale with the aim to optimize production systems to reach the target production cost at 0.5 euro/kg dry weight biomass. The AlgaePARC project harbours 18 industrial partners, focusing both on energy (e.g. Neste oil), green chemicals (e.g. BASF) and nutrition (e.g. DSM, Unilever,Roquette).

Algae biofuel industrial trends and development into other applications

Through strategic and consistent political consistent support over decades, there has been accelerated development the recent years. The algae biofuel industry has now entered commercialization with Solazyme already producing and selling large volumes of biodiesel using *heterotrophic* production technology. At the same time, Sapphire Energy is the main driver of *photosynthetic* produced green crude which is now being commercialized. Both these companies are scaling up their demonstration facilities to larger production plants. This means that significant milestone have been reached when algae can be grown like crop over longer time periods

Another milestone was recently met by a recent report from the *US National Academy of Sciences* on algal biofuels, concluding that *that sustainability concerns are not a barrier to future growth* (US Research Council, 2012). This will significantly increase the political incentive to drive development of the microalgae sector further. The US Department of Energy report 'National Algal Biofuels Technology Roadmap' demonstrate that the future of algae biofuels is bright in many ways (US DOE, 2012).

However, in order for autotrophic biofuel to become economically viable and competitive with petroleum fuels at 3-4 USD/gal, both CAPEX and OPEX must be reduce by 50%, and biomass productivity and oil content must increase (Davis, 2011; Richardson, 2012). Some market analysts think that the successful commercial implementation of algal biofuel must depend on the development of high-value co-products (renewable polymers or pigments). The market size of each co-product should monitor to avoid market saturation and value decrease. If the each of the valuable biomass components are collected, the total value of the individual products will exceed that of the production costs of one unit dry biomass (Wijffels, 2010b). The use of microalgae derived compounds such as highly concentrated EPA/DHA or pigments will require advanced biorefinery technology, and increase costs, but the end product is sold at a higher price. There seems to be a trend that many microalgae producing companies increase the focus on high-value, low volume market.

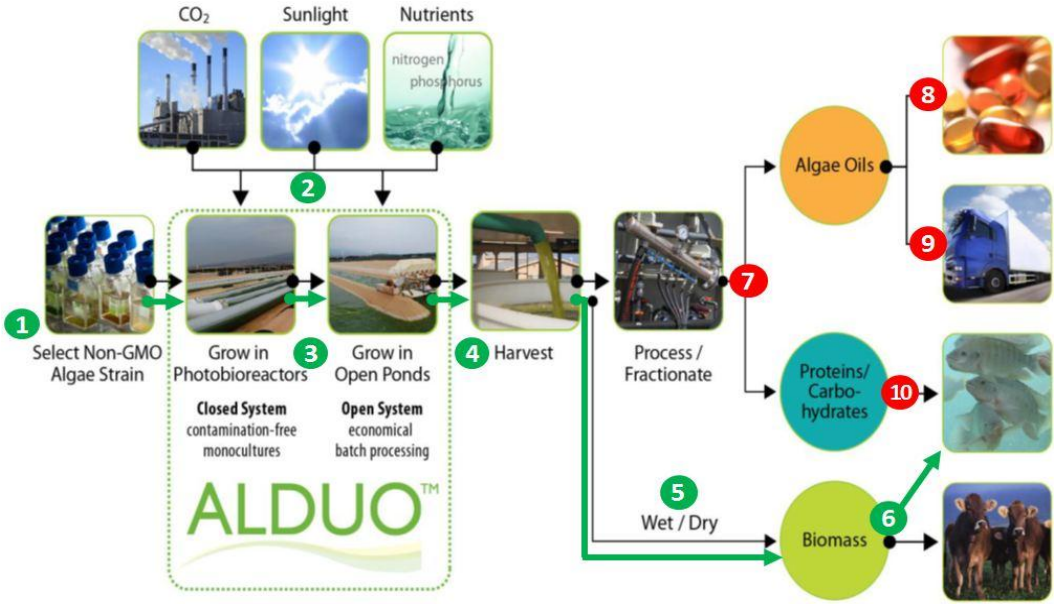

Fig. 23. A flow diagram of the production of microalgae products at Cellana. *The production of wet or dry microalgae biomass for use in aquafeed (**green line along steps 1-6**) are the same as for the production of refined products like high-value oils (EPA/DHA), biofuels and lipid extracted meal for feed (**steps 7-10**). Figure modified from Cellana (www.cellana.com).*

Currently, the largest volumetric co-product from the biofuels industry is the large volume of lipid extracted algae (LEA) that is left after most of the neutral TAG lipids have been removed from the microalgae. The LEA meal is used as a feed ingredient, rich in protein and carbohydrates and with some polar lipid. LEA meal has been used to replace soybean meal in animal feed and aquaculture. Agrifeed (Texas A&M, USA) has developed LEA as a feed ingredient for fish and shrimp, which is priced at about 265 USD/t (agriliferesearch.tamu.edu).

Cellana - developing the biorefinery business modell

There is a clear trend among biofuel companies to diversify their product, and explore synergetic opportunities to market co-products while at the same time developing larger scale production to meet the commodity market in the future. Cellana (Hawaii), formerly HR BioPetroleum, previously focused entirely on the development of algal oils for the conversion into biofuel. However, in 2012 Cellana announced that the company would develop a biorefinery approach to diversify the product range into omega-3, feed, fuel and personal care products. Figure 23 shows how the microalgae production line is the same until the biomass is harvested, before it is routed into wet/dry biomass, high-value oils (EPA/DHA), biofuels and lipid extracted meal for feed.

7.3 Development of microalgae biomass as a feed commodity

The *political will* and market pull to develop algal biofuels in the US have been the most important drivers of the industrial technology development to make *controlled* microalgae production more operational, more scalable and more cost-efficient in general. The biological and technological advances made in the biofuel sector will also enable the development of other sectors.

<u>Similarities</u> of phototrophic biofuel industry with EPA/DHA production

The development in the biofuel and feed sector is no in opposition (Figure 23). The initial technology barriers related to phototrophic algal biofuels are similar to the barriers that must be overcome to develop industrial production of microalgae biomass for use in aquafeed. Table 9 shows a comparison of the process steps of photoautotrophic microalgae production for development of biofuels with the development of EPA/DHA-rich biomass for use in aquafeed. There are ongoing research efforts relevant for both applications to increase the photosynthetic efficiency and the general biomass productivity, which will contribute to reduce the cost per unit.

<u>Differences</u> between phototrophic biofuel industry and EPA/DHA production

The main difference with respect to strain selection and development for the two application areas are related to the lipid content. Strains optimized biofuel production should have high lipid contents, but this may contribute to reduce the overall biomass productivity if nutrient limitation is used to increase the lipid content (see chapter 3.1). However, an increased production of EPA/DHA for use in aquafeed does not necessarily have to include higher lipid content, and may therefore not affect the overall biomass productivity.

While the strain selection and development will differ with respect to that single optimization criterion, the remaining process steps for production of wet or dry biomass for conversion to biofuel are therefore very similar to the production of EPA/DHA rich biomass for aquafeed application. Therefore, the technology developments and research advances related to phototrophic biofuels will directly benefit the development of low-cost EPA/DHA rich biomass for use in aquafeed.

Table 9. Comparison of the photoautotrophic microalgae production of biofuels and EPA/DHA rich biomass for aquafeed.

Process step	Algae biofuels	EPA/DHA biomass for aquafeed
I. Develop optimal algae strains		
a. Increase productivity (PE)	Very important	Very important
b. Increase neutral lipid content	*Very important*	*Not relevant - unless positive for EPA and DHA content*
c. Increase EPA/DHA content	*Not relevant*	*Very Important*
d. Optimize for production (tolerance to temp, pH and high cell density, robust)	Important	Important
e. Develop methods to optimize strains	Important	Important
II. Production/mass cultivation		
a. Improve photobioreactor design	Important	Important
b. Reduce cost on CAPEX	Important	Important
c. Reduce cost on OPEX	Important	Important
d. Optimize resource usage and integrate industrial side streams	Important	Important
III. Harvesting and Drying		
a. Reduce cost on CAPEX	Important	Important
b. Reduce cost on OPEX	Important	Important
IV. Commercial operations		
a. Successful scale-up	Important	Important
b. Stable, continuous production	Important	Important

Development of heterotrophic DHA for feed applications

The production of DHA by the thraustochytrids *Schizochytrium* and the microalgae *Crypthecodinium* were industrialized by Martek Biosciences during the last decade, targeting the high-value product applications in nutraceuticals and infant formula. The production principles relies on mature industrial technology, where the production costs are fairly predictable and well known (see chapter 4.2 and 8.1). DSM and Alltech are currently using the same technology, and are involved in the development of feed ingredients for the aquaculture sector.

DSM Aquaculture and Nutrition: This division of DSM is a leading supplier of vitamins, carotenoids, eubiotics, and feed enzymes to the global feed industry. The focus on aquaculture has not been on DHA supplements for aquafeed as the production price of *Schizochytrium* and *Crypthecodinium* is still prohibitively high. However, such freeze-dried biomass appears to be fully digestible (Carter, 2003).

Alltech (Winchester, KY, USA): Alltech is among the top ten animal health companies in the world, focused on natural scientific solutions to today's biggest agriculture, aquaculture and food industry challenges. The company is the largest producer of protected organic minerals in the world and primary in yeast, algae, and solid-state fermentation systems. Alltech Algae in Winchester, Ky., is one of the largest fully operational commercial algae production facilities in the world. The algae fermentation facility was acquired from Martek Bioscience Corporation (now DSM-Martek) in 2010, and houses a variety of sizes and types of fermenters for growing heterotrophic algae. It is equipped with a fully functional pilot plant - a scaled-down replica of its large production system - that enables our research and applications teams to experiment with new strains and production methods before rolling them out for commercial production. Alltech Algae has been in full commercial operation since April 2011 and is currently focusing on two types of heterotrophic algae, currently licensed from DSM-Martek.

Potential to upscale

The development of microalgae industry is continuous, and the global production capacity is increasing. As technology will progress and biological advances are made in terms of productivity, the new knowledge can be exploited relatively rapid. An annual productivity of 128 tonnes/ha/year may be achievable with good cultivation conditions in the South of Europe, and with a 100 ha plant the annual biomass productivity would be 12 800 tonnes (Draaisma, 2012). As the biological and technological bottlenecks are passed, the development of food and feed commodities are realistic in the 5 years perspective.

8 Techno-economic analysis of EPA/DHA production in microalgae

Chapter summary box

A main challenge for microalgae production is its economic feasibility. This chapter is taking an overall evaluation of three different technologies in two different locations. The input data are based on prior research and technological know-how. Among the three technologies, flat panel reactors, tubular reactors and more commonly known open pond raceways, the innovative flat panel reactors show highest production cost efficiency. Locations in the Netherlands and in Spain were compared as two base cases. The results show that Spain is a far better location due to significant higher irradiation levels, and also cheaper land costs. The base case in Spain for flat panel reactors shows a cost of 39.1 USD per kg EPA/DHA, and a cost of 2.34 USD per kg dry weight.

When evaluating measures to optimize operational efficiency, our scenario analysis points out that costs can be reduced to between 50% and 82% of the base case, given various scenarios such as increased output of EPA/DHA, free CO_2 and nutrients, reduced mixing cost to a technological minimum, or 60% increase in photosynthetic efficiency. A combination of these scenarios can give a cost level of 30 % base case cost, reducing the cost level to 11.88 USD per kg of EPA/DHA.

The main drivers for high production cost efficiency are bio-production factors such as irradiation levels, photosynthetic efficiency, and EPA/DHA outputs, but also capital costs in the form of interest rate can have a severe effect. Localization should be carefully considered as it has a huge impact on the production and design of the facility together with other possible synergies such as free CO2 and easy access to cooling water.

Research challenges:

- Further develop and validate techno-economic analyses.

This chapter will provide an evaluation of the production costs for the production of EPA and DHA in microalgae by both photoautotrophic and heterotrophic production systems based on chapters 1-7 of this report. However, because heterotrophic production systems are considered as a mature technology and the phototrophic production systems are still under development, the main efforts have been placed on analyzing the phototrophic production systems.

8.1 The production of microalgae EPA/DHA by photosynthesis

The objective of this chapter is to provide estimates of microalgae production costs. Our estimates should provide an understanding of the future competitiveness of EPA from microalgae for use in fish feed.

Base case estimates of microalgae production costs should be based on currently available knowledge and technologies. It can be argued that since the 1980s innovations in microalgae production technologies have also changed the cost structure. Several studies have been published in the past decades on microalgae production costs, but these have been based on technologies available at the time of publication; for example, in a manually operated flat panel reactor, labor costs and mixing energy were responsible for 75 % of the total cost of $ 90 per kg dry weight of *Nannochloropsis* sp (Cheng-Wu et al. 2001) or 32.16 USD/kg dry weight of *Phaeodactylum tricornutum* produced in tubular photobioreactors (Grima et al. 2003). The economy of scale also is difficult to assess as the no photobioreactor plants so-far has reached a scale larger than 1 h. In a 300 m^2 tubular pilot reactor plant, the actual biomass production costs were 90 USD/kg while a modest scale up (about 50 times) was shown to potentially reduce biomass production costs to 16.4 USD/kg dry weight (Acién et al. 2012) . Consequently, this should be taken into consideration when employing dated productivity

estimates. However, the estimates provided in this study are based on a combination of data published in peer-reviewed journals and unpublished studies.

8.1.1 Cost-effectiveness analysis

The analysis is limited to cost-effectiveness, and do not provide a net present value analysis. Estimating a net present value analysis would require assumptions on the development of market prices for EPA/DHA, as manifested indirectly in fish oil prices. However, this is outside the scope of this study.

This report provides annual capital and operating costs, cost per unit of dry weight algae biomass, and per kilo EPA/DHA.

8.1.2. Production technology alternatives

Three different production technologies are compared: (1) panel photobioreactors, (2) horizontal tubular photobioreactors, and (3) raceway open ponds.

Raceway ponds have been used in industrial scale (with total plant area >50 ha) for decades, while many pilot scale tubular reactor plants have been made but only a few of appreciable scale (500 – 5000 m^2). The flat panel photobioreactors is the most recent cultivation system developed and is considered to be the most promising in terms of volumetric and land area productivity, but is demanding in terms of required man power for operation and maintenance. To our best knowledge, no large-scale scale flat panel production facility has yet been established for industrial production.

8.1.3 Treatment of uncertainty in the analysis

Estimates of costs are affected by technological and economic uncertainties (or risks) because of imprecision in both underlying data and modeling assumptions. The effects of significant uncertainties should be analyzed and reported. This will be done by means of discussion and sensitivity analysis.

In our analysis we first specify base values for the stochastic variables in the production cost model (base assumptions). We then calculate the production cost – total and unit cost – using these base values. For each variable we assess if the uncertainty is of a magnitude that it deserves a sensitivity analysis, i.e. if the percentage effect on unit cost is significant enough. In the sensitivity analysis we analyze the effect of changing the value of one variable at the time on total and unit cost of production.

The following factors influence expected production costs and the uncertainty surrounding these costs: (1) Photosynthetic efficiency, (2) geographic location choice for plant, and its effect on land price, irradiation conditions, etc. (3) capital equipment purchase prices, economic lifetime, maintenance costs and actual physical capacities, (4) prices and efficiencies of variable inputs in production process, and (5) interest rate.

Photosynthetic efficiency (PE) as reactor specific productivity factor

It is assumed that the 3 reactor types differ in their ability to retain solar energy as energy conserved in the microalgae biomass produced. The current maximum theoretical energy conservation (PE) is 8-10 % (Melis, 2009). This value, however, has been approached only in laboratory photobioreactors whereas outdoor reactors generally are considerably less efficient. We use assumptions from Norsker et al (2011) in the base case. In the accompanying information to this paper, typical PE values for the three reactor types were argued to be

respectively 1.5%, 3% and 5 %. This was based on pooled literature values for different algal species and geographical locations. This approach obviously is biased by historical conceptual understanding and technological level and does not encompass more recent developments. It should also be emphasized that there are ongoing molecular engineering research activities to improve the thermodynamic efficiency of the photosynthesis and subsequent energy conservation over the current level.

Geographic location choice for plant

A critical decision related to the choice of production technology is the geographic localization of a plant. A commercial scale plant that exploits economies of scale will require a substantial land area, in the order of 100 ha to reach an acceptable economical scale of operation. Consequently, the price of land is an important determinant of unit production costs, as we will see later. But the location also influences several important cost drivers in autotrophic microalgae EPA/DHA production:

- Irradiation (sun & cloud conditions)
- Temperature (productivity, particularly of EPA/DHA is reduced at high temperatures)
- Land area prices and land use planning politics
- Access to / price of CO_2Energy supply and prices
- Access to cooling water
- Reclamation or disposal of waste water
- Price and skills of labor
- Price and quality of local/regional suppliers

There are substitution opportunities between factors of production (inputs), and the localization decision influences the composition of inputs. For example, many regions with high irradiation input levels also have high summer temperatures. The negative effect of high temperatures on production can be mitigated by higher input of energy to cool water.

As suggested above, there are tradeoffs between land prices and other costs influenced by the choice of plant location that requires careful consideration. In the analyses in this chapter we have selected candidate locations, but this is based on a preliminary an incomplete analysis of potential production locations.

We have selected for our analyses two “representative” locations for cloudy Northwestern Europe and clear-sky southern Europe; the geographic locations (Eindhoven, Holland and Huelva (Spain).

Capital equipment and operating costs in production process

Assumptions on capital equipment purchase prices, economic lifetime, maintenance costs and actual physical capacities are critical for the economic analyses. In particular the choice of discount rate and depreciation rate has significant influence on the unit cost. We use major equipment purchase prices and related project costing from Norsker et al (2011) in the base case. For operating costs related to non-capital inputs such as energy, materials and labor we use process lay-out and utilities prices from Norsker et al (2011) in the base case.

Interest rate and depreciation rate

The cost of capital invested is the sum of two components: (1) an interest rate which represents the opportunity cost of capital, and (2) depreciation for capital equipment.

An interest rate (or discount rate) of 5 % is used to calculate the opportunity cost of capital, representing the return on an equally risky investment. If the investment had been risk-free, one could have used the interest on a government bond or a risk free bank loan. However, in our case the investment is risky, and rational capital suppliers will demand a risk premium added to the risk-free interest rate which reflect the level of risk, or more precisely the market rate of return of equally risky alternatives if such were available.

In our microalgae production cases, however, it is difficult to find similar investment projects with reported financial returns to use as a basis for choosing a discount rate. Nevertheless, a risk premium should be added, and this is a candidate for sensitivity analysis. Interest rates for risk free investments are presently low. In North-America and Western Europe government treasury notes and bonds typically the rate of return is around one percent to around three percent depending on country and maturity, see for example typical interest rates from the US and EU.

We use a 10% depreciation rate for capital equipment. Since the analysis is done for a representative year, this can also be interpreted as a 10 year average life time for capital equipment.

8.1.4 Base case estimates

Appendix 8.A-8.C present central assumptions for the three alternative production technologies (flat panels, tubular open ponds) in Spain (Huelva) and the Netherlands (Eindhoven). We see in the appendixes that most of the variables have identical values. The main difference between Spain and the Netherlands is the productivity per ha, driven by differences in irradiation conditions. Table 10 shows the total production assumed for the different production technologies for Spain and the Netherlands. We see that flat panels have much higher production than tubular and open pond. Tubular photobioreactors have a productivity that is only 64-65 % of flat panels, while for open ponds productivity is even lower at only 32% of flat panels.

The productivity in Spain is around 80% higher than in the Netherlands for all three production technologies. In addition, land rental costs differ significantly, with a cost that is 67% higher in the Netherlands than in Spain. Equipment capital costs are identical, as well as operating costs.

Table 10. Total production of phototrophic microalgae biomass from a 100 ha plant (dry weight per year)

Technology	Spain	The Netherlands
Flat panels	12 170	6 730
Tubular	7 830	4 383
Open pond	3 915	2 192

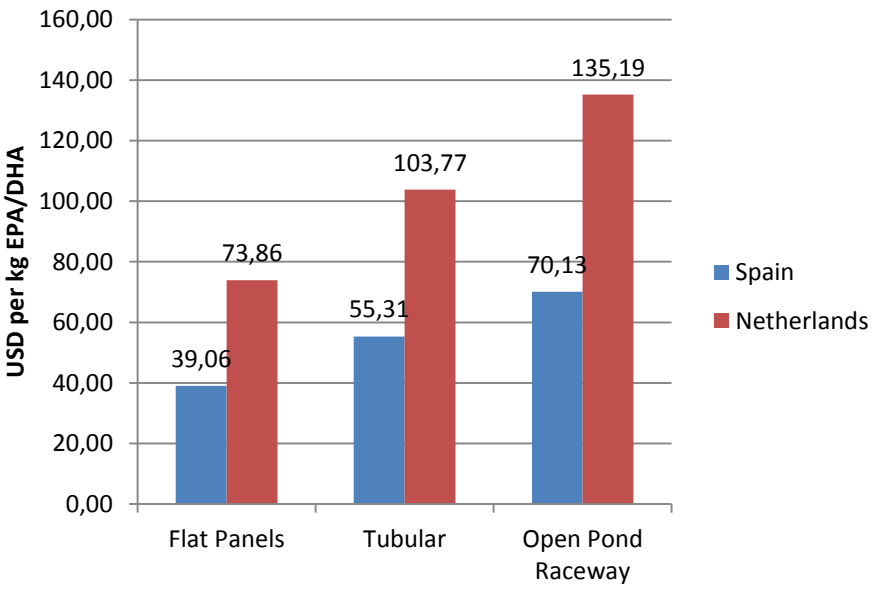

Figure 24. Base case estimates of production costs in USD per kg EPA/DHA equivalents..

Figure 24 shows the base case estimates of the three different production technologies in Spain and the Netherlands. We see that the costs in Spain are significantly lower in Spain under our assumptions, less than 50% of the costs in the Netherlands for all three technologies. Furthermore, flat panels have the lowest production cost of the three technologies in both countries, with 39.1 USD per kg EPA/DHA in Spain and 73.9 in the Netherlands. In both countries cost increase by around 40% as one moves from flat panes to tubular, and by around 80% as one move from flat panels to open pond raceways.

Figures 25 and 26 shows the base case production costs in Spain and the Netherlands measured in another unit of output, USD per kg of dry weight (DW) of biomass produced. Otherwise, all the same base case assumptions apply. Hence, the relative differences of production costs between the three production technologies and the two countries are the same as in figure 24.

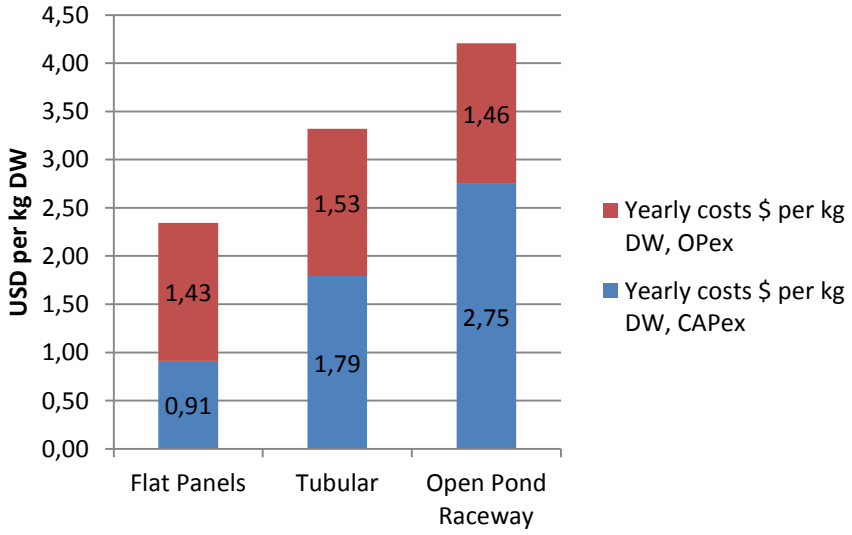

Figure 25. Production costs in Spain in USD per kg DW, split by capital costs and operational costs.

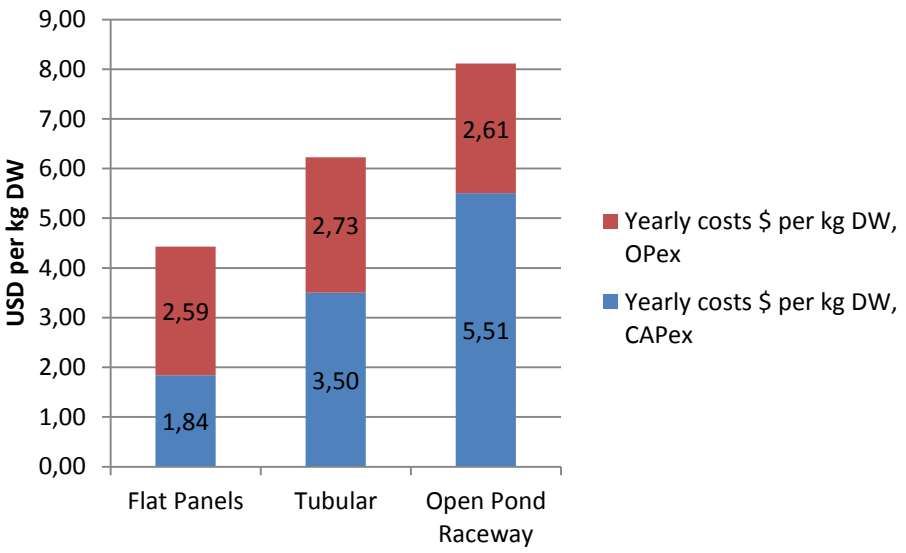

Figure 26. Production costs in the Netherlands in USD per kg DW, split by capital costs and operational costs.

Figures 25 and 26 also show the costs split into capital expenditures (CAPex) and operating expenditures (OPex). We see that the three technologies have different CAPex and OPex shares of total costs. Again they are fairly similar between the two countries. For flat panels CAPex is around 40% of total costs, for tubular CAPex is around 55%, and for open pond raceways CAPex is slightly below 70% of total costs. Furthermore, we see that tubular has almost double the CAPex per unit of output compared to flat panels, while for open pond raceways the CAPex is almost three times as high as for flat panels.

8.1.5 Sensitivity analysis on different optimizations

A sensitivity analysis was carried out using following 4 *individual* optimizations in the form of increased technical efficiencies ("innovations") or reduced prices:

1. PE increased 60% of base case values (1.5, 3, and 5%), while EPA/DHA remains at 6 % DW.
2. EPA/DHA content doubled from 6% to 12% DW, using base case PE.
3. Eliminating CO_2 and nutrient costs, assuming free waste CO_2 (for example flue gas) and nutrient streams (for example cattle manure digestate)
4. Reducing mixing to the minimum technically feasible in the tubular and flat panel reactors, by lowering tubular flow velocity from 0.5 to 0.3 m per second and aeration rate from 1 liter air per minute per liter culture volume

Given the lower irradiation levels and higher cost level in the Netherlands we focus our analysis on production in Spain. In relative (percentage) terms the effects listed above are identical and numbers from the Netherland case can be given upon request.

Figure 27 shows the sensitivity analysis for the four optimizations compared to the base case in Spain. The effects on production costs per kg of EPA/DHA are significant compared to the base case. A PE increase of 60% leads to a production cost which is only 63% of the base case production cost for all three production technologies. The lowest cost is obtained in Spain with 24.41 USD per kg EPA/DHA for flat panels.

When EPA/DHA content is doubled from 6% to 12% DW production cost is reduced to 50% of the base case production cost for all three production technologies. Again, the lowest cost is obtained with 19.53 USD per kg EPA/DHA for flat panels.

Eliminating CO2 and nutrient costs, assuming free waste CO_2 (for example flue gas) and nutrient streams (for example cattle manure digestate) have different percentage effects on production costs across the three production technologies,. For flat panels costs are reduced to 77-78% of the base case production cost, and the lowest cost is obtained with 30.24 USD per kg EPA/DHA.

Reducing mixing to the minimum technically feasible in the tubular and flat panel reactors, by lowering tubular flow velocity from 0.5 to 0.3 m per second and aeration rate from 1 liter air per minute per liter culture volume, have very similar percentage effects on production costs in flat panels and tubular production technologies. For flat panels costs are reduced to 80-82% of the base case production cost, and the lowest cost is obtained with 31.39 USD per kg EPA/DHA.

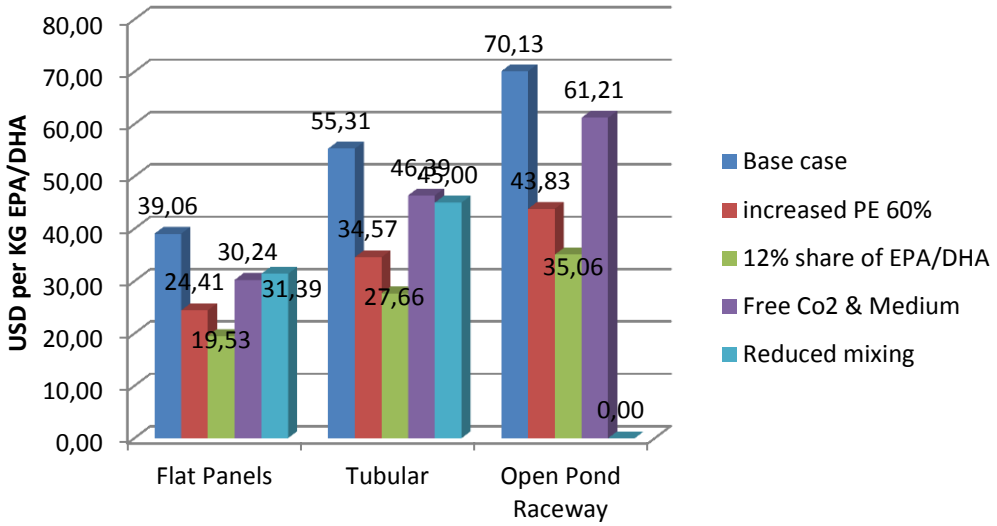

Figure 27. Sensitivity analysis of alternative values for four optimizations compared to the base case in Spain

Next, we examine the effects on production costs of:

1. PE increased 60% of base case values (1.5, 3, and 5%), while EPA/DHA remains at 6 % DW – eliminating CO2 and nutrient costs, and reducing mixing to the minimum technically feasible in the tubular and flat panel reactors.
2. Simultaneously doubling EPA/DHA content from 6% to 12% DW, eliminating CO2 and nutrient costs, and reducing mixing to the minimum technically feasible in the tubular and flat panel reactors.

Figure 28 compare these two alternatives to the base case for Spain. We see that there is a dramatic reduction in production costs. For flat panels alternative 1 has a production cost that is less than 40% of the base case. Alternative 2 leads to production costs that are only around 30% of the base case for flat panels. Also for tubular and open pond production technologies the reduction is significant, but in percentage terms not as much as for flat panels. Hence, the relative advantage of flat panels increases with these changes (Figure 28).

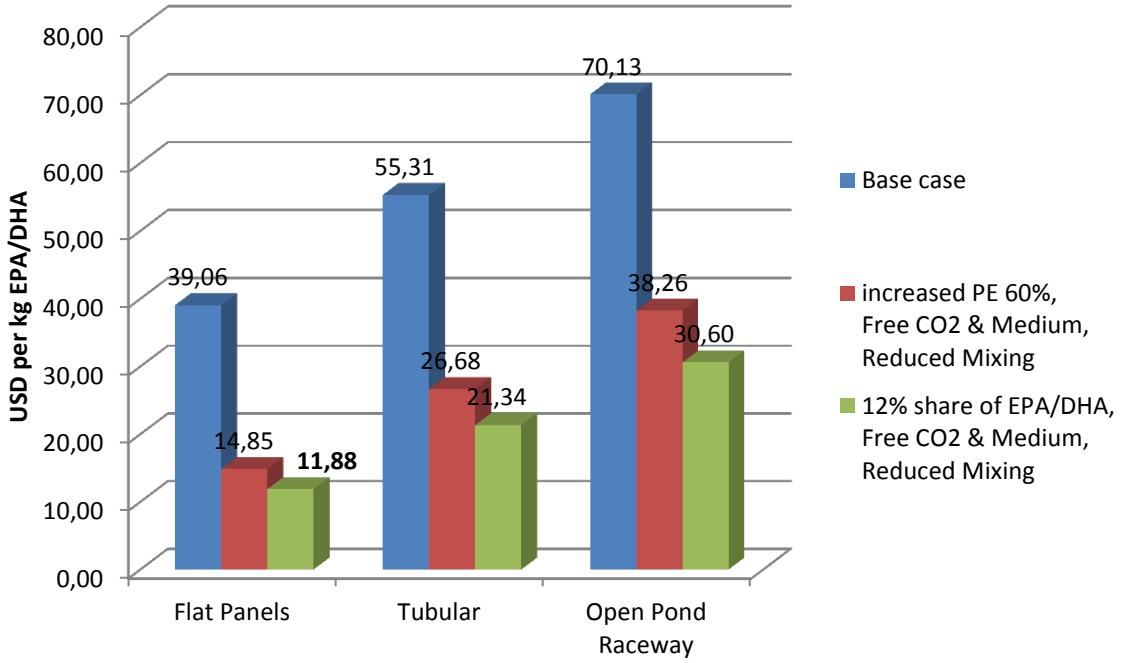

Figure 28. Sensitivity analysis of optimizing either the PE (60%) or doubling the EPA/DHA content - compared to the base case values upon production in Spain.

8.1.6 Sensitivity analysis on capital and land costs

The sensitivity of the production cost estimates to increases in capital and land costs were analyzed. In the following the effect on production cost per kg of EPA/DHA were investigated:

1. An increase of the required rate of return on capital invested from 5% to 15%, to reflect generally increased risk-free interest rates, generally higher returns in equity markets, higher perceived technical-economic risk of the investment projects or higher risk aversion among potential investors.
2. An increase in the cost of land of 50% to reflect a general increase in the property market.
3. An increase in investment cost of 30% to account for higher prices of raw materials and intermediate inputs used in capital equipment, etc.

Figure 29 show the sensitivity analysis of higher capital and land costs relative to the base case in Spain. We see overall that the effects are not dramatic. An increase in the interest rate from 5% to 15% has the most significant effect, and leads to a 21% increase in production costs for flat panels, 31% for tubular and 19% for open pond. An increase in the cost of land of 50% leads to an increase in production costs of 3-5%, depending on production technology. An increase in investment cost of 30% leads to increase in production costs of 11-17%, depending on production technology.

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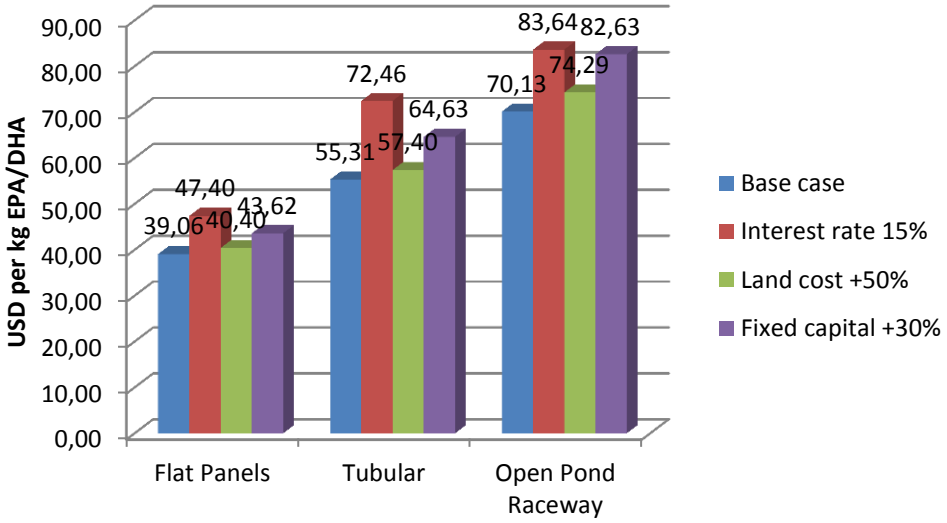

Fig. 29. Sensitivity analysis of higher capital and land costs relative to the base case in Spain.

8.1.7 Summary of findings

This chapter has compared three different production technologies - (1) panel photobioreactors, (2) horizontal tubular photobioreactors, and (3) raceway open ponds – in Spain (Huelva) and the Netherlands (Eindhoven).

The main difference between the two countries in our analysis is the irradiation conditions, which give rise to 80% higher productivity per ha in Spain for all three production technologies.

There are also very large differences in productivity between the three different production technologies, with tubular and open pond production technologies only providing 65% and 32% of the output we have for flat panels technology.

Costs are assumed to be identical in Spain and the Netherlands, except for land cost, which is lower in Spain.

For the base case our assumptions give rise to *flat panel production technology in Spain as being clearly the low cost alternative*. Production in the Netherlands is far from being cost competitive with Spain, as unit costs are around 90% higher in the Netherlands for all three production technologies. Flat panels also emerge as clearly the low cost alternative compared to tubular and open pond technologies. In the base case tubular has 41% higher cost and open ponds 30% higher cost than flat panels. The main driver of the differences between the two countries and the three production technologies is the difference in productivity caused by different irradiation conditions.

The effects of higher rate of return (interest rate) requirements, higher capital costs and higher land prices were analyzed. For the base case low cost alternative - flat panels in Spain - an increase in the interest rate from 5% to 15% has the most significant effect, as it leads to a 21% increase in production costs. An increase in the cost of land of 50% leads to an increase in production costs of only 3%. An increase in investment cost of 30% leads to increase in production costs of 11%. For the other production technologies the effects were of a similar size, and they were also similar for the Netherlands.

We also analyzed the effects of increases in technical efficiencies (or innovations) and reduced prices which are believed to be realistic. An increase in photosynthetic efficiency (PE) of 60% leads to a production cost which is only 63% of the base case production cost for all three production technologies. When EPA/DHA content is doubled from 6% to 12% DW production cost is reduced to 50% of the base case production cost for all three production technologies. When also eliminating CO2 and nutrient costs, this lead to a reduction in flat panels base case costs down to 78% of the original base level. An additional reduced mixing rate to the minimum, give a further total reduction to 82%.

A conclusion to draw from the analysis is that location choice is a critical determinant of production cost efficiency. Irradiation conditions and land prices of different locations have a direct effect on production costs. However, a location decision also requires careful assessment of factors such as access to and price of CO_2, challenges associated with high temperatures, access to cooling water, energy supply and prices, reclamation or disposal of waste water, price and skills of labor, and prices and quality of local or regional suppliers.

8.2 Economical considerations heterotrophic DHA production

For an economical feasible process (15 % internal rate of return on investments) a "value production" of 170-340 USD/m^3·d is required (experience-based value for large scale fermentation plants for amino acid production). Values in the lower range will be sufficient for processes with a simple downstream process. With 195 USD/m^3·d as basis and the highest reported productivity of DHA of 10-12 g/l·d (Table 5), a selling price of DHA of 15.5-19.5 USD/kg will give an economically feasible process (Table 11).

Table 11. Required selling price for DHA for a value production of 195 USD/m^3·d, with selected productivities.

Calculation basis	Productivity [g/l·d]		Required DHA selling price [USD/kg]
	Lipid (TFA)	DHA	
Current status (DSM/Martek)	24	10	19.5
Potential improvements, ex. 1	30	15	13.0
Potential improvements, ex. 2	24	17	11.5

The current price of fish oil is approximately 2.3 USD/kg (figure 2, chapter 1). The oils applied in fish feed typically contain 10 % DHA and 15 % EPA (Ackman, 2005). This makes an ω3 price of 9.2 USD/kg and a DHA-price of 23 USD/kg. However, the fish oil contains EPA as well. When the prices are calculated based on the total ω3 LC-PUFA, the microbial process will be competitive at a fish oil price of 3.9-4.9 USD/kg.

Table 12. Price of DHA from fish oil, with fish-oil prices in the range 1.3-5.20 USD/kg.

DHA content [%]	Fish oil price [USD/kg]			
	1.3	**1.95**	**2.60**	**5.20**
10 % (DHA only)	13	20	26	52
25 % (sum of DHA and EPA)	5.2	7.8	10.4	20.9

The current price and estimated production costs for microbial oils are far higher than this. Possible reasons may be smaller production scale (as a coarse estimate, increasing the production plant from 100 m^3 to 3000 m^3 will reduce the production costs by a factor of 2-4), or that the productivities given in the patent is not obtained in the full scale production process.

9 Risk analysis

Chapter summary box

A SWOT analysis was conducted for the phototrophic production of microalgae based EPA and DHA. The most important strengths: sustainable source at lowest trophic level, original source of EPA/DHA in marine food web and very high productivity. Among the indicated weaknesses: currently high CAPEX for closed systems, high OPEX for mass transfer optimization (circulation), and that a technology development is required to reduce processing costs. The major opportunities are that the technological development will decrease CAPEX & OPEX, that strain selection, domestication and GM strains will to increase productivity, and that the big biofuel industry drive technology development. The identified threats are an increased production of EPA/DHA in transgenic land plants, yeast, bacteria; the lack of strategic R&D perspective and funding, and contamination by grazers and disease organisms.

Risk reducing efforts:

- *Development of technology with positive impact on cost-efficiency, as guided by TEAs and LCAs.*
- *Improve process control and operationalize the biological cultivation process.*

9.1 SWOT analysis

A preliminary SWOT analysis was developed for the industrial production of marine microalgae as a source of EPA and DHA rich raw material in aquafeed (Table 13).

9.2 Risk reduction

The potential weaknesses described above need to be challenged at an early stage in order to meet the goals for industrial production of marine microalgae as raw material in fish feed, while closely monitoring the development of the potential threats. The weaknesses described are mainly in two categories: *i)* challenges that can be overcome by technology or biology developments, and *ii)* the challenges to develop reproducible process and systems control.

In the first category of challenges, the development of technology must have an impact on cost-efficiency of the production or the processing of the biomass. In this respect, the continuous refinement of comparative TEAs and LCAs will be important to give guidance on process design, technology development and research focus. The tight collaboration to internationally leading research and engagement of all stakeholders at an early stage, are important factors to overcome these challenges. Another factor that can indirectly reduce the demand for technological progress, is the parallel technology "race" driven by the big biofuel companies.

The second category of challenges related to process control, will require the multidisciplinary, coherent approach of both biologists and process engineers – in order to industrialize or operationalize the biological cultivation process. This will require bioprocess engineering competence.

Table 13. SWOT analysis.developed for the industrial production of marine microalgae as a source of EPA and DHA rich biomass for aquafeed applications.

Strenghts - internal	Weaknesses - internal
a. Sustainable source at lowest trophic level b. Original source of EPA/DHA in marine food web c. Very high productivity d. CO_2 is used as the carbon-source e. Production has a CO_2 remediating effect f. Production in arid regions possible g. Can be grown in seawater h. Minimal processing – no oil extraction i. Interplay with world leading aquaculture industry	a. Currently high CAPEX for closed systems b. OPEX for mass transfer optimization (circulation) c. Technology development required to reduce processing costs d. Dependent on irradiation e. Biomass production may be variable f. High price/kg due to immature technology g. Lack of interdisciplinary, coherent and coordinated research efforts
Opportunities - external	**Threats - external**
a. Technological development will decrease CAPEX & OPEX b. Strain selection and domestication to increase productivity c. GM algal strains to increase productivity d. Big biofuel industry drive technology development e. Strategic Research funding f. Process integration: greenhouses, waste water remediation, CO_2 bioremediation g. Added value compounds in; carotenoids, glucans, essential amino acids h. Organic feed component? i. Flexible physiology allows combination of cultivation technologies j. Co-production with biofuel lipid (saturated FA)	a. Increased production of EPA/DHA in transgenic land plants, yeast, bacteria b. Lack of c R&D perspective and funding c. Grazers and disease organisms

The climatic conditions may be challenging in that it should balance sufficient irradiation and moderate temperature. The optimal geographical location of the final production facility should be guided by comprehensive techno-economic analyses and comparative life-cycle assessments. As seen in the techno-economic analysis, irradiation has high effect on the production cost efficiency, and should be a key factor to consider. In addition, any potential benefit in form of access to free CO2, nutrients, or subsidized land cost should also be evaluated. Project management, industrial process control, security, engineering and public health and safety are also important factors to focus on for reducing the development of potential risks, and this can be managed by tight integration along the value chain partners.

PART IV
Future perspectives

10 Concluding remarks

Chapter summary box

At present, the price projections made for the heterotrophic production of DHA (19 USD/kg DHA eq) are competitive with the price levels of DHA equivalents in refined or concentrated fish oil. The production cost may be further reduced to 11.5 USD/kg DHA eq, based on a foreseeable productivity increase in the next 5 years.At present the estimated production cost for the phototrophic production of EPA and DHA is 39 USD/kg EPA&DHA eq, when using flat panel reactors in high irradiance regions. A future optimization of productivity, and reduction of production costs, which are realistic in a 5 year perspective have been described in the techno-economic analysis (chapter 8). Based on these projections, the production cost may be further reduced to 11.9 USD/kg EPA&DHA eq. Microalgae production of EPA and DHA has the potential to develop into a sustainable alternative to fish oil for use in aquafeed. This potential can be realized by establishing a *fit-for-purpose* research and development pipeline with integrated research along the value chain In light of the recent price development and the future fish oil price projections, the data presented in tables 14 and 15 suggest that microalgae can develop into an economically viable source to EPA and DHA.

10.1 Microalgae is a future economically viable EPA-and DHA-rich biomass for use in aquafeed

Currently, the best available benchmark values of price levels of various fish oil products are given by Wahren and Mehlin (2011) in table 14. When taking the EPA and DHA contents of the various fish oil products into account, the price of EPA&DHA equivalents (eq) pr kg can be determined.

Table 14. Price levels and volumes of different fish oil products.

Modified table from Wahren & Mehlin (2011). The table has been modified by converting NOK/kg into USD/kg- and by estimating the cost per EPA&DHA unit cost.

Fish oil product	EPA and DHA content	Estimated cost **USD/kg fish oil product**	Estimated cost **USD/kg EPA & DHA equivalent**
Refined oil	30 %	5-10	15-30
Concentrated oil	40-70 %	9-33	27-99
Concentrated oil	70-90 %	20-98	28-137
Concentrated oil	≥ 90 %	98-445	108-490

The outcome of the techno-economic analyses of phototrophic and heterotrophic production of EPA and DHA from chapter 8, are summarized in table 15.

Table 15. Comparison of production costs per unit EPA and DHA based on phototrophic and heterotrophic production

Production cost estimates based on techno-economic analysis and cost projections (chapter 8).

Production principle	Estimated production cost (USD per kg)		
	EPA+DHA	EPA	DHA
Phototrophic production			
Current production cost	39.1	48.8*	156.2*
Production cost after optimization	*11.9*	*15.8**	*47.52**
Heterotrophic production			
Current production cost	19.0	-	19.0
Production cost after optimization	*11.5*	-	*11.5*

**Assuming an EPA:DHA ratio of 3:1*

Phototrophic production of EPA and DHA

At present the estimated production cost for the phototrophic production of EPA and DHA is 39 USD/kg EPA&DHA eq, when using flat panel reactors in high irradiance regions.

A future optimization of productivity, and reduction of production costs, which are realistic in a 5 year perspective have been described in the techno-economic analysis (chapter 8). Based on these projections, the production cost may be further reduced to 11.9 USD/kg EPA&DHA eq.

The potential for improvements are several fold for the phototrophic production, reflecting that this technology is still under development and that unforeseen, innovative leaps can be made.

Heterotrophic production of DHA

At present, the price projections made for the heterotrophic production of DHA (19 USD/kg DHA eq) are competitive with the price levels of DHA equivalents in refined or concentrated fish oil. The production cost may be further reduced to 11.5 USD/kg DHA eq, based on a foreseeable productivity increase in the next 5 years. While the production technology is mature and strains are well developed, a further, significant improvement of the productivity is most likely to occur through strain improvement or genetic modification.

The report of the Scottish Aquaculture Research Forum (SARF, 2011) estimated the cost of heterotrophic produced DHA at 2 200 GBP per ton algal oil. With a normal content of 30% DHA this will equal a production cost estimate at around 12 USD/kg pure DHA equivalents, which compares well with the future cost estimate for DHA by heterotrophic production.

The projected costs for the phototrophic production are also assumed to be further reduced by optimization of output of EPA/DHA to 12%, and using wastestreams like CO_2, wastewater/waste nutrient sources, and other industrial sidestreams. In addition if power cost is reduced through reduced mixing by lowering tubular flow velocity from 0.5 to 0.3 m per second and reducing aeration, the cost can be as low as 15.84 USD/ kg pure EPA equivalent

10.2 R&D Challenges

The overall research challenges identified in this study are related to increasing the biological productivity, and to reduce the production costs. The strategies to meet these challenges are discussed in the respective chapters of this report.

Research challenges to improve the biology potential:

a) Screen the biodiversity to identify novel, productive strains with high EPA and DHA levels.
b) Establish robust and sustainable strains of the selected algae that can be used in industrial production

Research challenges to improve the biological productivity:

a) Develop model systems and molecular tools to allow genetic modification programs.
b) Combine optimal traits and coordinately channel energy into synthesis of EPA and DHA.
c) Develop improved strains with 2-4 times higher levels of EPA and DHA.
d) Develop model systems and molecular tools to allow genetic modification (aimed at light absorption optimizing and directing carbon flow to EPA and DHA production)

Research challenges to improve production systems and reduce costs:

a) Development of low-energy circulation systems for mass transfer
b) Establish cultivation systems using low-cost materials
c) Identify novel strains with optimal production characteristics
d) Ensure sustainability and improve process design through life cycle analysis
e) Improve process design through techno-economic analyses

Research challenges to improve harvesting and processing systems:

a) Development of low-cost dewatering of specific microalgae cultures with high content of EPA and DHA
b) Development of low-cost drying methods for dewatered microalgae biomass
c) Develop minimal processing procedure for EPA/DHA-rich microalgae for use in aquafeed
d) Identify the need for lipid extraction of microalgae biomass

Research challenges for the development of microalgae as a feed ingredient:

a) Selection of algae strains that have the right nutritional profile and high nutrient digestibility in carnivorous fish
b) Develop efficient processing method that ensure high digestion of all nutrients in the microalgae
c) Find optimum inclusion level of microalgae products into fish feed
d) Study effects of microalgae on physical quality of extruded fish feed
e) Define optimum feed production technology with use of microalgae as raw material
f) LCA analysis for using microalgae as fish feed

Industrial challenges:

a) Maximize product value
b) Develop cost-efficient production lines.
c) Develop novel value chains

Fig. 30. The development of an interdisciplinary research and development pipeline to develop microalgae as a aquafeed resource. *The integrated approach will ensure proper integration along the value chain, and connect basic research efforts with application experts.*

10.3 Recommendations

Microalgae production of EPA and DHA has the potential to develop into a sustainable alternative to fish oil for use in aquafeed. This potential can be realized by establishing a *fit-for-purpose* research and development pipeline with integrated research along the value chain (Figure 30), coupled to international centers of expertise in various fields. This should be integrated with ongoing development of industrial microalgae production efforts, to maximize any synergy effects. The continued research on more productive algal strains, more cost-efficient production pathways and dewatering techniques for the development of microalgae biofuels, will be directly relevant for developing microalgae biomass into an aquafeed resource. In light of the recent price development and the future fish oil price projections, the data presented in tables 14 and 15 suggest that microalgae can develop into an economically viable source to EPA and DHA.

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APPENDIX

8.A. Appendix: Base case assumptions for flat panels in Spain and the Netherland

Variable	Spain	Netherlands	Unit of measurement
Photosynthetic efficiency	5	5	%
Annual production per ha per year	24,34	13,46	new numbers
Annual production per ha per year	121,7	67,3	ton/ha ground/yr
Total production per year	12170	6730	ton/yr
CO2 fixation (ton CO2 / ton Biomass)	1,8	1,8	
Lipid production total per year	3651	2019	ton/yr
share of EPA/DHA	0,06	0,06	
EPA production total per year	730,2	403,8	ton/yr
Interest rate	5	5	%
Depreciation	10	10	%
Production area	100	100	ha
Total land area	125	125	ha
Land rental	1 954 545	3 257 575	USD/year
Area tube m2	15,65	15,65	m2
Price / m2	0,21	0,21	$ / m2
Power cost	0,07	0,07	$ / kWh
Power consumption	101 248 699	101 248 699	kWh
Labor, technicians	6	6	person
Labor, engineers	1	1	person
Wage, technicians	45606	45606	USD/year
Wage, engineers	65152	65152	USD/year
Payroll charges	25	25	% of wage
Maintenance cost	0,04	0,04	Per USD of capital equipment
Raw materials			
Polyethylene	3 678 571	3 678 571	m2
Culture medium	6 363 255	6 363 255	kg
Carbon dioxide	11 645	11 645	ton

8.B. Appendix: Base case assumptions for tubular photobioreactors in Spain and the Netherland

Variable	Spain	Netherlands	Unit of measurement
Photosynthetic efficiency	3	3	%
Annual production per ha per year	26,1	14,61	
Annual production per ha per year	78,3	43,83	ton/ha ground/yr
Total production per year	7830	4383	ton/yr
CO2 fixation (ton CO2 / ton Biomass)	1,8	1,8	
Lipid production total per year	2349	1314,9	ton/yr
Share of EPA/DHA	0,06	0,06	
EPA/DHA production total per year	469,8	262,98	ton/yr
Interest rate	5	5	%
Depreciation	10	10	%
Production area	100	100	ha
Total land area	1,3	1,3	ha
Land rental	1 954 545	3 257 575	USD/YEAR
Polyethylene data from Technogrow			
2.5 € /tube d=6 cm; L=83 m)	0,039247892	0,039247892	$/m
Area tube m2			m2
Price / m2			$ / m2
Power cost	0,07	0,07	$ / kWh
Power consumption	47869326,15	47869326,15	kWh
Labor, technicians	6	6	person
Labor, engineers	1	1	person
Wage, technicians	45 606	45 606	USD/year
Wage, engineers	65 152	65 152	USD/year
Payroll charges	25	25	% of wage
Maintenance cost	0,04	0,04	Per USD of capital equipment
Raw materials			
Photobioreator tubes polyethylene (replaced yearly)	17 543 860	17 543 860	m
Culture medium	4 141 138	4 141 138	kg
Carbon dioxide	7 578	7 578	ton
Media Filters	10 881	10 881	units
Air filters	5 775	5 775	units

8.C. Appendix: Base case assumptions for open ponds in Spain and the Netherland

Variable	Spain	Netherlands	Unit of measurement
Photosynthetic efficiency	1,5	1,5	%
Annual production per ha per year	26,1	14,61	
Annual production per ha per year	39,15	21,915	ton/ha ground/yr
Total production per year	3915	2191,5	ton/yr
CO2 fixation (ton CO2 / ton Biomass)	1,8	1,8	
Lipid production total per year	1174,5	657,45	ton/yr
share of EPA/DHA	0,06	0,06	
EPA/DHA production total per year	234,9	131,49	ton/yr
Interest rate	5	5	%
Depreciation	10	10	%
Production area	100	100	ha
Total land area	125	125	ha
Land costs	1 954 545	3 257 575	USD/year
Area tube m2	15,64513141	15,64513141	m2
Price / m2	0,20821653	0,20821653	$ / m2
Power cost	0,07	0,07	$ / kWh
Power consumption	12 650 104	12 650 104	kWh
Labor, technicians	6	6	person
Labor, engineers	1	1	person
Wage, technicians	45 606	45 606	USD/year
Wage, engineers	65 152	65 152	USD/year
Payroll charges	25	25	% of wage
Maintenance cost	0,02	0,02	Per USD of capital equipment
Raw materials			
Culture medium	2 070 569	2 070 569	kg
Carbon dioxide	3 789	3 789	ton
Media Filters	13 138	13 138	units
Air filters	0	0	units